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Synchronization Modulation of the Na/K Pump Molecules Can Hyperpolarize the Membrane Potential of PC12 Cells

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1 Introduction

Previously, we have developed a technique of synchronization modulation (SM) to electrically synchronize the Na/K pump molecules and activate their functions. This technique has been applied to intact skeletal muscle fibers and mammalian cardiomyocytes and successfully activated the pump functions and increased the ionic concentration gradients. Recently, we studied pheochromocytoma PC12 cell line and used confocal microscopic imagining techniques with a fluorescent probe to monitor the SM electric field-induced hyperpolarization in the membrane potential.

2 Materials and Methods

Cultured PC 12 cells were stained in PBS solution with fluorescent dye Tetra-Methyl Rhodamine Ethyl Ester (TMRE), a Nernstian dye. The high permeability and positive charge allows TMRE redistribution across the membrane when the membrane potential changes. The ratio of the equilibrium distribution of the dye molecules across the membrane can be used to calculate the membrane potential by the Nernst equation. TMRE is a slow dye which takes time to redistribute across the cell membrane in response to the membrane potential changes.

An Olympus IX81 inverting confocal microscope utilizing the Fluoview FV500 Tiempo V4.3 analysis package was employed for data collection. Standard Rhodamine optics were employed to graph the observed Fluorescence. When dye staining had reached a maximal effect reflecting an equilibrium state, the SM electric field is applied to the cells. The fluorescence both in the fiber and in the bathing solution was continuously measured every 2 minute.

The SM oscillating electric field was applied to the fiber through two Ag/AgCl electrodes. The electric potential was 30V, peak-to-peak on the two electrodes placed 1.1 cm apart. For a cell with a diameter of 30 μm, the field induced membrane potential was estimated as 40 mV, peak-to-peak. The electric field has a pulsed oscillating waveform with an initial frequency of 10 Hz. After a finite duration of 10 seconds of the stimulation, the frequency was gradually modulated up to a final value of 300 Hz in a stepwise pattern, taking approximately 5 minutes to reach this final value, and then remained at this value.

3 Results

The results are shown in the following Figures. After the fluorescent intensity reached steady-state, showing a plateau in the fluorescent intensity, the SM electric field was applied at 5^{th} minute. **Fig. 1** is a control (nice cells). It clearly shows that the fluorescent intensity was gradually increased due to the application of the SM electric field. **Fig. 2** shows the results (five cells) in the presence of ouabain, a specific inhibitor of the Na/K pumps. The field-induced increase in the fluorescent intensity was eliminated.

These results indicate that the SM electric field induced hyperpolarization in the membrane potential is due to activation of the Na/K pumps.

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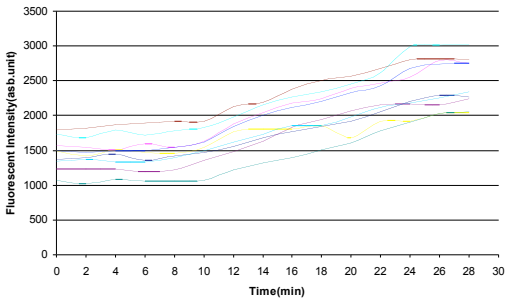

Figure 1 : Synchronization modulation electric field-induced hyperpolarization in the membrane potential.

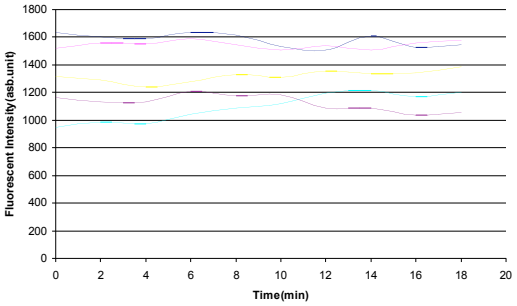

Figure 2 : The SM field-induced hyperpolarization in the membrane potential is eliminated by ouabain, a specific inhibitor of the Na/K pumps.