Effect of Increased Bathing Solution Salinity on the Threshold and Total Refractory Period of the Frog Sciatic Nerve

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Abstract

The proposed experiment tested the effect of increased bathing solution salinity on the threshold and total refractory period of the frog sciatic nerve. We hypothesized that an increased extracellular concentration of sodium would decrease the threshold and decrease the length of the refractory period. The refractory period was measured after bathing the sciatic nerve alternately in Leibovitz and a high saline Leibovitz-NaCl solution. Contrary to our hypothesis, we observed a 39.2% increase in threshold voltage and a 30% increase in the total refractory period. These results can be explained by the reduction of potassium concentration in the Leibovitz-NaCl solution. Other explanations for our results include osmolarity differences and changes in the concentrations of other ions.

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1 Introduction

1.1 Action Potentials

Action potentials in neurons result from the flow of ions due to an ionic concentration gradient across the cell membrane. The membrane potential depends on the permeability and concentration of sodium, potassium, and chloride ions. The potential is given by the Goldman constant-field equation [1]:

$$V = \frac{RT}{F} \ln \left(\frac{P_{K^+}[K_o^+] + P_{Na^+}[Na_o^+] + P_{Cl^-}[Cl_i^-]}{P_{K^+}[K_i^+] + P_{Na^+}[Na_i^+] + P_{Cl^-}[Cl_o^-]} \right) \tag{1}$$

At rest, the cell is considered to be selectively permeable to potassium [2]. Thus from the Goldman equation, a lower extracellular concentration of K^+ will lower the resting potential. During an action potential, the cell becomes more permeable to sodium. Therefore a lower extracellular concentration of Na^+ decreases the peak value of the action potential.

1.2 Refractory Period

The refractory period can be divided into the absolute and relative refractory periods. During the absolute refractory period, no amount of stimulus can generate an action potential. During the relative refractory period, an action potential can be generated only with a larger stimulus than normally required [2].

1.3 Hypothesis

Overton found that sodium ions in the extracellular solution are required for an action potential [2]. We decided to examine the effects of the extracellular concentration of sodium ions on the compound action potential (CAP) of the frog sciatic nerve: specifically, on the threshold stimulus value and the length of the total refractory period.

From the Goldman equation, increasing the extracellular concentration of sodium ions causes an increase in the membrane potential throughout the action potential, both during the onset and the refractory period. Thus, a smaller stimulus is needed to raise the membrane potential to threshold. Also, the relative refractory period is shorter as the resting potential is reached sooner.

Therefore, we hypothesized that increasing the concentration of Na^+ ions in the bathing solution would decrease the threshold and length of the total refractory period.

2 Methods

2.1 Preparation

We wanted a solution with a high concentration of NaCl. As 1 mole of NaCl is 58g, we used 29 g/L of NaCl in water, corresponding to 500mmol/L. To maintain an extracellular concentration containing reasonable levels of other ions necessary for cell survival, we mixed 100 mL of our 500 mmol/L pure salt solution with 100 mL of Leibovitz solution (see Appendix C for chemical composition) to obtain a Leibovitz-NaCl solution. Because Leibovitz has 8 g/L of NaCl, the resulting solution contains 18.5 g/L of NaCl or 318.96 mmol/L. The Leibovitz-NaCl solution has a total NaCl concentration 2.31 times higher than the Leibovitz solution.

2.2 Dissection

After dissecting the sciatic nerve from a frog, we stimulated the nerve and measured responses as outlined in the lab manual [3].

2.2.1 Assessing Condition of Nerve

To assess the condition of the nerve after dissection, we placed the negative electrode at the last recording electrode contacting the nerve, moved the positive electrode to different recording positions, and observed the nerve response to large inputs. Based on the assessed nerve condition, we determined the optimum location for electrode placement for the rest of the experiment.

2.3 Measurement Methods

2.3.1 Finding Threshold

We determined the threshold current using a train rate of 10/s. One experimenter slowly increased the input current starting from zero while the other experimenter looked for a change in the output waveform. The second experimenter was “blind” to the input being applied. When the person looking at the screen observed a deflection from baseline, the first experimenter controlling the input recorded the current applied as the threshold. Then the two experimenters switched positions, repeated the test, and then the average was recorded as the final threshold.

2.3.2 Finding Refractory Period

To find the refractory period, we set the input to be two pulses, both set to the threshold current measured above plus an additional fixed amount of current I. Adding the current I to the threshold made it easier to measure the refractory period. Starting from zero, we increased the delay between the two pulses until we saw a second threshold response. The delay between the two pulses was recorded as the refractory period.

2.4 Collecting Data

After bathing the sciatic nerve in Leibovitz solution for 10 minutes, we found the threshold and refractory period as specified above. Then the sciatic nerve was bathed in the Leibovitz-NaCl solution for 10 minutes and the above measurements were repeated. We let I be the initial threshold current measured for the sciatic nerve in Leibovitz solution.

To control for time, the nerve was placed back into pure Leibovitz solution and the measurements were repeated. A second measurement in Leibovitz-NaCl solution was also made.

Although we tried to add I to the base threshold in all measurements of the refractory period, adding the full I was sometimes impossible as the maximum output voltage for the signal generator was 15 volts. When the $threshold + I$ exceeded 15 volts, we used 15 volts. In an attempt to prevent the maximum voltage from being reached, we repeated the experiment using a smaller value for I.

Table 1: **Threshold and refractory period in different bathing solutions**

Solution	Measurement	Threshold (V)	Stimulating Voltage (V)	Refractory Period (msec)
Leibovitz	1	6.95	13.9	2.75
	2	7	14	2.9
	3	6.95	11.95	3.35
Leibovitz-NaCl	1	9	15	3.3
	2	10.4	15	4.5

3 Results

3.1 Condition of Nerve

While testing the condition of our nerve, we found the nerve was damaged between electrodes 2 and 3, corresponding to a length of about 3cm. As a result of our assessment, we proceeded with the rest of the experiment with the positive electrode at position 2 and negative electrode at position 5. The output response of the nerve with the electrode at various positions can be seen in Appendix A.

3.2 Measurements

Table 1 displays the data collected from the frog sciatic nerve over several sets of measurements within two different bathing solutions. The time sequence of measurements was L1, LN1, L2, L3, LN2 (L=Leibovitz and LN=Leibovitz-NaCl). The CAP threshold was first found, and then the nerve was stimulated with a voltage equal to the threshold plus a voltage increment. The last column shows the total refractory period, measured as discussed in section 2.3.2. An increased threshold voltage and a longer refractory period for the Leibovitz-NaCl solution over the standard Leibovitz solution can be seen.

Making these trends more apparent, Table 2 summarizes the data displayed in Table 1 with the average threshold and refractory period for the two different solutions. Bathing the nerve in the Leibovitz-NaCl solution resulted in a sig-

Table 2: **Average threshold and refractory period**

Solution	Avg. Threshold (V)	Avg. Refractory Period (msec)
Leibovitz	6.97	3
Leibovitz-NaCl	9.7	3.9

nificantly higher (39.2%) average threshold voltage. Also, the total refractory period increased by 30% when the nerve was bathed in Leibovitz-NaCl.

The graphs showing the nerve stimulation responses from our experiment are in Appendix B and the protocol is in Appendix D.

4 Discussion

4.1 Analysis of Results

The results obtained from this experiment neither support nor answer our hypothesis. Based on the Goldman equation (Equation 1), we expected a lower threshold voltage due to increased extracellular sodium concentrations. Consequently, we hypothesized that the refractory period would decrease. However, the data indicate the opposite occurred when the nerve was bathed in Leibovitz-NaCl. It is unlikely that the results were due solely to sodium concentration changes.

4.1.1 Threshold Voltage

We observed an increase in the average threshold voltage of the nerve CAP when the nerve was bathed in Leibovitz-NaCl, beyond a value possible due to experimental error, appearing to contradict the Goldman equation. However, if we examine our experimental method, the mixed Leibovitz-NaCl solution has half the ion concentration for all non-sodium ions compared with the plain Leibovitz solution. Changing the concentrations of these ions, especially potassium, probably caused this unforeseen result. The chemical composition of Leibovitz is given in Appendix C.

At rest, the cell is relatively impermeable to sodium. Consequently, the membrane potential is determined almost entirely by the balance of intra- and extra-cellular potassium, governed by the Nernst equation:

$$V = \frac{RT}{F}\ln\left(\frac{[K_o^+]}{[K_i^+]}\right) \tag{2}$$

Inadvertently reducing the extracellular concentration of potassium by half making the resting membrane potential more negative by:

$$\Delta V = \frac{8.314 \cdot 300}{96485}\ln(.5) = -18\text{mV} \tag{3}$$

Lowering the resting membrane potential made it more difficult for the nerve to reach threshold, increasing the threshold voltage.

4.1.2 Refractory Period

The action potential's characteristic shape is governed by the “switching” on and off of sodium and potassium membrane voltage channels. During the absolute refractory period, the sodium channels close and the potassium channels open to repolarize the membrane. Movement of ions across the membrane is determined by Fick's Law for diffusion:

$$\phi = -D\frac{\partial c}{\partial x} \tag{4}$$

If we have a greater concentration gradient for potassium across the membrane, we will have a greater flux of K^+ ions while the potassium channels are open. As the channels are open for a fixed length of time, a greater flux of potassium will result in a faster repolarization *but also* a greater hyperpolarization of the membrane. Sodium has no effect during this recovery phase, as the sodium channels are closed. Consequently, the overshoot in the low-potassium bathing solution is much larger than for the normal Leibovitz solution. As a result, the nerve takes longer to return to its resting potential and the total refractory period is longer.

4.2 Key Limitations of the Experiment

- We did not manage to isolate the effect of extracellular sodium ion concentrations on the compound action potential. The effects of lowered potassium concentrations probably confounded our results, as explained above. There may also be other ions that affected the result.

- We should have added a *fixed* value I to all threshold voltages to create a stimulating voltage, rather than having it equal the most recently measured threshold voltage. In addition, for the Leibovitz-NaCl solution, we were unable to add the correct I due to the instrument's output-voltage constraints. The variability of I made our data results incomparable and analysis difficult. For instance, the last data point for Leibovitz solution was generated by adding a value $I = 5$V, whereas in the previous two measurements, I had been 6.95 and 7V respectively. Using variable values of I weakened the power and usefulness of the average.

- The nerve experienced obvious trauma during the dissection process: CAP conduction was completely terminated beyond 3 cm along the axon. Failure to constantly bathe the nerve in Leibovitz solution, rupturing of the nerve, or other possibilities could well have interfered with the data collection process.

- The osmolarity of Leibovitz and Leibovitz-NaCl are different, perhaps leading to a significant change in cell volume. This would change the intracellular ion concentrations, which we assumed to be constant during the experiment. To test whether osmolarity differences caused the results we observed, we could run the experiment using Leibovitz and Leibovitz with added biochemically inert molecules.

4.3 Conclusions

The effect of increased extracellular sodium ion concentrations, when all other ions (potassium, in particular) are decreased by half, is to significantly increase the stimulus needed to reach threshold and to increase the length of the total refractory period. For further work, the *isolated* effects of sodium concentration should be examined by maintaining fixed concentrations of all other ions, perhaps by adding NaCl directly to Leibovitz solution. In addition, we could confirm the results here were due to changes in potassium by studying the isolated effects of extracellular potassium concentration. The other concerns discussed should also be addressed in future experiments.

References

[1] W. F. Ganong, *Review of Medical Physiology.* Appleton and Lange, 14th ed., 1989.

[2] L. R. Johnson, ed., *Essential Medical Physiology.* Lippincott-Raven, 2nd ed., 1998.

[3] MIT EECS, “The compound action potential of the frog sciatic nerve,” 2001.