Simulation study of the effect of *Mitragyna speciosa* on Hybrid current in rat Hippocampus CA3 Pyramidal Neuron.

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**Abstract**— We have simulated the hybrid current using Neurons in Action software to observe the hypothetical effect of *Mitragyna speciosa* in the model rat hippocampus CA3 pyramidal neuron at different Calcium ion conductance and at different temperature. Previous studies using the patch clamp technique showed that *Mitragyna speciosa* or Ketum could block long lasting Calcium channel (L-type Ca channel) currents in N1E-115 neuroblastoma cells. There is currently no such study of the effects of ketum on pyramidal neurons using the patch clamp technique. Our team is interested to study the ability of Ketum alakaloid to block L-type Ca channel in a rat's brain. The results of the simulation study gave us valuable information and guidelines for our future study to replicate the Ca channel blocking nature of the alkaloid of the *Mitragyna speciosa*.

**Keywords-component:** Simulation, Hybrid Current, *Mitragyna Speciosa*, CA3 Pyramidal Neuron

I. INTRODUCTION

Neurons in Action (http://neuronsinaction.com) is a tutorials and simulations software of nerve functions developed by John W. Moore and Ann E. Stuart based on NEURON (http://neuron.duke.edu and http://www.neuron.yale.edu) which is another professional software simulation environment for computational neuroscience developed by M.L.Hines, J.W.Moore, and N.T. Carnevale from Duke and Yale University. It is also excellent software for learning and understanding the neurophysiology of the generation of single neuronal activities and in plotting the voltage, ionic current and conductance. Since the computer simulations in Neuron in Actions before and after real experiments: it predicts remarkably the nerve function where we have conducted a simulation study of Ca ion action potential in the hypothetical presence of *Mitragyna speciosa* in the model of rat CA3 pyramidal neuron [1]

*Mitragyna speciosa* (Ketum in Malaysia /Kratome in Thailand) is grown mostly in the tropical and sub-tropical regions of South-East Asia mainly in Thailand and Malaysia. Mitragynine is the major alkaloid found in this plant has inhibitory function of vas deferens contraction of rat, most likely through its blockage of neuronal Ca2+ channels [2]. Using the patch-clamp technique, mitragynine was found to block T- and L-type Ca2+ channel currents in N1E-115 neuroblastoma cells [2]. There are several studies on opioid receptors mediated *Mitragyna speciosa* activity, such as opioid receptors mediated antidiarrheal effect on rat gastrointestinal tract [3], and analgesic action through inhibition of guinea pig ileum contraction *in vitro* via the opioid receptors have been studied [4]. Through opioid receptors pathway an antinociceptive action of mitragynine when administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) in mice has also been studied [5]. One study showed the involvement of descending noradrenergic and serotonergic receptors system in antinociception in mice when mitragynine was administered supraspinally [6]. There are several studies on *Mitragyna speciosa* as an alternative of drug withdrawal symptoms. For example, administration of the aqueous extract of *Mitragyna speciosa* significantly inhibited ethanol withdrawal induced behaviors [7], and to manage opioid withdrawal symptoms [8, 9]. *Mitragyna speciosa* blocks neuromuscular junction. Lows concentration Kratom extract produced muscle relaxation and High concentrations of kratom extract and mitragynine blocked the nerve conduction, amplitude and duration of compound nerve action potential [10]. Other results suggest that chronic administration of mitragynine can altered the cognitive behavioral function in mice [11]. Another studies on toxicity showed that oral administration of standardized methanolic extraction of *Mitragyna speciosa* Korth increases rat blood pressure after an hour of drug administration. The highest dose of extract also induced acute severe hepatotoxicity and mild nephrotoxicity [12]. One case study in human showed decrease mental status and seizure after ketum exposure may be the suggested mechanism of the involvement of the modification of adenosine binding or stimulation of adrenergic and/or serotonergic receptors [13]. However, all those studies on opioid, adrenergic, serotonergic receptors mediated and Ca channel mediated, but on either smooth muscle or neuroblastoma cells. There is no study on the effects of *Mitragyna speciosa* alkaloid on L-type Ca channel current either in simulation study or in real experiment with pyramidal neuron. From this view point we have simulated in this study a model neuron cell using Neurons in Action.

II. METHODS

Hodgkin and Huxley model is the model for study of electrophysiology based on four differential equations also stated in Neurons in Action and [14]. The total current across the membrane is the sum of capacitive current, Na current, K current and leakage current. Each type of ionic currents, \(I_{ion}\) further quantified by the conductance of ion \(g_{ion}\) and its driving force (the difference between membrane potential \(V\) and equilibrium potential of ion, \(E_{ion} \), \(V - E_{ion}\), this
relationship can be expressed by Ohm’s law, \( I_{ion} = g_{ion}(V - E_{ion}) \).

The equation for calculating the equilibrium potential (Nernst equation) of ion is as follow:
\[
E_{ion} = \frac{RT}{F} \ln \left( \frac{[ion]}{[lon]} \right),
\]
where \( R = \) the gas constant, \( T = \) temperature, Kelvin, \( z = \) valence of ion, \( F = \) the Faraday constant.

The equation for the Capacitive current is as follow
\[
C_{m} \frac{dV}{dt} = \frac{g_{ion}(V - E_{ion})}{R}
\].

Sodium channel current

The equation for the Na current is as follow
\[
I_{Na} = g_{Na} m^{3} h (V - E_{Na}),
\]
where \( g_{Na} = \) membrane conductance for Na, \( V = \) membrane potential, \( E_{Na} = \) equilibrium potential for Na, \( m = 1 - e^{-t/\tau_{m}} \) and \( h = e^{-t/\tau_{h}} \) are the activation (rising) and inactivation (falling) gates, respectively.

Calcium channel current

Below is the equation for the Ca current
\[
I_{Ca} = g_{Ca} m^{2} h (V - E_{Ca}),
\]
where \( g_{Ca} = \) membrane conductance for Ca, \( V = \) membrane potential, \( E_{Ca} = \) equilibrium potential for Ca, \( m = 1 - e^{-t/\tau_{m}} \) and \( h = e^{-t/\tau_{h}} \) are the activation (rising) and inactivation (falling) gates, respectively.

Potassium channel current

The underneath equation is for the K current
\[
I_{K} = g_{K} n^{4} (V - E_{K}),
\]
where \( g_{K} = \) membrane conductance for K, \( V = \) membrane potential, \( E_{K} = \) equilibrium potential for K, \( n = 1 - e^{-t/\tau_{n}} \) is a rising (activation) exponential function.

Leakage current

The following is the equation for the leakage current
\[
g_{l} (V - V_{l})
\]

The initial parameters of simulation experiments are

- Resting membrane potential -65mV,
- Membrane Capacitance 1 \( \mu F/cm^{2} \),
- Ca channel density 0.12 S/cm^{2}, Na channel density 0.12 S/cm^{2}, K channel density 0.036 S/cm^{2},
- reversal potential (mV) of Ca, Na, K are 134.74, 50, -77 respectively.
- The conductance and reversal potential of leak current are .0005 S/cm^{2} and -54.3mV respectively.
- Stimulation parameter in patch current clamp mode was set to delay 0.1 ms, duration 0.1ms and amplitude 0.2 nA. Later we have run the experiments at different temperature from 6.3 degree C to 36.3 degree C in steps of 10 fold. Since the effect of Mitragyna speciosa is dose dependent, the changes of Ca channel conductance was in steps of .03 S/cm^{2} and the Na and K channel conductance was constant.

III. RESULTS

We have presented the action potentials at different temperature at different Ca ion channel density or conductance in Fig 1 and Fig 2 when Na and K ion channel density or conductance is constant. The amplitude of action potential and duration decreasing linearly with the decrease of Ca conductance at low temperature at 6.3 and 16.3 degC (Table I). Fig 2 depicts the amplitude and duration of action potentials at 26.3 and 36.3 degC which are almost stable at different Ca conductance and the amplitude of action potentials at 36.3 deg C is subthreshold (Table I). Fig 3 and 4 showed the traces of K, Na and Ca current at different Ca ion conductance when K and Na ion conductance are constant. The Ca ion Current (ICa) is decreasing with the decrease of its conductance at low temperature at 6.3 and 16.3 deg C (table), the value of Na Current (INa) and K current (IK) is not shown in the table. At the temperature of 26.3 deg C the ICa is less evoked and at the high temperature of 36.3 degC the ICa does not evoked (Fig 5 and Fig 6). We have demonstrated the sum of IK and ICa at Fig 7 at 6.3, 16.3 and 26.3 deg C and at high temperature at 36.3 deg C in Fig 8.
Figure 3. Traces of Potassium Current (IK), Sodium Current (INa) and Calcium Current (ICa) at the different level of Ca channel density (conductance, gCa) at 6.3 degC

Figure 4. Traces of Potassium Current (IK), Sodium Current (INa) and Calcium Current (ICa) at the different level of Ca channel density (conductance, gCa) at 16.3 degC

Figure 5. Traces of Potassium Current (IK), Sodium Current (INa) and Calcium Current (ICa) at the different level of Ca channel density (conductance, gCa) at 26.3 degC

Figure 6. Traces of Potassium Current (IK) and Sodium Current (INa) at the different level of Ca channel density (conductance, gCa) at 36.3 degC. Calcium Current (ICa) does not evoked.

Figure 7. Sum of Potassium (IK) and Calcium Current (ICa) at 26.3 degC, 16.3 degC and 6.3 degC (left to right group of traces) at the different level of Ca channel density (conductance, gCa)

Figure 8. Sum of Potassium (IK) and Calcium Current (ICa) at 36.3 degC at the different level of Ca channel density (conductance, gCa)

IV. DISCUSSION

We have simulated the effect of *Mitragyna speciosa* on Ca current at different temperature and at different Ca ion conductance on CA3 Pyramidal neuron hypothetically.
TABLE I: THE VALUE OF ACTION POTENTIAL AND CURRENT AT DIFFERENT LEVEL OF CA CHANNEL DENSITY (CONDUCTANCE, GCA) IN S/CM², VOLTAGE IN MV, TIME IN MS, CURRENT IN MA/CM², ICA AND IK ARE CALCIUM AND POTASSIUM CURRENT RESPECTIVELY.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>I Ca</th>
<th>Sum of ICa &amp; IK</th>
</tr>
</thead>
<tbody>
<tr>
<td>gCa ampl time</td>
<td>ampl time</td>
<td>ampl time</td>
</tr>
<tr>
<td>0.12</td>
<td>0.58</td>
<td>4.4</td>
</tr>
<tr>
<td>0.09</td>
<td>0.62</td>
<td>4.1</td>
</tr>
<tr>
<td>0.06</td>
<td>0.67</td>
<td>3.9</td>
</tr>
<tr>
<td>0.03</td>
<td>0.74</td>
<td>3.7</td>
</tr>
<tr>
<td>0</td>
<td>0.85</td>
<td>3.68</td>
</tr>
</tbody>
</table>

The rising phase of action potential is due to the opening of Na ion channel. Due to the characteristic of L-type Ca channel that is more slowly open than Na channel during depolarization the Ca current appears during plateau of action potentials with the competition of K ion current. Since we are decreasing the gCa by changing the density of open channels in the patch the plateau of action potential is not sustained and due to the imbalance of IK and I Ca the repolarization of action potentials emerge by dint of K current. Similarly we have observed the long plateau of summed IK and I Ca when gCa decreasing. The rates of channel opening, closing and inactivation depends on the changes of temperature. Changes of temperature also affect the rate at which the ionic conductance turn on and off. The durations of the Ca conductance affect the durations of the currents, gCa decreasing with the duration of summed current diminishing. This duration of change is temperature dependent. But the temperature dependence of the long-lasting calcium channel (L-channel) current in vascular smooth muscle cells was investigated using the patch clamp technique that is little bit different from our simulation study which may be due to the different cells studied [15]. However the simulation study using Neuron in Actions is useful for education and it may possibly give us valuable guidelines and information before the real experiment is done.

V. CONCLUSION

The results of simulation study provides us with valuable information and guidelines about the temperature dependence of the long-lasting calcium channel (L channel) current in the rat hippocampus CA3 pyramidal neuron model at different Calcium ion conductance using patch techniques. In the future our team will be interested to study the ability of Ketum alkaloid to block L-type Ca channel in rat brain.

ACKNOWLEDGMENT

This study has been supported partly by FRGS Grant (MOHE) (203/PPSP/6171137) for author T.B.

REFERENCES


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