Monte Carlo Simulations of catecholamine neurotransmitters using cell-attached patch amperometry

June 15, 2007

Akrita Bhatnagar, George Mason University

Mentor: Dr. Joel Stiles, Carnegie Mellon University and University of Pittsburgh

Introduction:

Catecholamines are charged neurotransmitters found in the neuroendocrine cells called chromaffins. The release of catecholamine, through a narrow fusion pore of a chromaffin cell requires a charge compensation by other ions to maintain an osmotic balance of charges. The aim of this research is to show that the exocytotic catecholamine release is not associated with the cation flux through the channels in the vesicle membrane, but the Na^+ influx (which is present outside the cell) through the fusion pore.

This is studied by using the technique of cell-attached patch amperometry, which serves a dual purpose: the pore openings can be characterized by cell attached patch-clamp capacitance measurements while neurotransmitter (catecholamine) release is recorded simultaneously by an amperometric current[4].

Tools such as a patch clamp/pipette and a carbon fiber electrode are used to measure the voltage that is created by a net outward current due to the ion flow, known as the amperometric current [3]. Simultaneously, patch clamp capacitance measurements are also made which are synchronized with the amperometric current [1]. The capacitance of the cell increases as the surface area of the cell membrane increases. Hence, the surface area increases when the vesicle membrane fuses to the cell membrane to form a fusion pore. Therefore, a change in capacitance

is an indication of changes in the surface area of the plasma membrane which occur due to endocytosis or exocytosis (creation of a fusion pore).

The calcium ion concentrations in the pipette can be varied and hence the effect can be measured on the change in flux of catecholamine particles. Also, an external voltage is applied to the patch clamp to measure changes in the membrane capacitance. So the catecholamine ions move under the influence of the presence of both a concentration gradient and a potential gradient, which represents the ‘Electrodiffusion Theory ‘, and it can be physically measured by the Nernst-Planck equation [1].

The release of catecholamines from single vesicle can be detected electrochemically as an amperometric spike. The initial flux of the catecholamines through the narrow fusion pore is measured as an amerometric foot signal which precedes the spike [5].

To represent a suite of models of the patch clamp and carbon fiber electrode with varying dimensions, and for mesh generation a blender is used. A blender is an open source, free of charge tool which is then exported as a Model Description Language (MDL) and run in a MCell3 (Monte Carlo Cell) program to create simulation objects using specialized algorithms. The MCell3 results, that is, meshes are visualized, imported and edited in DReaMM, which is a computer aided design software [1].

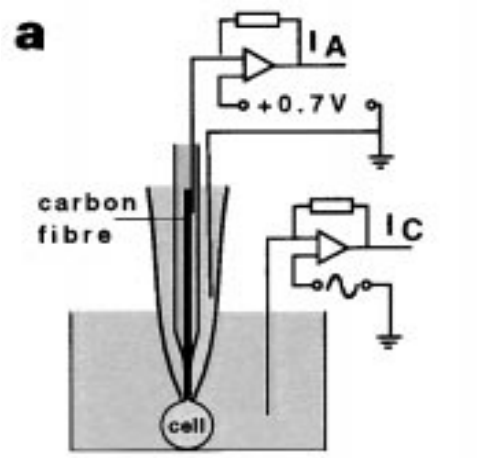

Figure 1: A patch clamp and a carbon fiber electrode attached to a chromaffin cell [2]

Methodology:

First in Blender, a basic model of the chromaffin cell, fusion pore, carbon fiber electrode and patch clamp is created, using basic shapes such as spheres, cubes and so on. Using DreaMM and Mcell, the simulations and 3D structures are formed. Assume that non charged particles flow through the fusion pore. Dimensions such as the distance of the carbon fiber electrode, the size of the vesicle and the size of the pore can be varied. This is done to measure the impact of each factor on the simulations of particles reaching the carbon fiber electrode.

To account for charged neurotransmitter, i.e., catecholamine, the fusion pore is replaced with a transporter, which is attached to an external voltage source. The inside of the cell or vesicle is filled with a matrix.

To analyze the influence of extracellular ion concentration on fusion pore conductance, fill the patch clamp/pipette with varying concentrations of NaCl solution. Maintain the osmolarity by adding glucose and check the effect on the patch amperometry recordings.

Expected results:

As the distance of the carbon fiber electrode is changed, the diffusion distance of the catecholamines to the patch clamp increases, and hence the rate of catecholamine release should decrease, that is, the foot signal should be extended. The ion flux (the amperometric current) should therefore decrease. During the foot, the capacitance should be lower as the fusion pore has a low conductance.

As the size of the vesicle is decreased, the rate of catecholamine release should increase as the emptying rate of a small vesicle is expected to be more. Hence, the amperometric current and the capacitance should also increase, as the conductance increases.

As the size of the fusion pore is increased, more catecholamines are expected to pass through. Again, the amperometric current and the capacitance should also increase, as the conductance increases.

As the concentration of NaCl is decreased, a reduced catecholamine flux is expected, as explained by the electrodiffusion theory. Hence the amperometric current and the capacitance should also decrease, as the conductance decreases.

References:

[1] Center for Quantitative Biological Simulation: The MCell/DReaMM Environment for Microphysiological Simulation. **MCell and DReaMM v. 3.0** http://www.mcell.psc.edu/

[2] Albillos, A., Dernick, G., Horstmann, H., Almers, W., Toledo, G.A. & Lindau, M. The exocytotic event in chromaffin cells revealed by patch amperometry. *Nature* **389,** 509-512 (1997)

[3] Ales, E., Tabares, L., Poyato, J.M., Valero, V., Lindau M. & Toledo, A. High calcium concentraions shift the mode of exocytosis to the kiss-and-run mechanism. *Nature* **1,** 40-44 (1999)

[4] Dernick G., Toledo, G.A. & Lindau, M. Exocytosis of single chomaffin granules in cell-free inside-out membrane patches. *Nature* **488,** 1-5 (2003)

[5] Gong, L.W., Toledo, G.A. & Lindau, M. Exocytotic catecholamine release is not associated with cation flux through channels in the vesicle membrane but Na^+ influx through the fusion pore. *Unpublished*