Using Hodgkin-Huxley Equations to Determine the Inhibitory Effects of Neurotoxins that Lead to Multiple Sclerosis

Rajvi Babaria

Abstract— Multiple sclerosis (MS) is caused by the degeneration of myelin sheath, which leads to the reduction of axonal action potential conduction, which is initiated by dysfunctional sodium and potassium channels where ions are blocked. Neurotoxins, chemicals that hinder activity of nerve tissue, inhibit the ions' conductances across the neuronal membrane, leading to the development of symptoms associated with MS. However, neurotoxins, such as tetrodotoxin and tetraethyl-ammonium, are used for cancer pain therapies as they block channels to prohibit the transmission of pain signals. An experimentation was conducted that investigated the inhibitory effects of those potent neurotoxins to determine the toxins' effects on voltage-gated channels using the Hodgkin-Huxley differential model. The hypothesis was that of all possible neurotoxin concentrations, increasing concentrations of tetrodotoxin in a mammalian neuron would result in fewer displayed spikes with smaller amplitudes in one sinusoidal period on a voltage-clamp amplifier. The experimentation showed that since there was a decrease in the neuronal membrane's current, the blockages in the channels led to the development of MS symptoms. The derived conclusion also showed that the usage of Pronase as a therapeutic option to treat multiple sclerosis is effective as the proteolytic enzymes served as antagonists to the neurotoxins' impacts.

Index Terms— Biochemistry; Neurophysiology; Neuroimmune disease; Multiple Sclerosis; Voltage-gated channels; axonal action potential

1 Introduction

All neuronal cells have a plasma membrane wherein action potentials, or the crossing of varying ions across the membrane, occur. The stimulation of action potentials allows for the transmission of electrical signals throughout the entirety of the nervous system, which in turn, allows for a living organism to function. Each neuron has "voltage-gated sodium and potassium channels deployed experimentally along unmyelinated axons" that consist of "different amino acid sequences and physiological characteristics" which prevents the occurrence of "persistent neurological deficits (Craner)." The opening of sodium channels "results in membrane hyper-polarization, or a more negative potential", the opening of potassium channels "results in membrane depolarization, or a less negative potential", and the opening of chloride channels "results in membrane depolarization, or a less negative potential (Lodish)." These ionic channels play significant roles in the rate by which electrical impulses travel along a neuronal axon, the prevention of neurological disorders and diseases, and the maintenance of immune functionality in the nervous system, specifically the central nervous system.

One autoimmune disease that is associated with the deterioration or destruction of such ionic channels and other neurological structures is Multiple Sclerosis. The progressive neurological deterioration of nerve cells and the alteration of sodium and potassium ionic channel expression have been considered the two major pathophysiological causes of the evolution of the disease. The "deployment of additional sodium channels to demyelinated parts of the axon" provides a molecular substrate and ensures the improvement of ionic channel expression (Waxman). Action potential propagation is necessary for neuronal excitation in order to effectively establish adequate connection and communication between diverse structures in the nervous system. Therefore, the differentiation and dysregulation of voltage-gated sodium channels and potassium channels can trigger aberrations in the excitability of neuronal membranes. Furthermore, "failure of axonal action-potential conduction" can exacerbate multiple sclerosis symptoms, including the "remission of clinical deficits", which results in the deterioration of voltage-gated ionic channels that are concentrated at the Node of Ranvier, the site of action potential propagation (Lodish). Footer. Click inside the text box to type the name of the journal the article is being submitted to and the manuscript identification number. Click the forward arrow in the pop-up tool bar to modify the header or footer on subsequent pages.

2 Rationale

The expansion of research regarding this disease is imperative to both the scientific and medical society as insight can be provided that describes the aftermath effect of cancer pain therapies and how the attempt to eliminate one disease can lead to the development of symptoms associated with another disease. The most effective way to investigate the applications of this research is to use the hodgkin-huxley model; the Hodgkin-Huxley is a calculus-based model that consists of analytic formulas that describe the underlying mechanism for the firing of action potentials through which information is propagated in the nervous system. The model implements theoretical physics, the path integral, and is dependent on the functionality of ionic channels. The mathematical model investigates the synergistic action of ionic channels, which are inclusive of sodium and potassium ions, and provides an asymptotic analysis of the level of "rapidity of action potential initiation (Colwell)." The usage of the non-linear differential equation model allows for the further exploration of ionic conductances in neuronal cells to inevitably understand ionic channels' functions as molecular targets.

3 HYPOTHESIS

This research was designed for three prime purposes: conduct virtual experimentation to investigate the characteristics of neurotoxins by inducing concentrations of the toxins on a functional neuron, analyze the biochemical processes that occur in mammalian neuroimmune systems to determine the ionic changes that result after the stimulation of neurotoxins, and to determine which drug or combination of enzymes can be used to prevent the negative implications of the neurotoxins. To investigate the relationship between multiple sclerosis and cancer pain-relief therapies, a hypothesis was formulated being that increasing concentrations of tetrodotoxin, an alkaloid neurotoxin that blocks the voltage-sensitive sodium channels, in a mammalian neuron will result in fewer displayed spikes with smaller amplitudes in one sinusoidal period on a voltage-clamp amplifier. This would indicate that the usage of tetrodotoxin for the treatment of cancer pain can potentially result in the development of systems associated with multiple sclerosis.

4 PROCEDURE

4.1 Part 1: Setup on Virtual Simulator

1. Placed Mammalian brain slice in saline solution under microscope to keep cell membranes preserved
2. Placed two voltage clamps on the brain slice and applied suction to effectively record conductance of cell membrane

4.2 Part 2: Simulated Conductance for Resting Potential (Controlled neuron)

1. Set the intracellular and extracellular ionic concentrations for Sodium (Na+), Potassium (K+), and Chloride (Cl-) based on generalizations of the Nernst equilibrium in a temperature of 37 degrees Celsius
2. Calculated the membrane potential of each ion (Na+, K+, and Cl-) using the Goldman-Katz equation
   Part 3: Simulated Conductance for Action Potential (Controlled neuron)

4.3 Part 3: Simulated Conductance for Action Potential (Controlled neuron)

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current that derived the action potential was stimulated
2. Created a stimulus that generated a current and excited the axon of the neuron to successfully activate or polarize the neuron
3. Calculated the ionic membrane current of the neuron using equation from Hodgkin-Huxley model after 1 ms.

4.4 Part 4: Induced 50% tetrodotoxin concentration on the neuron in its active potential stage (neuron affected by stimulus 1 current)

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current was stimulated to derive the action potential with 50% tetrodotoxin
2. Calculated the ionic membrane current of the neuron using equation from Hodgkin-Huxley model after 1 ms.

4.5 Part 5: Induced 100% tetrodotoxin concentration on the neuron in its active potential stage (neuron affected by stimulus 1 current)

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current was stimulated to derive the action potential with 100% tetrodotoxin
2. Calculated the ionic membrane current of the neuron using equation from Hodgkin-Huxley model after 1 ms.

4.6 Part 6: Induced 50% tetraethyl-ammonium concentration on the neuron in its active potential stage (neuron affected by stimulus 1 current)

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current was stimulated to derive the action potential with 50% tetraethyl-ammonium
2. Calculated the ionic membrane current of the neuron using equation from Hodgkin-Huxley model after 1 ms.

4.7 Part 7: Induced 100% tetraethyl-ammonium concentration on the neuron in its active potential stage (neuron affected by stimulus 1 current)

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current was stimulated to derive the action potential with 100% tetraethyl-ammonium
2. Calculated the ionic membrane current of the neuron using equation from Hodgkin-Huxley model after 1 ms.

4.8 Part 8: Determined the top two percent concentrations of either tetrodotoxin or tetraethyl-ammonium that result in the most impactful effects of multiple sclerosis

1. Using the collected data and voltage clamp amplifier graphs, the two toxin concentrations with the least spike amplitude and least number of spikes were found (this indicates the extremities of the concentrations)

4.9 Part 9: Using results from part 8, induced a 100% concentration of the drug pronase on the active neuron (neuron affected by stimulus 1 current) with a combined concentration of the determined toxin concentrations

1. Using the same intracellular concentrations and extracellular concentrations of Sodium, Potassium, and Chloride as the concentrations from the resting potential of the controlled neuron, a current was stimulated to derive the action potential with combined concentration of 100% tetrodotoxin and 100% tetraethyl-ammonium and 100% pronase concentration.

2. Determined the amplitude and number of spikes from the voltage clamp amplifier graph that described the effects of pronase

5 FIGURES

By utilizing the Virtual Amrita Laboratories Universalizing Education Software, images of voltage clamp graphs were created to then categorize data into computational-based and experimental-based tables.

5.1 Resting Neuron Data

	Intracellular Ionic Concentration (millimeters)	Extracellular Ionic Concentration (millimeters)	Membrane Potential (millivolts)
Na+ (Sodium ion)	5	145	90
K+ (Potassium ion)	140	5	-89.06
Cl- (Chloride ion)	4	109	-88.34

Fig. 1. Intracellular and extracellular ionic concentrations derived using goldman-katz equation

Figure 1 displays the values of each membrane potential for each ion given the intracellular and extracellular ionic concentrations, which were calculated using the Goldman-Katz equation. The computed values represent the typical membrane potential, measured in millivolts, for a mammalian neuronal cell, The voltage-clamp graph of the neuron in its resting potential state expresses a constant membrane voltage for each ionic channel, thus suggesting a lack of neuronal activity. The absence of neuronal activity is a result of electrochemical signals not being fired by the neurons. This state is similar to the state of a neuron that has damaged nerve cells due to Multiple Sclerosis.

5.2 Active Neuron Data - No Toxin Induction

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-65	0	-0.001	0.014
0.1	-66	0	-0.001	0.013
0.2	-67	0	-0.001	0.013
0.3	-68	-0.001	-0.001	0.012
0.4	-69	-0.001	0	0.011
0.5	-70	-0.001	0	0.01

Fig. 2. The ionic current values during 0.5 ms interval for an activated neuron with no toxin induction

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
0	0.007	-0.002	0.005	-7.995

Fig. 3. The ionic membrane current of an active neuron

Because Figure 2 and 3 display the ionic membrane current for the action potential state with 0% neurotoxin induction after 0.5 ms, which was -7.995 mV: this suggests that there was an outward current or an influx of outwardly flowing potassium ions. The excess potassium ions caused the derivative of the ionic membrane current to become more negative, thus causing the neuron to polarize and become more active.

5.3 Active Neuron with 50% TTX Induction

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-65	0	-0.001	0.014
0.1	-66	0	-0.001	0.013
0.2	-67	0	-0.001	0.013
0.3	-68	-0.001	-0.001	0.012
0.4	-69	-0.001	0	0.011
0.5	-70	-0.001	0	0.01

Fig. 4. The ionic current values during 0.5 ms interval for an active neuron with the induction of 50% TTX Concentration

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
-0.001	0.007	-0.002	0.004	-7.996

Fig. 5. The ionic membrane current of an active neuron with 50% TTX concentration

Figure 4 and 5 display the ionic membrane current for the action potential state with 50% Tetrodotoxin induction after 0.5 ms was -7.996 mV: since the value was greater than the ionic membrane current for the 0% neurotoxin induced neuron, this neuron had a reduction in sodium ions and an increase in potassium ions. There was a decrease in the voltage for this action potential and a spike amplitude of 133 mV with a spike frequency of 2 over a 47/30 sinusoidal interval.

5.4 Active Neuron with 100% TTX Induction

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-65	0	0	0.014
0.1	-66	-0.001	0	0.013
0.2	-67	0	0	0.012
0.3	-68	-0.001	0	0.012
0.4	-69	-0.001	0	0.011
0.5	-70	-0.001	0	0.01

Fig. 6. The ionic current values during 0.5 ms interval for an active neuron with the induction of 100% TTX Concentration

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
0	0.007	-0.002	0.005	-8.995

Fig. 7. The ionic membrane current of an active neuron with 100% TTX concentration

Figure 6 and 7 display the ionic membrane current for the neuron with 100% tetrodotoxin induction after 0.5 ms was -8.995 mV, which suggests the greatest reduction of sodium flow or a significant blockage in the sodium channel. There was a decrease in the voltage for the action potential and a spike amplitude of 4 mV with a spike frequency of 4 mV over a 4pi/3 sinusoidal interval. The blockage was more severe than the one expressed with the active neuron with 50% TTX induction

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-64	0	-0.003	0.007
0.1	-64	0	-0.002	0.007
0.2	-65	0	-0.002	0.007
0.3	-65	0	-0.002	0.007
0.4	-66	0	-0.002	0.006
0.5	-66	0	-0.001	0.006

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
-0.001	0.005	-0.001	0.003	-3.997

5.5 Active Neuron with 50% TEA Induction

Fig. 8. The ionic current values during 0.5 ms interval for an active neuron with the induction of 50% TEA Concentration

Fig. 9. The ionic membrane current of an active neuron with 50% TEA concentration

Figures 8 and 9 display the ionic membrane current for this neuron after 0.5 ms was -3.997 m V. which suggests that although it is negative, it has a greater influx of sodium ions in comparison to the other data values because the value is more positive. There was a decrease in the voltage for the action potential with a spike amplitude of 144 mV with a spike frequency of 3 over a 4pi/3 sinusoidal interval.

5.6 Active Neuron with 100% TEA Induction

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-63	0	-0.003	0
0.1	-63	0	-0.003	0
0.2	-63	0	-0.003	0
0.3	-62	0	-0.003	0
0.4	-62	0	-0.003	0
0.5	-62	0	-0.003	0

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
-0.006	0	0.001	-0.005	2.995

Fig. 10. The ionic current values during 0.5 ms interval for an active neuron with the induction of 100% TEA Concentration

Fig. 11. The ionic membrane current of an active neuron with 100% TEA concentration

Figures 10 and 11 display the ionic membrane current for this neuron after 0.5 ms was 2.995, which suggests that the usage of this concentration results in the greatest reduction of potassium flow or a significant blockage in the potassium channel. There was an increase in the voltage for the action

Time (ms)	Voltage (mV)	Leak Ion	Sodium Ion	Potassium Ion
0	-63	0	0	0
0.1	-63	0	0	0
0.2	-63	0	0	0
0.3	-63	0	0	0
0.4	-63	0	0	0
0.5	-64	0	0	0

potential and a spike amplitude of 18 m V with a spike fre-

Current of Sodium Ion	Current of Potassium Ion	Current of Resting Potential Membrane	Total Ionic Current Across the Membrane	Derivative of Ionic Membrane Current
0	0	0	0	-1

quency of 2 over a 47/30 sinusoidal interval. The blockage was more severe than the one expressed with the active neuron with 50% TEA induction.

5.7 Active Neuron with 100% Pronase, TEA, and TTX Induction

Fig. 12. The ionic current values during 0.5 ms interval for an active neu-

ron with the induction of 100% Pronase, TTX, and TEA

Fig. 13. The ionic membrane current of an active neuron with 100% Pronase, TTX, and TEA

There was a decrease in the voltage for the action potential with a 100% TTX, 100% TEA, and 100% Pronase induction and a spike amplitude of 46 mV with a spike frequency of 4 over a 4p/3 sinusoidal interval. There is no change in the voltage, which is due to the combined effects of TX and TEA, However, since the proteolytic agents of Pronase were combined in this trial, there are was a dramatic increase in the spike amplitude, thus delineating the fact that Pronase caused the firing of electrochemical signals

5 Conclusion

As per the data tables, the induction of tetrodotoxin concentrations on an active neuron resulted in a distinct decrease in membrane current values, delineating the fact that there was a significant reduction in sodium current flow. The decrease in the membrane current and the blockage of the sodium channel suggests the gradual occurrence of demyelination, which is a process where the axons of a neuron start to lose their ability to transmit signals, a key aspect in multiple sclerosis. The induction of tetraethyl-ammonium concentrations on an active neuron resulted in a profound increase in membrane current values, which shows an extensive reduction of potassium flow or a sizable blockage in the potassium channels. The analysis of each neurotoxin led to the conclusion that the usage of tetrodotoxin and tetraethyl-ammonium to inhibit the transmission of pain signals in cancer pain relief therapies leads to the gradual development of symptoms associated with multiple sclerosis, thus proving my hypothesis to be correct. This is because the induction of the neurotoxin concentrations blocked the passage of ions across the ionic membrane of neurons, which in turn, slows down the transmission of electrochemical signals through the neuronal axons. Furthermore, the injection of 100% Pronase drug concentration, which consists of proteolytic enzymes, reverses the effects of the tetraethyl-ammonium and tetrodotoxin, modifying the sodium and potassium channels; this in turn, prohibits the development of symptoms associated with multiple sclerosis. The results of this experiment showed that there is a correlation between chemicals used in cancer pain relief therapies and chemicals that lead to the derivation of multiple sclerosis symptoms; the research conducted for this project to investigate the impacts of Pronase allows for scientists and other professionals to develop more efficient therapeutic modes to essentially treat multiple sclerosis.

7 End Sections

7.1 Acknowledgments

I would like to thank Amrita Virtual Labs for providing me with the resources to conduct my simulation using my own independent procedure at home.

7.1 References

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