Lab 7 Report: Frog Heart Study

I. Introduction

The cardiovascular system is an efficient transport mechanism faster than diffusion. In the cardiovascular system is the heart, a muscular pump that drives the flow of blood through blood vessels. Unlike a human heart that contains four chambers, a frog heart only contains three chambers: two atria and a ventricle. Frogs also have a sinus venosus, which is a large cavity incorporated into the wall of the right atrium. Blood returns to the sinus venosus from the body via two anterior and a posterior venae cavae. Blood then drains from the sinus venosus into the right atrium and empties into the ventricle. The ventricle then pumps blood to the pulmonary arteries. Blood returns from the lungs through the pulmonary veins to the left atrium and empties into the ventricle. Heartbeat originates in muscle cells and is regulated by the autonomic nervous system. The parasympathetic system releases acetylcholine, which decreases heart rate, and the sympathetic system releases norepinephrine, which causes an increases in heart rate. The purpose of this lab is to investigate how different temperatures, neurotransmitters, and electrolytes affect the heart rate and strength of contraction. My hypothesis is that warm temperature and epinephrine will increases heart rate while cold temperature, acetylcholine, potassium, and calcium will decrease heart rate.

II. Materials and Methods

The subject of this experiment was a pithed frog, meaning that the frog had the central nervous system destroyed by a probe, while maintaining the autonomic nervous system. Our materials were a transducer, an amplifier, string, a staple, LabScribe software and the treatments we were introducing to the frog’s heart. With the frog lying on its back, we made a cut with scissors from the abdominal area all the way to the mouth of the frog. We cut through the muscle layer and sternum until the heart was exposed. We attached a staple through the apex of the ventricle. The other end of the staple was tied to string. Once we hooked the heart with the
staple, we pulled the string up and tied it to the transducer. The heart was lifted up from the chest but remained attached to the body. The transducer was connected to the amplifier, which was connected to the computer. We used LabScribe to record the frog’s heartbeat at baseline. The small peaks represented atria contraction while the taller peaks represented ventricle contraction. Heart rate was determined by measuring the number of tall peaks per minute. Strength of contraction was determined by measuring the amplitude of the taller of the two peaks. We measured three amplitudes and took the average.

Each group was given two treatments to test on the pithed frog. We were assigned warm and cold temperatures. We added room temperature Ringer’s solution to the heart to get a stable baseline recording. After recording heart beats for 3 minutes, we added a few drops of warmed Ringer’s solution to the heart and recorded heartbeat for 3 minutes. We then again added room temperature Ringer’s solution to the heart and recorded a second baseline for 3 minutes. Finally, we added a few drops of Ringer’s solution that had been chilled on ice. We recorded the heartbeat of the heart with the chilled Ringer’s solution for 3 minutes. Other groups repeated the same procedure but instead of measuring how cold and warm temperatures affect heart rate and strength of contraction, they measured the effect of added acetylcholine, epinephrine, potassium chloride solution, and calcium chloride solution. The results are summarized in the following section.

III. Results

Figure 1 shows how temperature, neurotransmitters, and electrolytes affected heart rate. In our group, the frog had a heart rate of 48 beats per minute prior to adding any solution. After adding warm Ringer’s solution, heart rate increased to 64 beats per minute. Adding cold Ringer’s solution decreased the heart rate from a baseline of 44 BPM to 30 BPM. A second group tested acetylcholine and epinephrine. Adding acetylcholine greatly decreased heart rate from a baseline of 54 BPM to 10 BPM. Epinephrine increased heart rate from a baseline of 48 BPM to 60 BPM. Another group tested the effects of potassium chloride solution and calcium chloride solution on heart rate. Potassium chloride insignificantly decreased heart rate from a baseline of 57 BPM to 54 BPM. Calcium chloride decreased heart rate from a baseline of 55 BPM to 44 BPM.
Figure 2 shows how temperature, neurotransmitters, and electrolytes affected strength of contraction. After adding warm Ringer’s solution, the strength of contraction decreased from a baseline of 108 mV to 91 mV. When we added cold Ringer’s solution, the strength of contraction increased from a baseline of 103 mV to 112 mV. Adding acetylcholine greatly decreased strength of contraction from a baseline of 201 mV to 88 mV. Epinephrine greatly increased strength of contraction from a baseline of 71 mV to 126 mV. Potassium chloride insignificantly increased strength of contraction from a baseline of 44 mV to 45 mV. There was almost no change. Calcium chloride increased strength of contraction very little from a baseline of 44 mV to 47 mV.
IV. Discussion

Conclusions:

My hypothesis, stating that warm temperature and epinephrine will increase heart rate while cold temperature, acetylcholine, potassium, and calcium will decrease heart rate, was partially correct. As expected, warm temperature increased heart rate by 16 BPM and epinephrine increased heart rate by 12 BPM. Chemical reactions are sped up by heat and the heartbeat is a function of chemical reactions. Therefore, a higher temperature makes the heart beat more times. Epinephrine is secreted by the adrenal medulla in response to increased sympathetic activity and therefore, it makes sense for epinephrine to increase heart rate. Cold Ringer’s solution decreased heart rate by 14 BPM indicating that cold temperatures slow chemical reactions, which results in the reduction of beats per minute. Acetylcholine did decrease heart rate by 44 BPM. This was expected since parasympathetic neurons release ACh, which binds to mAChR on the SA nodal cells. This decreases the frequency of action potentials and lowers heart rate. Calcium chloride results were as expected, with the heart rate decreasing by 11 BPM. Calcium makes it harder to open the sodium channel, leading to slower firing of action potentials and a slower heart rate. Potassium only lowered heart rate by 3 BPM, which is not the significant change we were expecting. Heart rate and strength of contraction remained almost stable when potassium was added. Perhaps the amount of potassium added to the frog’s heart was not enough to affect heart rate. The general trend for temperature and electrolytes was that if heart rate increased, strength of contraction decreased. If heart rate decreased, strength of contraction increased. For neurotransmitters, both heart rate and strength of contraction changed in the same direction, either decreasing or increasing together. Muscarinic receptors of acetylcholine inhibit contraction of cardiac muscle fibers. Epinephrine increases myocardial contractility, increasing stroke volume and increasing cardiac output.

Physiological Relevance:

Cardiac muscle does not require commands from the central nervous system to contract. Cardiac muscle contractions are triggered by signals originating from the muscle itself and this contractile activity is said to be myogenic. Although pacemaker cells control heartbeat, other factors affect the electrical activity of pacemaker cells. Regulation of the heart by neural input, circulating hormones, or any other factor originating from outside the organ is extrinsic control.
Neural control of the heart is carried out by the autonomic nervous system. Increased activity in sympathetic neurons increases the frequency of action potentials in the pacemaker cells. Sympathetic neurons release norepinephrine increasing heart rate. Epinephrine, secreted by the adrenal medulla in response to increased sympathetic activity, increases heart rate. Parasympathetic neurons release acetylcholine, decreasing the frequency of action potentials and lowering heart rate. Electrolytes are significant to heart rate because the normal state of cardiac cell membrane polarization is dependent upon the maintenance of a normal ionic balance across the membranes. Temperature affects the speed of chemical reactions and since the heartbeat is a function of chemical reactions, cold and warm temperatures alter heart rate. In this lab, we were able to observe first hand how neurotransmitters, electrolytes, and temperature affect heart rate and heart contraction.

Problems/ Suggestions:
A problem we encountered was that not all trends were easily seen due to leftovers. Baseline after treatment would not resemble the baseline prior to treatment. When we treated the heart sequentially with different electrolytes or drugs, the accumulating waste solution surrounding the frog may have absorbed through its skin, and activated other physiological pathways that counteracted the direct effect of the drug on the heart. Next time this experiment is repeated, perhaps the heart needs to be wiped with gauze or a dry cotton ball before hydrating the heart with water and reestablishing baseline. Also, it was strange that the groups reported very different values for the strength of contraction for the baseline prior to any treatment. The initial baselines were 108 mV, 201 mV, and 44 mV. Perhaps this vast difference was caused by not all frogs being the same age or in the same physical condition.

Future Experiment:
A future experiment can measure what levels of poisonous substances, in particular nicotine and arsenic, would stop a frog’s heart from beating. Nicotine activates nicotinic acetylcholine receptors on postganglionic neurons. This activates the parasympathetic system and slows down the frog heart. Arsenic is a chemical element found in many minerals and in conjunction with sulfur and metals. A high amount of arsenic in drinking water or in the environment can be toxic and lead to arsenic poisoning. My hypothesis is that it would take a
smaller dose of nicotine to stop a frog’s heart from beating than the dose of arsenic required to poison it because a frog’s natural environment is more likely to contain arsenic than nicotine.

The required materials are a pithed frog, string, a staple, a transducer, an amplifier, lump arsenic, and laboratory grade nicotinic solution. Because nicotine and arsenic are toxic if you are exposed at high levels, other safety equipment may be required, such as a procedure mask and gloves. Each group should only test one of the two substances to reduce the number of frogs that have to be used. First, have the frog laid out on a tray and cut with scissors from the abdominal area all the way to the mouth of the frog. Cut through the muscle layer and sternum until the heart is exposed. Tie the string to one end of the staple and use the other end of the stable to hook through the apex of the ventricle. Tie the other end of the string to the transducer, which is connected to the amplifier. Record the heartbeats for 3 minutes before you add any substance. If you are testing with the nicotinic solution, add three drops of the solution. Record the change in heartbeat for three minutes. Count beats per minutes and measure amplitude by taking the average of three peaks. Add three more drops of nicotinic solution and record the heartbeat for three minutes again. Continue adding 3 drops every three minutes until the heart stops beating. If you are testing the arsenic, repeat the procedure described previously but instead of adding nicotinic solution, add 10 mg of arsenic every 3 minutes to the surface of the heart. Continue until the heart stops beating. Convert the amounts of nicotine and arsenic added into moles. If my hypothesis is correct, the expected result is that it takes a larger concentration of arsenic compared to nicotine to poison the frog’s heart.