UNC PEMBROKE COPYRIGHT AND AVAILABILITY FORM

Student Name: Savannah Melvin

Title of Project: 3,4-Diaminopyridine as a Treatment for Lambert-Eaton...

Degree (Circle one): Undergraduate  Masters  Doctorate

Date of Graduation (Month Year): May 2019  Degree Received: B.S.

Major Subject: Biology with a Biomedical Emphasis

Advisor (print name): Robert Monge

AVAILABILITY OPTION (check one)

☑  Release the work immediately for worldwide access on the Internet.

☐ (Patent Hold)  Secure the work temporarily for patent and/or proprietary purposes, then release the work for worldwide access on the Internet.

☐ (Journal Hold) Hold the work for one year, then release the work for worldwide access on the Internet. (One* year extension on request, if needed)

UNCP COPYRIGHT AGREEMENT

I hereby certify that, if appropriate, I have obtained and attached hereto a written permission statement from the owner(s) of each third party copyrighted matter to be included in my thesis, dissertation, or record of study, allowing distribution as specified below.

I certify that the version I submitted is the same as that approved by my advisory committee.

I hereby grant to UNCP or its agents the non-exclusive license to archive and make accessible, under the conditions specified below, my thesis, dissertation, or record of study in whole or in part in all forms of media, now or hereafter known. FERPA. To the extent this thesis, dissertation, or record of study is an educational record as defined in the Family Educational Rights and Privacy Act (FERPA) (20 USC 1232g),

I consent to disclosure of it to anyone who requests a copy.

I retain all other ownership rights to the copyright of the thesis, dissertation or record of study.

I also retain the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or record of study.

16  Revised January 2016
STUDENT AVAILABILITY & COPYRIGHT AGREEMENT

I have read and fully agree to the UNCP copyright agreement regarding my thesis/dissertation. I agree to the thesis/dissertation availability option I selected above. I understand that the availability option is my choice and that there may be publishing consequences to my selection.

Student Signature:  

Thesis Advisor/Faculty Mentor's Signature

I have discussed the availability choices with my student, and I am aware of the choice my student has made.

Advisor/Mentor's Signature:  

(Only One Signature Required)

UNCPembroke  
Electronic Theses and Dissertations (ETDs)  
How to Choose an Availability Option

UNCP's Policy

Your Electronic Thesis/Dissertation (ETD) will be made available immediately after graduation worldwide on the Internet via The Mary Livermore Library, unless you choose to delay release for publishing, patent or proprietary reasons.

Why would I choose "Journal Hold"?

If you are (or will be) submitting material to a journal that restricts Internet access to material prior to publication, a "Journal Hold" is the option you need to select. This gives you time to get published, and your ETD is released one year after graduation to the Internet. This hold may be extended one additional year if an email is sent before the initial hold ends in order to give you time to finish publishing your material.

What is a "Patent Hold," and when would I choose it?

If you have patent and/or proprietary reasons for having information in your ETD held from the public domain, UNCP will hold your document until your patent has been secured, or the proprietary restriction is no longer necessary.

What if I have more questions about availability options?

If you still have questions or concerns about availability options, please call (910) 521-6834, (910) 521-6369, or email us at anne.coleman@uncp.edu, june.power@uncp.edu

Revised January 2016
3,4-Diaminopyridine as a Treatment for Lambert-Eaton Myasthenic Syndrome (LEMS)

Senior Project

In partial fulfillment of the requirements for
The Esther G. Maynor Honors College
University of North Carolina at Pembroke

By

Savannah Melvin
Biology
May 1st, 2019

Savannah Melvin
Honors College Scholar

Robert Poage, Ph.D.
Faculty Mentor

Teagan Decker, Ph.D.
Senior Project Coordinator

5-1-2019
Date

4-29-19
Date

5/1/19
Date
Acknowledgements

I would like to thank Dr. Poage for serving as my mentor and providing guidance for my research. He was instrumental in designing the research parameters with me, and he was easy to work with. I would also like to thank the Pembroke Undergraduate Research Committee for providing funding through the SURF grant.

The Animal Physiology class (BIO 4610) was also crucial to my research project because they helped collect the data. Finally, I would like to thank my family and friends for being supportive in my academic endeavors and for providing moral support when the project was stressful.
Abstract

Lambert-Eaton Myasthenic Syndrome (LEMS) is a debilitating autoimmune disease where the neuromuscular junction (NMJ) does not function normally. A patient with LEMS has damaged voltage-gated calcium channels (VGCC), which in return does not allow their NMJ to release an adequate amount of acetylcholine for muscle contraction. A novel treatment for LEMS is administering 3,4-Diaminopyridine (3,4-DAP), because the drug blocks presynaptic potassium channels and broadens presynaptic action potentials. Since presynaptic action potentials are broadened, more influx of calcium through VGCC occurs, and additional acetylcholine is released, reducing the muscle weakness associated with LEMS. To further LEMS research, we used a frog model (gastrocnemius muscle and the sciatic nerve). The frog muscle and nerve were soaked in different calcium concentrations to simulate LEMS. After we collected control data, we treated the muscle-nerve preparation with 3,4-DAP and investigated the effects 3,4-DAP has on muscle excitability and nerve excitability. We predicted that muscle twitch (force) generated by nerve stimulation would be enhanced by 3,4-DAP treatment but that direct muscle stimulation would be unaffected. We find that the research supports our original hypothesis.
3,4-Diaminopyridine as a Treatment for Lambert-Eaton Myasthenic Syndrome (LEMS)

Lambert-Eaton Myasthenic Syndrome (LEMS) is a rare autoimmune disease that is caused by defects in the neuromuscular junction (NMJ). In addition to being an autoimmune disease, LEMS is also characterized as a paraneoplastic disorder in approximately half of the patient’s affected (Gilhus 1). Individuals with an autoimmune disease have a defective immune system caused by the body attacking its’ own tissues. A paraneoplastic disorder is a condition that is caused by the patient’s immune system responding to a cancerous tumor in the body. Approximately 50% of LEMS patients also have small cell lung carcinoma (SCLC), which is what gives LEMS the paraneoplastic disorder classification (Gilhus 3). The remaining 50% of patient’s diagnosed with LEMS have an idiopathic form; researchers cannot definitively say what caused the autoimmune disorder (Tarr et al. 458).

Lambert-Eaton Myasthenic Syndrome (LEMS) can cause numerous side effects such as dry mouth and constipation, but the most common and serious side effect is muscle weakness. The muscle weakness is debilitating because every day tasks such as walking and lifting objects becomes difficult. Nearly 80% of patient’s with LEMS experience muscle weakness in both their arms and legs (Gilhus 2). As previously mentioned, LEMS is an autoimmune disorder; in this disorder, antibodies attack voltage-gated calcium channels (VGCC), specifically the P/Q-type. The attack on VGCC causes a reduction in the number of calcium channels and a reduction in the calcium influx. Since calcium influx is the trigger for neurotransmitter release,
damage to the VGCC causes a reduction in the amount of acetylcholine, the neurotransmitter needed to initiate muscle contraction. The insufficient amount of acetylcholine is what causes a weaker muscle contraction in a person with LEMS compared to an individual without LEMS.

Since LEMS affects every day tasks, receiving the appropriate treatment with the least amount of side effects is crucial for a patient’s quality of life. Patient’s who have SCLC receive cancer treatment first. Since the patient’s have a paraneoplastic disorder (LEMS), the successful removal of the cancer will alleviate symptoms (Tarr et al. 459). However, the disappearance of symptoms with cancer treatments is only possible if the cancer is what caused LEMS. For the patients that have the idiopathic form of LEMS or their symptoms just do not go away there are other drug therapy options available. The standard treatment for LEMS is a drug called 3,4-Diaminopyridine (3,4-DAP). The drug blocks presynaptic potassium channels and broadens presynaptic action potentials. Since presynaptic action potentials are broadened, more influx of calcium through the remaining VGCC occurs, and additional acetylcholine is released, reducing the muscle weakness associated with LEMS.

The research I performed this past school year focused on 3,4-DAP as a treatment for LEMS. My research mentor Dr. Robert Poage and I designed the experiment and set up the research parameters in the Fall 2018 semester. The data collection and analysis took place in the Spring 2019 semester with the help of the BIO 4610
(Animal Physiology) class. Dr. Poage and I were interested in whether muscle twitch (force) generated by nerve stimulation and muscle twitch (force) generated by direct muscle stimulation would be enhanced by 3,4-DAP treatment. We predicted that muscle twitch (force) generated by nerve stimulation would be enhanced by DAP treatment but that direct muscle stimulation would be unaffected.

The model animal used for our research was an adult leopard frog (*Rana pipiens*), because frogs are the standard animal model when performing experiments with the neuromuscular junction (NMJ); frogs are the animal model for this type of research because their NMJ is well understood and similar to humans. Additionally, the BIO 4610 class generally does a similar lab to the one we performed, so the frogs were more readily available than other options. Finally, frogs are easier to care for in a laboratory setting compared to other animals like mice. The frogs were cared for by Dr. Poage and other lab technicians at the University of North Carolina at Pembroke. Our lab protocol was approved by UNC-Pembroke’s Institutional Animal Care and Use Committee; the purpose of the committee reviewing our research plan was to ensure that the frogs were treated humanely and in accordance with federal research guidelines.

**Methods**

Adult leopard frogs (*Rana pipiens*) were decapitated and pithed after anesthesia in 0.1% tricaine methane sulfonate solution. The gastrocnemius muscle and sciatic nerve preparation was removed bilaterally and bathed in normal frog Ringer’s
solution (NFR) (in mM: 111 NaCl, 2 KCl, 1.0 mM TrisCl, 1.8 CaCl₂, pH 7.3-7.5). After 1-2 hours sitting unstimulated at room temperature, the preparation was affixed with pins to a wax or cork substrate. The calcaneal tendon was tied off with surgical suture thread and cut from its distal attachment. The other end of the thread was attached to a force transducer (FT-104, iWorx) which was mounted horizontally on a ring stand above the muscle preparation. Tension was applied to the thread by raising the force transducer to 10-30 cm above the muscle preparation. The output of the force transducer was routed into an analog-digital converter (IWX-214, iWorx) which was controlled by Labscribe3 software (iWorx) through a PC-interface. The integrated stimulator of the IWX-214 unit was directed to a sleeve-type electrode (BNC-SE) attached to the sciatic nerve of the preparation for stimulation of sciatic nerve motor axons. Finally, a separate set of needle electrodes (BNC-N2) were inserted, one into either end of the gastrocnemius, for direct electrical stimulation of the muscle cells. The students stimulated either the muscle or nerve at 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, and 5.0 volts.

The output (contractile force) was measured in grams.

**Results**

The data was collected by the Animal Physiology students during two separate two hour lab sessions, January 31, 2019 and February 7, 2019. The data collected in January was strictly control data; the gastrocnemius muscle and sciatic nerve was soaked only in normal frog Ringer’s solution (NFR) (in mM: 111 NaCl, 2 KCl, 1.0 mM TrisCl, 1.8 CaCl₂, pH 7.3-7.5). The data collected in February consisted of more control data as well as the muscle and nerve preparation treated with 3,4-
Diaminopyradine. Both sets of data were collected by six groups of students; each group had 2-3 students, and the groups were put together at random. The names of the groups were: Drosophila, Leech, Rana, Mus, Rattus, and Squid. In January, there were two different ringer's solutions used. Each solution had three groups of students assigned to it at random; the solution that best simulated LEMS was the 0.3 millimolar calcium solution instead of the 0.5 millimolar calcium solution. The data collected in February was collected by six groups; three of the groups used the same control solution (0.3 millimolar calcium), and the other three groups had the control solution and 3,4-DAP.

As previously mentioned, the students stimulated the muscle or the nerve at different increments between 0.0-5.0 volts. When sufficiently stimulated, the muscle contracted and a contractile force (grams) was recorded. Each group generated a full set of data, but for comparison purposes, we averaged all of the 0.3 millimolar calcium control data together (6 groups, over the 2 lab sessions) and the set of data that consisted of 0.3 millimolar calcium solution and 3,4-DAP (3 groups, over 1 lab session). Figure 1A (below) shows the stimulus and averages for when the students stimulated nerve directly.
Figure 1A. Left column, voltage at which nerve was stimulated. Middle column, average force (g.) generated when muscle contracted in control (0.3 mM Calcium, blue line and triangles in Figure 1B). Right column, average force (grams) generated when muscle contracted in the DAP experiments (orange line and squares in figure 1B).

Nerve Averages Compared (Contractile Force)

Figure 1B. Effect of DAP on contractile force by nerve stimulation. Nerve stimulation control data (0.3 millimolar Calcium solution: 1/31/2019 & 2/07/2019, blue line/triangles) versus DAP data (0.3 millimolar Calcium solution plus 1 mM 3,4-Diaminopyridine 2/07/2019, orange line/squares). The data on this graph corresponds with Figure 1A.
The control data for nerve stimulation (middle column in Figure 1A) has a peak contractile force of 10.524 grams at a stimulus of 0.8 volts. The data for the 3,4-DAP groups has a peak contractile force of 29.678 grams at 0.2 volts. 3,4-DAP allowed a significant increase (182.0% increase) in contractile force and also allowed the muscle to hit its' peak at a much lower stimulus (0.2 volts versus 0.8 volts). The higher contractile force reflects a stronger muscle contraction, meaning patients with LEMS should be able to complete day to day tasks easier.

<table>
<thead>
<tr>
<th>Stimulus [Volts]</th>
<th>Avg_Muscle_Cntrol</th>
<th>Avg_DAP_Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.003</td>
<td>0.000</td>
</tr>
<tr>
<td>0.2</td>
<td>0.430</td>
<td>0.027</td>
</tr>
<tr>
<td>0.3</td>
<td>2.043</td>
<td>9.618</td>
</tr>
<tr>
<td>0.4</td>
<td>4.957</td>
<td>17.880</td>
</tr>
<tr>
<td>0.5</td>
<td>7.899</td>
<td>20.340</td>
</tr>
<tr>
<td>0.6</td>
<td>11.705</td>
<td>22.790</td>
</tr>
<tr>
<td>0.7</td>
<td>15.276</td>
<td>23.975</td>
</tr>
<tr>
<td>0.8</td>
<td>18.616</td>
<td>24.990</td>
</tr>
<tr>
<td>0.9</td>
<td>21.738</td>
<td>26.042</td>
</tr>
<tr>
<td>1</td>
<td>24.750</td>
<td>27.236</td>
</tr>
<tr>
<td>2</td>
<td>32.616</td>
<td>30.933</td>
</tr>
<tr>
<td>3</td>
<td>34.373</td>
<td>31.268</td>
</tr>
<tr>
<td>4</td>
<td>35.271</td>
<td>32.281</td>
</tr>
<tr>
<td>5</td>
<td>28.919</td>
<td>33.673</td>
</tr>
</tbody>
</table>

Figure 2A. Data from Figure 2B. Left column, voltage at which muscle was stimulated. Middle column, average force (grams) generated by muscle contraction in control (0.3 mM 2B Calcium, blue line in Figure 2B). Right column, average force (grams) generated by muscle contraction in the DAP experiments (orange line in figure 2B).
Figure 2B. Effect of 3,4-DAP on contractile force by muscle stimulation. Comparison between two experimental conditions: muscle control data (1/31/2019 & 2/07/2019, blue line/triangle) and the 3,4-DAP data (orange line/squares).

The control data (middle column in Figure 2A) has a peak contractile force of 35.271 grams, and the 3,4-DAP data has a peak contractile force of 33.673 grams. The control data, where the frog tissue was not treated with 3,4-DAP actually had a slightly higher peak contractile force. The peak contractile force decreased by 4.5%.

**Conclusion**

3,4-DAP had a significant effect on how strongly muscle contracts when the nerve is stimulated, but the medication had little or no effect on muscle contraction when the muscle was stimulated directly. Our research supports our original hypothesis because muscle twitch (force) generated by nerve stimulation was enhanced by 3,4-DAP treatment but direct muscle stimulation did not see an enhancement of greater than 10%.
If 3,4-DAP had significantly affected peak muscle contraction with direct muscle stimulation then that would be an area for concern. It would be an area for concern because scientists only want 3,4-DAP to increase neurotransmitter release and not additionally effect the muscle directly. If the results had turned out differently, we would have looked at how 3,4-DAP affects cardiac muscle and smooth muscle.

For future directions, we would like to repeat the experiment but incorporate another drug called GV-58, which is a calcium channel agonist. GV-58 and 3,4-DAP work synergistically together to completely reverse the muscle weakness associated with LEMS (Tarr et al. 461). The two drugs together is a safer option that would be available to more patient’s. 3,4-DAP is not an available option for some patient’s because once the drug gets to a high dose (generally > 80 mg per day), there are too many side effects. The drugs work synergistically together because 3,4-DAP increase the amount of calcium channels open and GV-58 allows each the channel to stay open longer; the combination of these two factors allows a greater calcium influx, specifically at the LEMS-damaged structure, the NMJ (Tarr et al. 460).

In conclusion, 3,4-DAP enhances muscle contractile force by nerve stimulation but not by muscle stimulation. The research we performed supported our original hypothesis. 3,4-DAP is able to enhance muscle contractile force, because the drug blocks presynaptic potassium channels and broadens presynaptic action potentials. Since presynaptic action potentials are broadened, more influx of calcium through VGCCs occurs, and additional acetylcholine is released, reducing the muscle weakness associated with LEMS.
References

