

Electrical and Mechanical Properties of the Frog Heart

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Introduction:

The heart is one of three components that make up the circulatory system, a system that is made up of two distinctive loops: systemic and pulmonary; the other two components are blood vessels and blood. The heart is located at the center of the chest cavity and acts as the mediator between the pulmonary circulation (mainly made up of the lungs) and the systemic circulation (the body but excluding the lungs). The human heart is a dual pump that is made up of four main components: left and right atrium and left and right ventricle. The atria make up the upper portion of the heart while the ventricles make up the lower portion. When people speak of the right side of the heart, they are referring to the collection of the right atrium and the right ventricle. Both are involved in returning blood from the systemic circulation to the pulmonary circulation. When people speak of the left side of the heart, they are referring to the collection of the left atrium and left ventricle. The left atrium and left ventricle are engaged in pumping blood from the pulmonary circulation into the systemic circulation. A more detailed description of the mechanism of blood flow will be discussed in the next paragraph.

The heart, made up of striated cardiac muscle, is the “pump” that governs the flow of blood, being an important player in maintaining body homeostasis. The main purpose of the heart pumping blood throughout the body is to supply oxygen to and at the same time, get rid of carbon dioxide (waste) from body tissues. Deoxygenated and CO₂-rich blood from the systemic circulation returns back to the right atrium via the venae cavae: inferior vena cava and superior vena cava. This blood then flows to the right ventricle and ultimately enters the lungs. At the lungs, the deoxygenated blood replenished with oxygen and the CO₂ is rid. The now oxygenated and CO₂-free blood returns back to the heart through the left atrium. The blood flows from the left atrium and into the left ventricle where it is pumped into the systemic

circulation and distributed to different parts of the body through the aorta. The above described process is how oxygen is transported to all areas of the body.

The heart is able to do the above mentioned and pump blood because of the contractions it undergoes when pacemaker cells are depolarized. The pacemaker cells in humans are located at the sinoatrial node (SA node), atrioventricular node (AV node), bundle of His (atrioventricular bundle), and Purkinje fibers. The main, or fastest, pacemaker cell is at the SA node and it is these fast pacemaker cells that initiate action potentials to stimulate muscle contractions.

In this exercise, we are studying the frog heart with the goal of better understanding the human heart. The frog heart is actually very similar to the human heart but they do have some major differences. Unlike the human heart, a frog heart has 2 atria and only 1 ventricle. The frog heart does not have a vena cava nor does it have an aorta. Instead, it has a sinus venosus and a conus arteriosus, analogous to the vena cava and aorta, respectively. Hence, blood returns from the systemic circulation returns to the right atrium via the sinus venosus instead and the blood leaves from the frog heart and enters the systemic circulation via the conus arteriosus. Since there is only one ventricle, one would be concerned that the oxygenated and the deoxygenated blood would mix together but in reality, the frog heart has trabeculae, something the human heart does not have. Trabeculae are spongy folds inside the ventricular wall and they along with the spiral valve, located inside the conus arteriosus, help to ensure that the 2 types of blood do not mix.

One of the two general goals of this exercise was to examine the basic properties of the heart. We examined the electrical and mechanical activities of the heart as well as the heart's responses to agonists associated with sympathetic and parasympathetic input. For observing the electrical activity of the heart, we expected to see that the electrical trace to look like an ECG

where the sharp peak corresponds to the QRS. The portion of the trace prior to the peak is expected to correspond to the P wave while the portion of the trace following the peak corresponds to the T wave. For observing the mechanical trace, we expected to see that there are two kinds of peaks (or humps) that alternate from one to the other. The high peaks correspond to ventricular contractions while the low peaks correspond to atrial contractions. As for the responses to parasympathetic and sympathetic input, we expect to see, respectively, a decrease in contractile force and an increase in contractile as a result of the type of input. High voltage is used to stimulate the vagus nerve, a nerve that is part of the parasympathetic nervous system, and so a decrease in contractile force and rate is expected. That is because the stimulation of the vagus nerve activates the parasympathetic system. An increase in contractile and heart rate is expected after adding a dose of epinephrine because epinephrine binds to the B-1 adrenergic receptors, which are part of the sympathetic nervous system. That is because the binding epinephrine with B-1 adrenergic receptors causes an increase in adenylyl cyclase. That in turn causes an increase in CAMP. CAMP activates PKA and active PKA leads to an increase in intracellular Ca^{2+} concentration. The more intracellular Ca^{2+} there is, the stronger the contraction. Therefore, the addition of epinephrine is associated with an increase in contractile force.

The other goal was to observe the responses to direct ventricular stimulation. In the instance of an extrasystolic contraction, we expected to see that an extra high peak contraction is seen right after the ventricular contraction, forming a sort of “M” shape on the mechanical trace. We expect to see that the extra peak to be lower than the ventricular contraction because of less filling time and hence, less filling; The Frank-Starling Law states that the more filling, the stronger the contraction and vice versa. A compensatory pause, the time between the

extrasystolic contraction and the normal contraction, allows for more filling time for the next normal ventricular contraction. As a result, we expect that contractile force for the next normal muscle contraction to be stronger than the contractile force before and during extrasystole because of the Frank-Starling Law, which states that more filling equals stronger contraction.

Materials and Methods:

The subject of this lab is the study and understanding of the heart. We are to examine the electrical, mechanical and different responsive properties of heart. Details about the materials/methods for this particular exercise can be found in Exercise 5 (pages 43-53) in the NPB101L Physiology Lab Manual (2nd edition) by Erwin Bautista and Julia Korber. We first opened up the frog so to expose the heart and hooked up all the appropriate connections so that the recording machine is able to record heart activity. After the setup, the first part we did was recording the heart activity so to examine the electrical and mechanical traces of the heart. Then, we moved onto examining the properties of extrasystolic contractions. We stimulated the heart during late diastole and early diastole and recorded our observations. Following the part about extrasystolic contractions was vagal stimulation. We first increased the voltage and caused bradycardia, slowing of the heart, and we increased the voltage even more to get cardiac arrest, stoppage of the heart. The heart beat returned after a short period of time and that demonstrated the concept of vagal escape where the next fastest pacemaker cell takes over after the one at the SA node is affected and does not function properly. The last part of the exercise we did was adding epinephrine to the heart and observing the effects it had on the heart's activity. We recorded data for 6 minutes in total for this portion of the exercise. Of the above mentioned protocols, my group and I really only exposed the frog heart. We had problems with the connections as well as the frog heart itself since the wiring of the frog heart ventricle was too

close and was about to rip the heart at any moment. As a result, we joined another group to complete the rest of the lab, following the instructions as listed in the lab manual.

Results:

Part 3: Electrical & Mechanical Activity of the Heart

After opening up the frog and exposing the heart, we set up our apparatus so to record normal frog heart activity. From the mechanical and electrical trace of heart activity, the information in table 1 was obtained. As expected, ventricular contraction is much greater than atrial contraction. The latency is determined to be 120msec, meaning that it takes 120msec after stimulation before a contraction is seen. In the human reflex lab, the latency for the H wave was 30-45 msec and so a latency of 120msec for heart contraction is very reasonable and expected. Also, the average heart rate of the heart was recorded as 37.260 BPM.

Table 1: General Data on Heart Activity

	Heart Data (averages)
Atrial Contraction Force	0.591 grams
Ventricular Contraction Force	2.522 grams
Heart Rate	37.260 BPM
Latency	120 msec

Part 4: Extrasystolic Contractions

We determined the point of late diastole on the heart activity mechanical trace and stimulated the heart at that point in hope to elicit an extrasystole. We did this multiple times while slowly increasing the stimulus voltage until an extrasystole contraction was observed. This threshold stimulus voltage, 2.8V in this case, is the minimum voltage that can elicit an

extrasystolic contraction during late diastole. We then increased the stimulus voltage to 2x the threshold, 5.6V in this case, and elicited an extrasystolic contraction during late diastole as well. Lastly, we increased the voltage to determine the threshold voltage that would elicit an extrasystole at early diastole. The threshold for that turned out to be 8.0V. The observed trend is that a much lower threshold is needed to elicit an extrasystole at late diastole than at early diastole. A threshold that is almost 3x that of the threshold during late diastole was needed to elicit an extrasystole. The observed results are, for the most part, consistent with the expected. The slight difference would be regarding to the extrasystole elicited during early diastole. During early diastole, a much greater threshold voltage is needed before a contraction is seen. The threshold voltage during early diastole is more than 5.2 V greater than the threshold voltage during late diastole. Such an elevated threshold during early diastole is due to the refractory period that directly follows ventricular contraction. Without the high voltage, there would not be an extrasystole contraction.

Table 2: Extrasystole Data: Stimulus voltages for different thresholds

	threshold during late diastole	2x threshold during late diastole	threshold during early diastole
Stimulus Voltage	2.8 V	5.6 V	8.0V

Table 3: Contractile Force before, during, and after extrasystole (late diastole)

	Contractile Force
Before extrasystole	2.47 grams
During extrasystole	2.20 grams
After extrasystole	2.506 grams

Table 4: Contractile Force before, during, and after extrasystole (early diastole)

	Contractile Force
Before extrasystole	2.451 grams
During extrasystole	0.948 grams
After extrasystole	2.257 grams

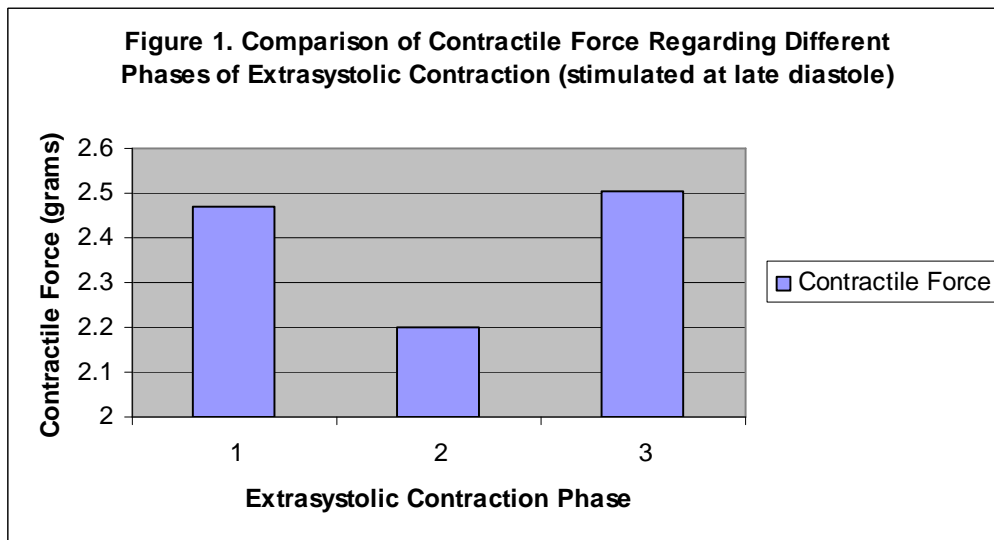


Figure 1 A plot comparing the contraction force 1) before extrasystole 2) during extrasystole and 3) after extrasystole when extrasystole was stimulated at late diastole. Contraction force of the heart is measured at different instances. The observed trend is that the contraction forces before and after extrasystole is notably greater than during extrasystole. Contraction force after extrasystole is the greatest of the three, slightly greater than before extrasystole.

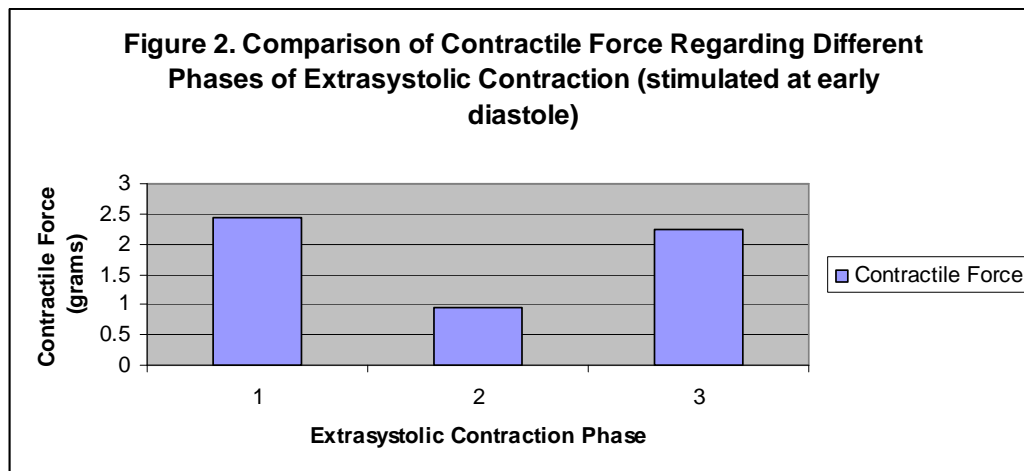


Figure 2 A plot comparing the contraction force 1) before extrasystole 2) during extrasystole and 3) after extrasystole when extrasystole was stimulated at early diastole. Contraction force of the heart is measured at different instances. The observed trend is that the contraction forces before and after extrasystole is notably greater than during extrasystole. However, contraction force after extrasystole is the less than that of before extrasystole.

The compensatory pause is the time period between an extrasystolic contraction and the next normal ventricular contraction. From the data collected, we determined the compensatory pause for late diastole, 2x late diastole, and early diastole. We simply looked at the mechanical trace of the heart, located the extrasystole and next normal ventricular contraction and determined the difference in time between the two points. We expected to see that the compensatory pauses be the same for the two instances of late diastole. However, the observed is that the compensatory pause for 2x late diastole is longer than that of late diastole. That could be due to the fact that the contraction was stimulated earlier for the 2x late diastole, resulting in a longer waiting time. The compensatory pause for early diastole is expected to be higher or rather, highest because the contraction is stimulated the earliest during early diastole. That means that

there is a longer wait time before the next normal ventricular contraction. The observed trend, does comply with that there is an increase in compensatory pause.

Table 5. Comparison of Compensatory Pauses

	Compensatory Pause (msec)
Late Diastole	131 msec
2x Late Diastole	144.2 msec
Early Diastole	174.8 msec

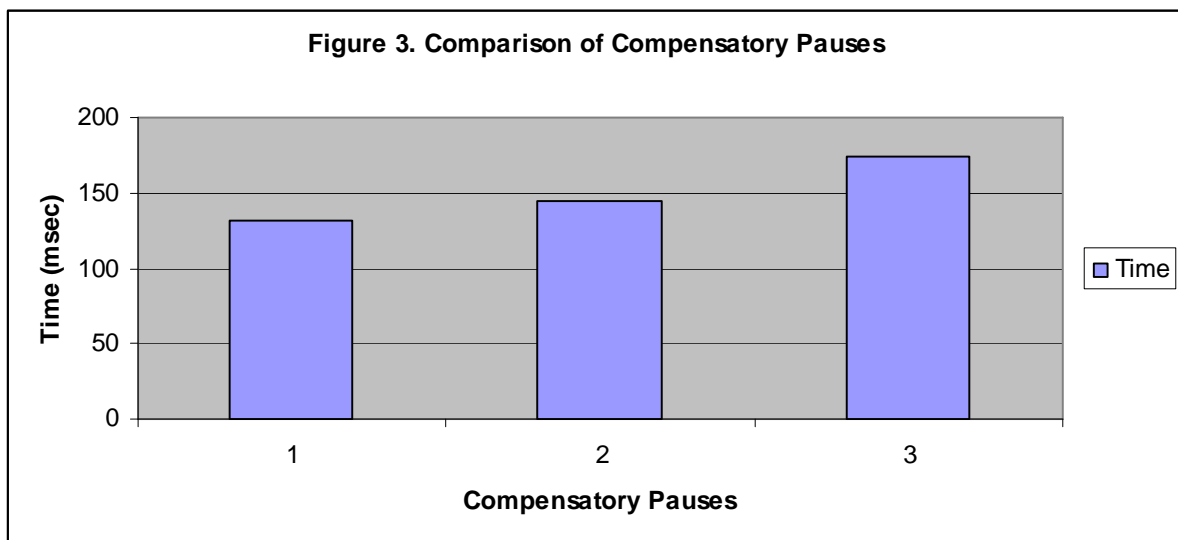


Figure 3 A plot comparing compensatory pauses at 1) late diastole, 2) 2x late diastole, and 3) early diastole. Compensatory pauses determined at each of the three types of extrasystolic stimulation. The observed is that there is an increase in compensatory pause with an increase in voltage. The compensatory pause at early diastole should be the longest one because extrasystolic contraction stimulated at late diastole has the longest filling time due to early stimulation.

Part 5: Vagal Stimulation- BRADYCARDIA

As part of the vagal stimulation portion of this exercise, we performed bradycardia on the heart. We stimulated the vagus nerve and the voltage was raised up until the first sign of the heart slowing down. The voltage at which the first sign of a slow heart is displayed is the threshold stimulus voltage for bradycardia. After bradycardia, the recording machine continued to record for 30secs more. We then used our recorded data and compared the heart rate and contractile force before and after bradycardia. The observed trends for heart rate and contractile force are opposite of one another. It is seen that heart rate prior to bradycardia is 1.265BPM greater than that of after bradycardia. However, it is seen that the contractile force after bradycardia is 0.042grams greater than that of before bradycardia.

Table 6: Bradycardia HR and Contractile Force before and after bradycardia

	Heart Rate	Contractile Force
Before Bradycardia	39.268 BPM	2.569 grams
After Bradycardia	38.003 BPM	2.611 grams

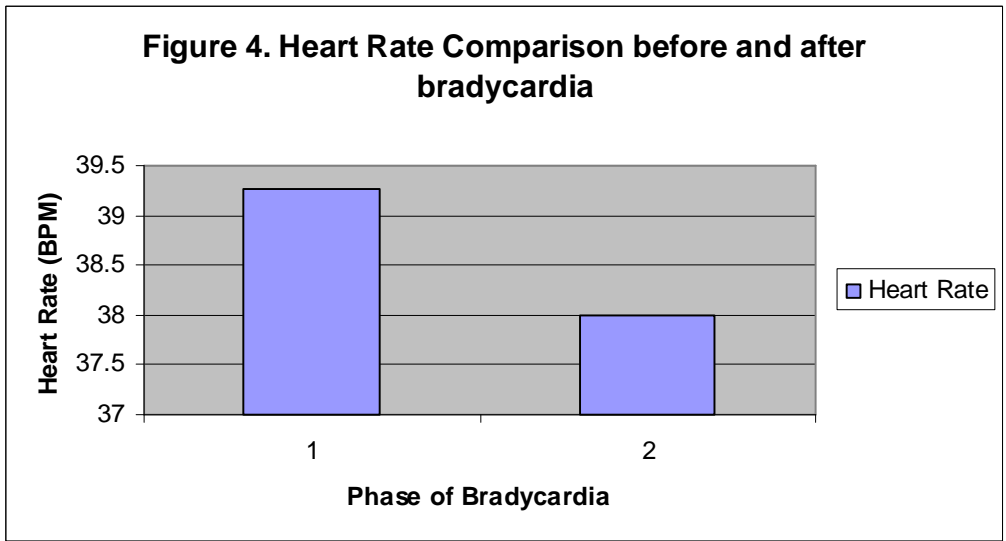


Figure 4 A plot comparing the heart rate 1)before and 2)after bradycardia. Frog heart stimulated at 1.0V and that resulted in bradycardia. The heart rate before and after bradycardia were recorded. The observed trend is that the heart rate for before bradycardia is greater than after bradycardia, slowing of the heart.

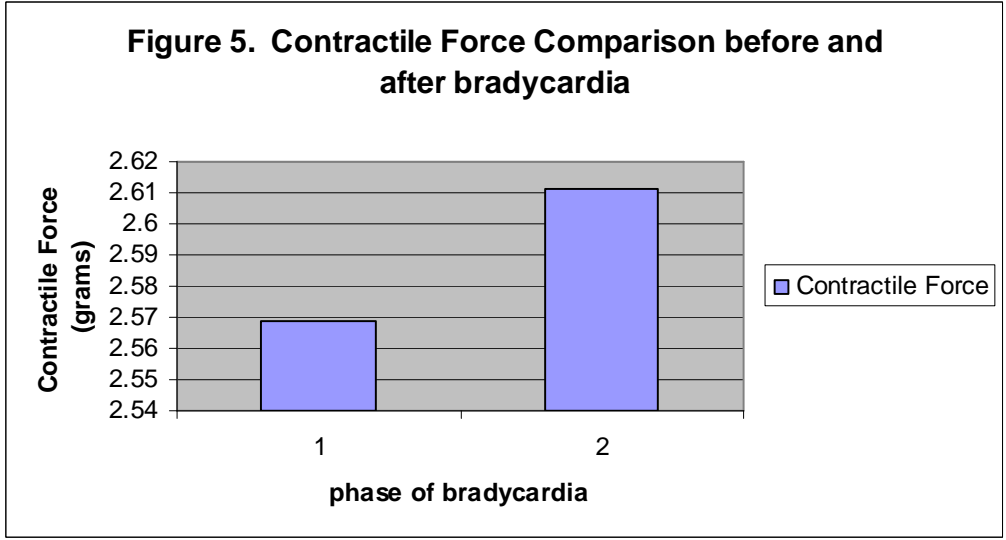


Figure 5 A plot comparing the contraction force 1)before and 2)after bradycardia. Frog heart stimulated at 1.0V and that resulted in bradycardia. Contraction force before and after

bradycardia were recorded. The observed trend is that contraction force is greater after bradycardia as compared to before.

Part 5: Vagal Stimulation- VAGAL ESCAPE

Vagal Escape was part of the later portion of the vagal stimulation section. Vagal escape is where the heart stops beating but after some time, resumes to essentially normal beating. We first stimulated the vagus nerve and gradually increased the voltage until the heart stopped beating; 2.4V was the stimulus voltage that corresponded to cardiac arrest. Heart activity was recorded throughout this process. Heart activity was recorded for 60 seconds after a heart beat returned. One of the two observed trends is that heart rate prior to vagal escape is significantly greater than the heart rate recorded after vagal escape. There is a 2.769 BPM difference between the two heart rates. This relationship is consistent to the expected. The other observed trend is that heart contractile force is greater after vagal escape. This is also consistent with the expected.

Table 7: HR and Contraction Force before and after vagal escape

	Heart Rate	Contraction Amplitude
Before Vagal Escape	37.22 BPM	3.014 grams
After Vagal Escape	34.451 BPM	3.396 grams

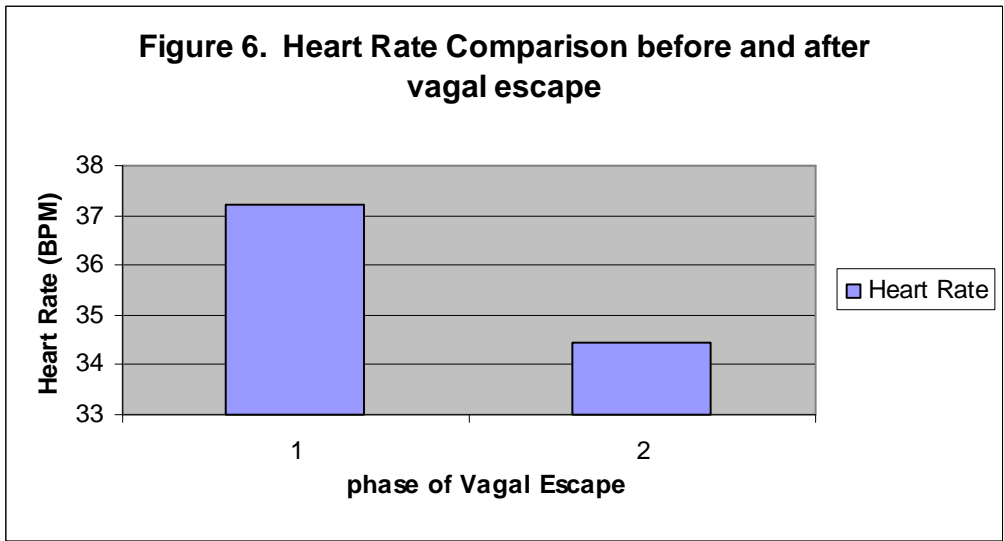


Figure 6 A plot comparing heart rate 1) before and 2) after vagal escape, the return of the heart beating after cardiac arrest. Frog’s vagus nerve was stimulated at a high voltage of 2.4V and that resulted in cardiac arrest. Heart rate was recorded for before and after vagal escape. The observed trend is that the heart rate before vagal escape is greater than the heart rate after vagal escape.

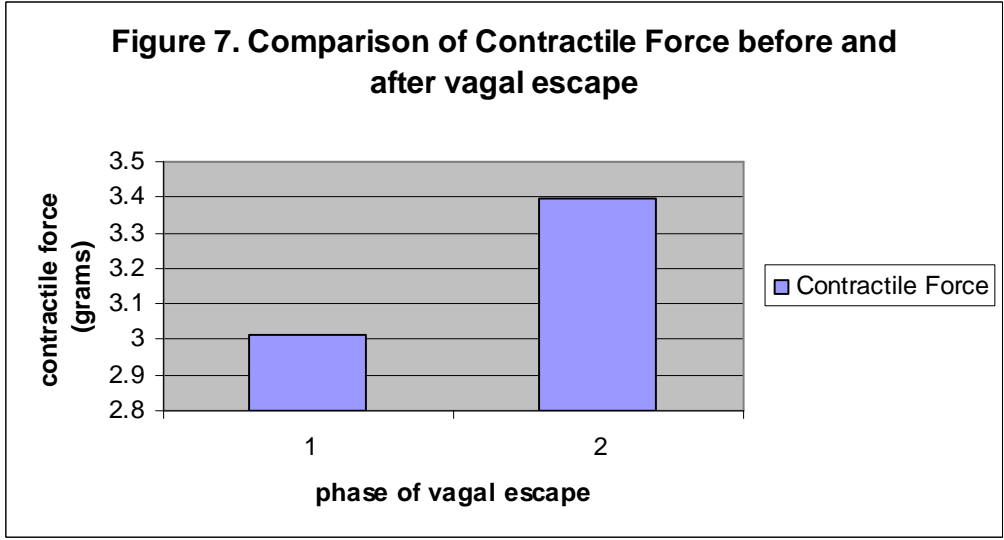


Figure 7 A plot comparing contractile force 1) before and 2) after vagal escape. The observed trend is that contraction force before vagal escape (when the heart was in bradycardia and about

to enter cardiac arrest) is less than the contraction force after vagal escape (when the heart began beating again).

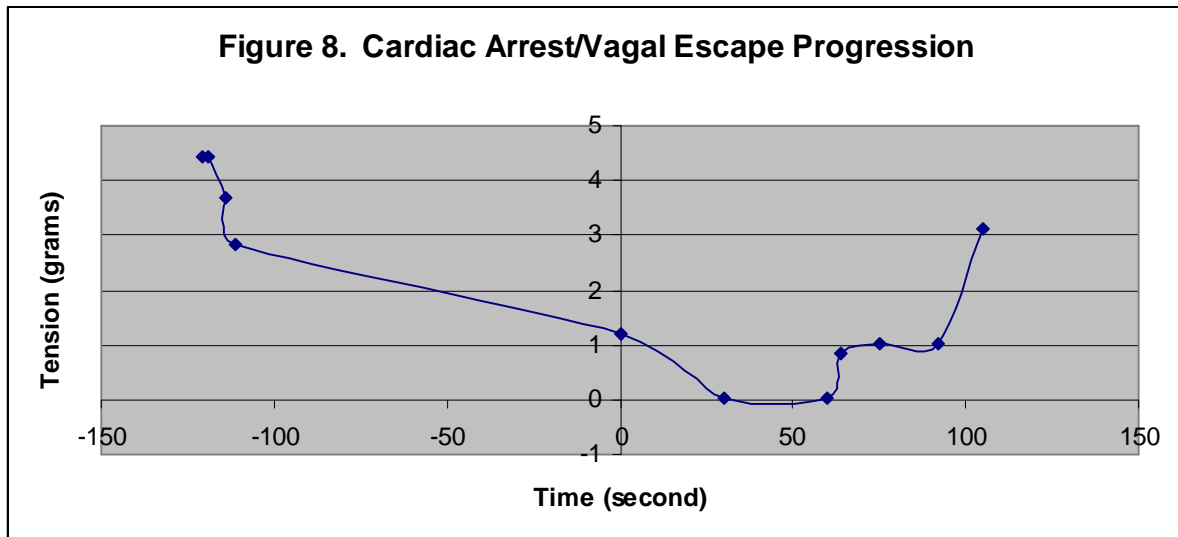


Figure 8 A plot showing the change in contraction force during which the heart was in cardiac arrest. X-axis represents the time that passed after the heart stopped. Y-axis represents muscle tension. Time = 0 represents the beginning of cardiac arrest. Contraction force was measured at 30 second intervals. The observed is that contraction force decreases during cardiac arrest, as shown with a dramatic dip on the curve, and contraction force gradually increases after the heart beat returned.

Part 6: The Effects of Epinephrine

Contractile force was recorded throughout vagal escape. We allowed the heart to beat normally and then we stimulated the vagus nerve and that led to bradycardia. When we increased the voltage, the heart arrested, resulting in a complete stoppage. In figure 6 below, time 0sec corresponds with the heart being in “late” bradycardia and time 12sec corresponds to

when the heart came to a complete stop. At both those times, the tension was recorded to be around 1gram. The dip after 12sec illustrates the decrease in tension to essentially zero. Starting at around 60sec, the curve begins to rise again, indicating that a heart beat is returning. At 120sec, the contractile force drastically increases from the 1.018 grams at 90sec to 3.336 grams. By 150sec, the contractile force reaches 3.591 grams. From the point when the heart arrested at 12sec to at 150sec, there is a 2.4grams difference in contractile force. Overall, the observed trend is that contractile force, over time, increases and heart activity resumes to normality after the heart has been arrested. The contractile force even exceeds the contractile force prior to cardiac arrest.

We added a few drops of epinephrine to the ventricle of the frog heart so to observe the response the heart has to sympathetic input. After adding epinephrine, we collected data for 6 min. With the data collected, we compared the heart rate and contractile force before and after epinephrine application. As shown in the two figures below, it is observed that both heart rate and contractile force are greater after epinephrine application. This observation agrees with what is expected. An increase in heart rate and contractile force are supposed to result with the sympathetic input.

Table 8: HR and Contraction Force before and after epinephrine

	Heart Rate	Contraction Amplitude
Before Epinephrine	37.736 BPM	2.009 grams
After Epinephrine	41.836 BPM	2.287 grams

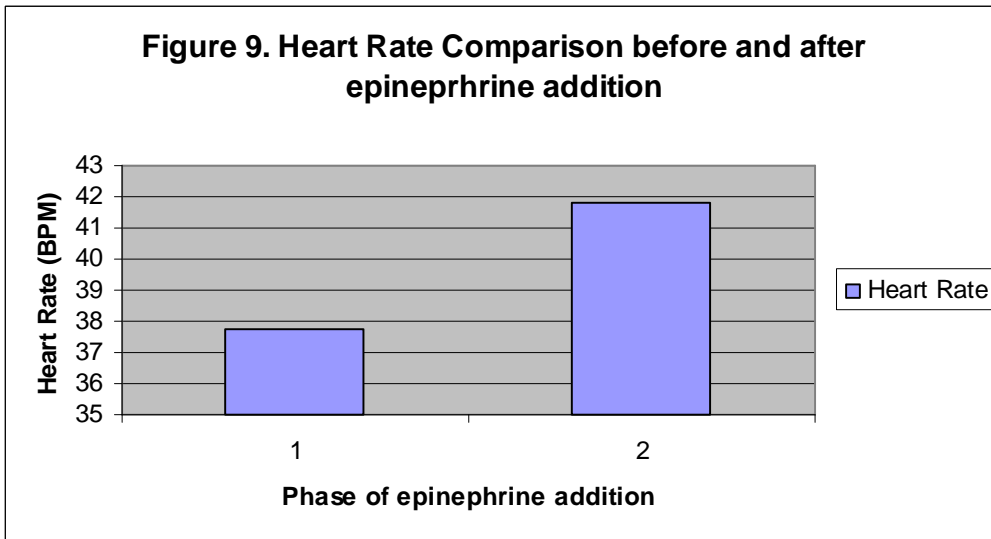


Figure 9 A plot comparing heart rate 1) before and 2) after the addition of epinephrine to the heart. Between 4 and 6 drops of epinephrine were added to the frog's ventricle. Data was collected for a total of 6 minutes. The heart rate for both cases were recorded and compared. The observed trend is that a higher heart rate is seen after the addition of epinephrine. In contrast, a relatively low heart rate is seen before the addition of epinephrine.

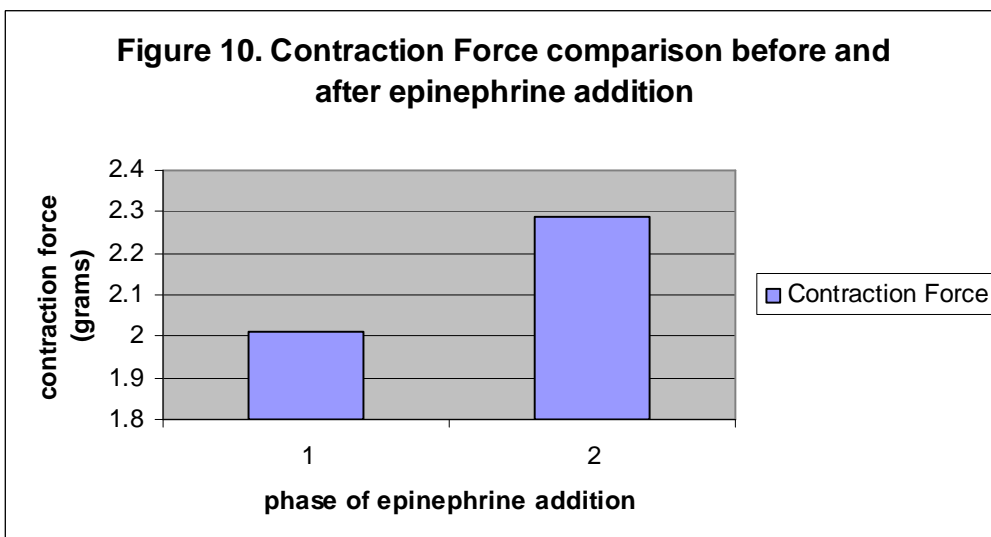


Figure 10 A plot comparing contraction force 1) before and 2) after the addition of epinephrine. Between 4 and 6 drops of epinephrine were added to the frog's ventricle. Data was collected for a total of 6 minutes. The observed trend is that contraction force is greater after the addition of epinephrine.

Discussion:

In the heart, there are autorhythmic or pacemaker cells that regulate the beating of the heart. Pacemaker cells only make up a very minor portion of cardiac cells because they are only responsible for initiating and conducting action potentials needed for heart contraction.

Pacemaker cells don't have resting potentials. Instead, they have what is known as pacemaker potential. According to Sherwood, a pacemaker potential is an autorhythmic cell membrane's slow drift to threshold. Three different ion movements, together, are responsible for pacemaker potential: an increase in Na^+ influx, decrease in K^+ efflux, and increase in Ca^{2+} influx. Na^+ first enter through special voltage gated channels. Then, K^+ channels start to close, resulting in a decrease in K^+ efflux. Lastly, there is an increase in Ca^{2+} influx due to the opening of T-type Ca^{2+} channels. All three ion movements ultimately contribute to the membrane reaching threshold for an action potential (Sherwood p. 309-310).

There are four locations in which pacemaker cells can be found and these locations are 1) SA node, 2) AV node, 3) Bundle of His (atrioventricular bundle), and 4) Purkinje fibers. The SA node is located near the superior vena cava in the right atrial wall. The AV node is located at the bottom of the right atrium. The bundle of His, right and left, extends from the AV node to the interventricular septum. The Purkinje fibers go from the bundle of His and extend all over the ventricular myocardium. All these pacemakers have different rates of depolarization to threshold and the fastest pacemaker cells are located in the SA node. The pacemaker cells at the other

locations have to be activated by the SA action potentials before they are able to reach threshold, at a slower rate, on their own. With that said, the pacemaker cells at the SA node are the primary regulators of the autorhythmic activity of the heart (Sherwood p310-311)

The excitation-contraction coupling process for heart (cardiac muscle) contraction is very similar to that of skeletal muscle contraction. A stimulus causes a ventricular action potential in cardiac contractile cell. That action potential travels down T tubules and opens up L-type Ca^{2+} channels, leading to an influx of Ca^{2+} . This influx of Ca^{2+} , in turn, causes the release of a great amount of Ca^{2+} from the sarcoplasmic reticulum. The end result of the multiple influx of Ca^{2+} is high cytosolic Ca^{2+} . With sufficient cytosolic Ca^{2+} , there is Ca^{2+} to bind to Troponin C so that it can no longer bind to Tropomyosin. Without Troponin C attached to it, Tropomyosin can no longer attach to and block the myosin binding site. The myosin binding site is now vacant for myosin to attach and when myosin attaches, cross-bridge cycling occurs. The resulting power strokes of cross-bridge cycling causes sliding of thick and thin filaments. Cardiac muscle contraction is then the end result (Sherwood p.315).

The main difference between skeletal muscle contraction and cardiac muscle contraction is that Ca^{2+} influx during phase 2 of ventricular action potential is required for cardiac contraction. As mentioned earlier, there is not always enough cytosolic Ca^{2+} in cardiac muscle and so in instances when there is a lack of cytosolic Ca^{2+} , Troponin C will still be bound to Tropomyosin, allowing Tropomyosin to continue blocking the myosin binding site. Myosin needs to bind to the myosin binding site to initiate cross-bridge cycling and so with the site blocked, cross-bridge cycling is not possible and that means that there won't be cardiac muscle contraction (Sherwood p.315).

The ventricular action potential is the mechanism behind the excitation of the heart. There are multiple phases to this mechanism and each phase is symbolic of different ion activity. In phase 0, there is a steep increase in membrane potential and that is due to the influx of mainly Na^+ and Ca^{2+} because Na^+ and Ca^{2+} channels opened up. As a result, depolarization occurs in phase 0. Phase 1 is where a quick repolarization, as indicated by the curve sloping down, takes place and that is due to the inactivation of Na^+ channels. Phase 2 is where the curve plateaus as a result of more Ca^{2+} influx. The Ca^{2+} in phase 2 are coming through L type Ca^{2+} channels. In phase 3, there is more repolarization but this due to Ca^{2+} channel inactivation and K^+ efflux as a result of K^+ channel activation. Phase 4 is the last phase and that is where resting membrane potential is resumed (Sherwood p. 314-315)

The heart acts as the organ pump that is responsible for pumping blood to all over the body. In doing so, the heart is supplying oxygen and eliminating waste, like CO_2 , from body tissues. Blood from the body flows back to the heart and by then, the blood is deoxygenated (since oxygen has been delivered to tissues) and CO_2 -rich (since CO_2 has been picked up, as waste, from the tissues). The blood enters the right atrium through the venae cavae. So to oxygenate the blood again, the blood must enter the pulmonary system and get oxygenated in the lungs. The blood leaves the right atrium and enters the right ventricle where it then enters the lungs to get rid of the CO_2 and be supplied with O_2 . The oxygenated blood enters the left atria and then left ventricle and ultimately goes into the system circulation by exiting through the aorta.

Our ultimate goal for this exercise is to better understand the properties of the human heart although we are using the heart of a frog, amphibians that are able to supply oxygen and nutrients to the cardiac muscle from blood due to the anatomy of the heart: 2 atria and 1 ventricle,

for the experiment. The heart is a complicated and extremely essential part of the body. It is made up of cardiac muscle. Cardiac muscle, like skeletal muscle, is striated, meaning that the muscle is made up of alternating thick and thin filaments. Cardiac muscles are made up of cardiac muscle fibers and these cardiac muscle fibers are connected to each other by intercalated discs. The two types of membrane junctions that are inside an intercalated disc are desmosomes, those responsible for mechanical connections, and gap junctions, those responsible for electrical connections (Sherwood p. 308).

Part 3: Electrical & Mechanical Activity of the Heart

In this exercise, we studied the properties of the frog heart, which is fairly similar to the human heart and the first thing we did after dissecting the frog and exposing its heart is exploring the normal activity of the heart. It is expected to see that ventricular contraction be greater than atrial contraction. As shown in Table 1, atrial contractile force is significantly less than that of ventricular contractile force. That is because the left ventricle is actually the part of the heart that pumps blood to the rest of the body. A larger contraction is needed to achieve that, hence the 1.931 gram difference between atrial and ventricular contraction force. Also, the ventricles are larger than the atria and so the ventricles are capable of larger contractions. The latency for heart contraction is expected to be greater than that of human reflexes as explored in lab 3. The latency for heart contraction is recorded as 120msec while the latency for a H wave is 30-45 msec. The latency for heart contraction was observed to be longer and that's because there are more processes that the body has to go through, as compared to a reflex, before a contraction can occur.

Part 4: Extrasystolic Contractions

Extrasystolic contractions are premature ventricular contractions. They appear right after a ventricular contraction when stimulated at either late or early diastole. In Table 2, note the threshold stimulus voltage for extrasystolic contraction at late and early diastole. The threshold for early diastole is significantly greater than the threshold during late diastole. That is because of the difference in the refractory period that each was stimulated in. During early diastole, the muscle is in the effective/absolute refractory period. That is the time period where no action potential can occur. In the case of late diastole, the muscle is in the relative refractory period. Unlike with effective refractory period, an action potential can occur. As seen in Table 4, the contractile force for extrasystole in early diastole is less than that of late diastole. Technically speaking, there should not even be a contraction seen in effective refractory period. The values for late diastole are greater than those at there is more filling time for late diastole and less filling time for early diastole. As expected, a stronger contraction corresponds to a greater filling of the ventricle. This is known as the Frank-Starling Law of the Heart (Sherwood p. 328). Often times, the extrasystolic contraction is of lesser contractile force because there is less filling time. The ventricular contraction after the extrasystolic contraction and the compensatory pause should be greater than that of before and during extrasystole. That is because there is more filling time and more filling time equals increased end diastolic volume which ultimately leads to stronger contractions. The data collected during late diastole, as seen in figure 1, complies with the expected. The data collected during early diastole, as seen in figure 2, show that contraction after extrasystolic contraction is of lesser intensity than before extrasystolic. That is probably due to the continual twitching of the frog heart after extrasystole and before the next normal

contraction. The multiple twitches interrupted the elongated filling time and so not only did we not see an increase in contractile force in post extrasystole, we saw a decrease instead.

Like mentioned earlier, the heart goes into 2 types of refractory period after ventricular contraction. Effective refractory period is the one directly after ventricular contraction while relative refractory period is the one after effective refractory period. During the effective refractory period, no action potentials can occur and the purpose of that is to prevent the heart from going into tetany (Sherwood p. 316). The cardiac muscle, in this sense, is really different from that of skeletal muscle, because tetany in muscle is promoted and seen as the ultimate contraction goal. There are no protective factors, like the effective refractory period, that protects the muscle from reaching tetany. The effective refractory period is what causes the compensatory pause when extrasystole is induced. In addition to effective refractory period, cardiac muscles also have relative refractory period and the purpose of a relative refractory period is simply to have a longer filling time and a stronger contraction.

In table 5 and figure 3, a comparison between the compensatory pauses from late diastole, 2x late diastole, and early diastole was conducted. It is expected to see that the compensatory pauses at late diastole be about the same because the muscle is stimulated at the time period and so the filling time between the extrasystolic contraction and next ventricular contraction should be the same. As evidently seen in figure 3, the compensatory pauses for the two late diastole instances are not the same. That 13.2 msec difference could be that the extrasystolic contractions were not stimulated at the same exact time and so slight difference in compensatory pause resulted. The compensatory pause for early diastole is observed to be longer than the other two and that is consistent with the expected because stimulated an extrasystolic contraction earlier on allows for a longer filling time, hence, a longer compensatory period.

Part 5: Vagal Stimulation- BRADYCARDIA

During bradycardia, the heart is slowed as a result the increased voltage that stimulated the vagus nerve, a nerve that's part of the parasympathetic system. It is defined as heart rate < 60bpm. Some of the symptoms that point toward bradycardia are fatigue, lethargy, nausea, dizziness, confusion, pre/syncope, and shortness of breath. (Ahsley et. al, ch 8 Bradycardia). Our heart rates are ~40bpm, well below 60bpm, and so can be classified as bradycardia. It is expected that the heart rate before bradycardia be greater than after bradycardia because bradycardia is the concept of slowing of the heart. The heart rate after bradycardia should be lower because bradycardia is part of the parasympathetic nervous system and the mechanism governing it is the chain of reaction that starts with the release of Ach. The parasympathetic system releases Ach and Ach binds to muscarinic Ach receptors. Pacemaker cell activity in the SA node is slowed as a result of Ach binding to the receptor. That leads to a decrease in HR. The observed HR data, as seen in figure 4, agrees with the expected. The same expected results go with contractile force. Contractile force after bradycardia should be lower because of parasympathetic activity. As described above, the SA node pacemaker activity is affected but in addition, the AV node pacemaker activity is also affected in the way that Ach makes it hard to spread action potential. In other words, that makes a contraction harder to elicit. The observed data actually goes against the expected; figure 5 shows that contractile force after bradycardia is greater than before. The difference between the two contractile forces is only 0.042 grams, not very much at all and so a simple yet plausible explanation would be that human errors while analyzing the data caused for such disagreeing results.

Since bradycardia is part of parasympathetic activity that affects heart activity, any sort of sympathetic activity would have a counteract effect and inhibit bradycardia. A study shows that galanin, a cardiac sympathetic co-transmitter has an inhibiting effect on bradycardia. Galanin reduces acetylcholine release and as described earlier, Ach binds to muscarinic Ach receptors so to slow the pacemaker cell activity at the SA node. When galanin is present, there will be a decrease in Ach due to decrease Ach release and the chain of parasympathetic reactions that follow is made impossible. The end result of that would be no effect on SA node pacemaker cell activity and so no bradycardia (Herring et. al p.1)

Part 5: Vagal Stimulation- VAGAL ESCAPE

Vagal Escape is where the heart stops beating and after a period of time, the [less intense] heart beat returns. It is the result of prolonged vagal stimulation in which bradycardia and then cardiac arrest is the end result. In the process, the SA node pacemaker activity is affected and so that's why we see the stopping of the heart. The return of the heart beat is when the next fastest pacemaker cells take over. This concept of taking over the role of the SA node is supported Wallace and Daggett. They say that the pacemaker cells at different areas other than the SA node usually take over the pacemaker role. The atrium, AV node, or ventricles have been suggested as sites of pacemaker activity when the SA node is no longer available for rhythmic regulation (Wallace et. al p.93). In our preparation, I do think that there is tonic vagal inhibition of the heart because in the beginning, the heart seemed to beat at a more normal pace. As more activities were done because after stimulating the heart for a while, our frog heart seemed to beat slower, showing signs of bradycardia and perhaps progressing to cardiac arrest.

When regarding heart rate and contractile force, it is expected that both will be lower for after vagal escape and that is because of slower pacemaker cells taking over the SA node. From figure 6, we can see that HR after vagal escape is lower. That agrees with the expected result, meaning that parasympathetic activity affected heart activity. In figure 7, opposing results are shown for contractile force before and after vagal escape. Contractile force is supposed to be less after vagal escape but the observed shows that contractile force after vagal escape is greater than before vagal escape. This odd result is probably due to the fact that the data for the vagal escape section is not very consistent or accurate. In my case, the contractile force before vagal escape actually corresponds more to that of bradycardia because normal heart activity was not recorded. If normal heart activity was recorded, the contractile force after vagal escape would indeed be lower because normal activity is regulated by the SA node pacemakers (fastest) while after-vagal-escape activity is regulated by other slower pacemakers, hence, weaker contraction.

Figure 8 is an illustration of how the vagal escape works. At the far left side of the curve, the tension is relatively high and as time passes by, the tension begins to drop, signifying bradycardia. At time= 0 was when the heart stopped. Tension drastically dropped as indicated by the sharp dip in the curve. The duration of cardiac arrest was calculated to be 62.76 seconds. At ~60sec, or 1min, after the heart stopped, a heart beat returns and that's when the next fastest pacemaker cells take over. Tension continues to gradually increase.

Part 6: The Effects of Epinephrine

Epinephrine is released by the sympathetic nervous system. Epi binds to B-1 adrenergic receptors and that increases the heart rate. With that said, it is expected that an increase in heart rate be seen after applying epi. In figure 9, we see, as expected, that an increase in HR did result

after epi addition due to increase in sympathetic activity. The application of epi also increases Ca^{2+} influx. As discussed in the cardiac muscle excitation-contraction coupling section, Ca^{2+} influx is responsible for generating heart contractions. The more cytosolic Ca^{2+} there is, the greater the heart contractions because of more cross-bridging. With that in mind, it is expected that after the application of epi, there will increase the contractile intensity. Like with HR, contractile intensity also agrees with the expected result.

Norepinephrine, like epinephrine, is part of the sympathetic nervous system and so the application of it will also lead to increase HR and contractile force. According to a study, the stimulation of adenosine A1 receptors has effects that protect the heart. These receptors are able to protect the heart through the inhibition of norepinephrine (Schutte et. al, p. 1). As said before, norepinephrine increases sympathetic activity and so adenosine A1 receptors act like buffers for the heart.

Conclusion:

In conclusion, through this exercise, we explored the properties of the heart and found that there are many factors that can affect heart contraction. Factors like degree of ventricular filling, compensatory pause, parasympathetic activity, sympathetic activity, cytosolic Ca^{2+} concentration, types of pacemaker cells, etc. all contribute in determining whether there will be a contraction and if so, how strong it might be. Because the heart is a complicated organ, the mechanisms associated with it are too. We found that a higher voltage is needed to stimulate an extrasystolic contraction during early diastole because of the effective refractory period. We also found that the compensatory pause is the longest for when during early diastole because the early extrasystole stimulation allowed for more filling time. Very importantly, we got a better

understanding of how parasympathetic and sympathetic activities affect heart rate and contractile force. Parasympathetic activity includes stimulation of the vagus nerve and release of Ach, neurotransmitter that will bind to muscarinic receptors to decrease HR and contractile force. Sympathetic activity includes the release of epinephrine, or norepinephrine, by sympathetic nerves and the binding of the either neurotransmitter to B1-adrenergic receptors so to increase HR and contractile force. The findings from this laboratory exercise are beneficial in researching for cures or preventions for heart diseases and other illnesses relating to the heart. With heart disease accounting for the most deaths in the U.S., better understanding of the heart and is always amongst the goals researchers strive for.

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