The Effect of Gramicidin on the Membrane Potential of Neurons in the CNS of *L. stagnalis*

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***Abstract*—Gramicidin is a pore-forming peptide which exhibits lethal properties against a large spectrum of cells. It forms monovalent cation-specific channel in the lipid bilayer of a cellular membrane with limited permeability to anions or polyvalent cations. Both ions and water move through the pore which is formed by the peptide backbone. We detected formation of pores induced by the dimerization of gramicidin molecules by monitoring changes in the membrane and action potentials of neurons in the central nervous system of *Lymnaea stagnalis*. This methodology could be used for the study of peptide interactions with neuronal cellular membranes.**

I. Introduction

Selective ion permeability and conductance are essential functions in all cells. Various agents including peptides can compromise membrane permeability. Gramicidin is a 15 residue polypeptide antibiotic complex that is assembled in helical dimers and forms pores (channels) in the lipid bilayer (Fig. 1) [1-3]. Two monomeric β-helices are joined via their N-termini in the center of the membrane to form a transmembrane dimer with an internal pore diameter of about 3-4Å [4-5]. Gramicidin channel is selective to monovalent ions such as Na^+ and K^+, passage of which through the channel occur together with several water molecules.

The sensitivity of the membrane potential to the ionic concentration gradient provides a measurable indication that the gramicidin channel has been formed. The formation of pores prevents maintenance of the cellular membrane potential [1]. It was observed by monitoring the time series recordings from sharp microelectrodes impaled into the target cell. The methodology could be used to study the peptides interaction with cell membranes..

II. Materials and Methods

A mixture of gramicidins A, B, C, and D form *Bacillus aneurinolyticus* (*Bacillus brevis)* was purchased from the Sigma-Aldrich chemical supply company.

The dissection of *L. stagnalis* begins with the administration of 0.36M $MgCl_2$ to anesthetize the animal. The snail is removed from its shell and pinned to a Sylgard lined Petri dish in a solution of snail saline (NaCl 51.3 mM, KCl 1.7 mM, $CaCl_2$ 4.1 mM, $MgCl_2$ 1.5 mM, with an adjusted pH of 7.4). Micro dissection techniques are used to expose the CNS, and the ganglia are removed intact and transferred to a smaller Sylgard lined Petri dish. The structure is stretched and pinned securely for ease of insertion of the glass microelectrode [7].

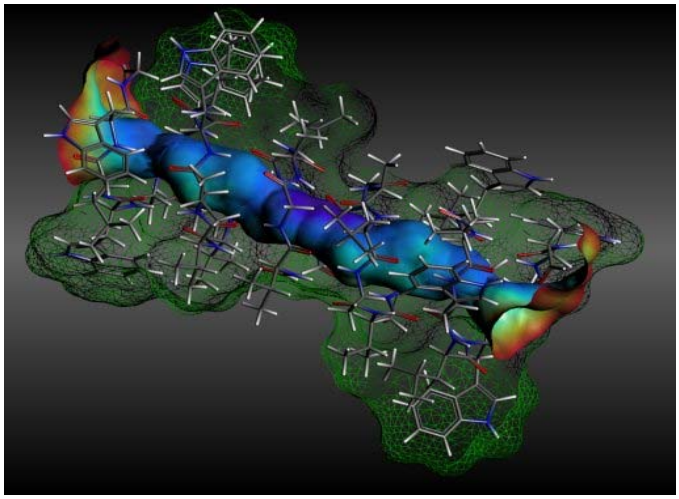

Figure 1. The pore opening of the helical structure of gramicidin can easily be seen in the image (right side) [6]. The channel is formed when the helix translocates across the cellular membrane. The diameter of the central pore is approximately 4 Å and is monovalent cations selective. The blue region represents hydrophilic potential and the red region represents lipophilic potential. (*Image courtesy of MOLCAD GmbH, Darmstadt, Germany*)

The neuron VD4, a known neuron in the dorsal presentation of the visceral ganglion, was identified by visual inspection and was impaled with a glass sharp microelectrode filled with 3M KCl and the membrane potential was measured.

We weighted the gramicidin and dissolved it in DMF. Gramicidin is a hydrophobic peptide and therefore can not be dissolved directly in aqueous solution. It was dissolved in DMF to have 18mg/ml concentration and was added to the 30 ml vessel containing snail saline and the CNS of *L. stagnalis*. Time series recordings were made before the addition of gramicidin, after 10 minutes of adding gramicidin, and after 20 minutes of adding gramicidin. Recordings are made using the Gene Clamp 500 (Axon Instruments) amplifier/stimulator

and the PMD-1608FS Analog to Digital (A/D) converter (Measurement Computing).

As a control, a 200µL solution of DMF, the hydrophilic solvent which was used to dissolve the gramicidin, was used to verify that the results were not due to the interaction between DMF and the cellular membrane.

III. Results

A baseline recording was made before the gramicidin was added to the Sylgard lined dish containing the CNS of *L. stagnalis* and the snail saline (Fig. 2, top). A 200µL solution containing 20µL of 10mM gramicidin dissolved in 180µL DMF was added to the dish. Approximately 10 minutes post addition of the DMF-gramicidin solution, another recording was made (Fig. 2, middle).

What is immediately apparent from the recordings from top to bottom is that the membrane resting potential is fluctuating more as the time in contact with gramicidin increases. The wild fluctuations seen in the bottom recording are most likely due to the fact that more channels are opening in the membrane and monovalent cations (primarily sodium and potassium) are constantly moving down their respective concentration gradients. This diffusion is possible because gramicidin channels are not gated. Another noteworthy effect is the nature of the action potentials themselves. Notice that the height of the action potential decreases from approximately 50 mV initially to just less than 40 mV. In addition to the height, the firing rate increases substantially the longer the gramicidin interacts with the cell. This is due to the trend of an increasing resting potential, as the cell perceives the increase as a signal to fire more rapidly.

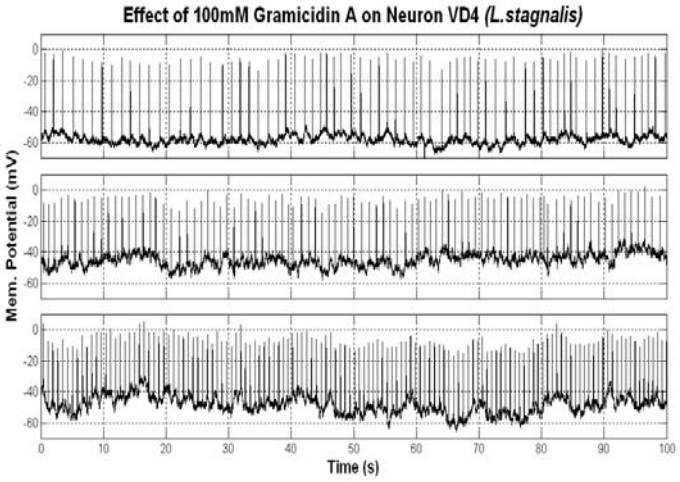

Figure 2. Sharp microelectrode recordings from neuron VD4, a neuron in the dorsal presentation of the visceral ganglion of *L. stagnalis*. The top recording is the baseline recording from the neuron. The middle recording was taken 10 minutes after introducing the solution containing the gramicidin. The resting potential has increased by approximately 10 mV. Note the increase in firing rate, consistent with the increased resting potential, and a reduction in the AP height. The bottom recording was taken 20 minutes post gramicidin. While the resting potential appears to be about equal to the potential taken at 10 minutes, the firing rate has increased, indicating an increase in permeability.

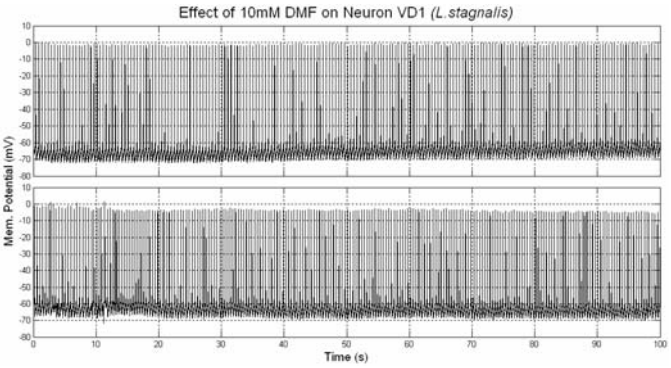

Figure 3. The top sharp microelectrode recording is the baseline time series from neuron VD1. The bottom trace is the neuronal output after adding DMF without gramicidin to the snail saline. This is a different effect than the gramicidin-DMF solution. Note that the action potential height remains constant and the resting potential is only marginally affected.

As indicated, a control experiment involving only DMF was performed to verify that the results seen previously were not an effect of the DMF. Fig. 3 shows the time series recording from the neuron VD1 after adding 200µL of DMF to the 30 ml vessel containing the snail saline and the CNS of *L. stagnalis.* The cellular signal after the addition of DMF remains consistent with the baseline recording. There are other effects observed when adding DMF to this preparation, such as an induced bursting pattern, but the effect on action potential height and resting potential are negligible.

IV. Discussion

The effects of gramicidin on the cellular membrane permeability of a neuron in the visceral ganglion of the pond snail *L. stagnalis* have been presented. We have shown that the effects of gramicidin are detectable using established sharp microelectrode methodologies. The developed protocol could be used to study membrane and amphipatic peptide interactions with the lipid bilayer of cell membrane.

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