

Lab 5: Electrical and Mechanical Properties of The Frog Heart

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Introduction:

The heart is considered one of the most vital organs in organisms. Heart disease is the No.1 cause of death in the United States. The heart is nothing more than just acting like a pump which is composed of muscles, delivering blood which carries all essential nutrients, vital oxygen and remove waste metabolic products cells don't need..Without the function of heart, none of living cells could survive. Therefore, the knowledge of the electrical and mechanical process behind heart contraction and heart rate should be learned in order to monitor and prevent abnormal heart activities.

The cardiac muscle is striated, involuntary, and size is shorter than skeletal muscle cells. The cardiac muscles are connected by the structure called intercalated disks which contains desmosomes mechanically connecting cells and gap junctions electrically coupling cell together. With the help of intercalated disk ,the depolarizing current flows through cell to cell and reaches its threshold to fire action potentials to contract heart. For this experiment, the heart used came from a double-pithed frog It contracts electrically similar to the human heart which will help in correlating the properties of human heart. however, there were some difference in the anatomical structure .Unlike human heart, the frog heart has three-chambers which included two atria and only one ventricle. Within the single ventricle, oxygenated and deoxygenated bloods were separated by a spongy folds wall called trabeculae. Similar to human heart, the blood is oxygenated in the lungs, travel through the pulmonary veins, and back to ventricle. The spiral valve in aorta will further ensure the separation of blood flow. The heart activity can be described by cardiac cycle which included diastole (ventricular filling and isovolumetric relaxation) and systole (isovolumetric contraction and ventricular ejection) .

In this experiment, mechanical and electrical activity of pacemaker cells and ventricle cells were measured to understand the effects of direct ventricular stimulation, and the stimulation of sympathetic and parasympathetic nervous systems on heart rate and ventricular contractile force. Extrasystolic contractions are premature ventricular contractions that occur when the cardiac muscle cells depolarize before the completion of repolarization of the previous action potential (Sherwood, 2010, pp. 319). An extrasystolic contraction should be expected when the heart is stimulated during late systole because the heart is in relative refractory period. During this time, the ventricle force produced from SA node fail to produce because the heart is in the absolute refractory period of extrastole. This missing of 1 beat produces the compensatory pause (Sanoop, 2012, pp. 295). A greater ventricular contraction force after the compensatory pause should be expected due to excess calcium remaining. When double the intensity of the threshold stimulus, the force of the ventricular contraction are expected the same as the force generated during late diastole at threshold. When stimulate the heart during early diastole, the heart could be in beginning of relative refractory which need larger intensity to initiate a response or absolute refractory period that there should be no response at all. The tension after the compensatory pause should be greater compared with the tension during late diastole due to Frank-Starling Law. The law states that that an increase in the preload to the heart will result in increased stroke volume and contractility strength (Konhilas et al., 2002, pp. 305-306). For last part, a decreased heart rate and force contractility are expected when direct stimulate vagal nerve and a increased heart rate and force of contractility are expected when add epinephrine due to the fact parasympathetic pathway decrease the heart rate by releasing acetylcholine to decrease pacemaker activity of SA node, and epinephrine released from sympathetic innervations increase heart rate and force of contractility.

Materials and Method:

The detail procedure performed and material needed in this experiment were found in the 2nd Edition of “NPB 101L Physiology Lab Manual” (Bautista et al, 2009, pp. 43-53). For the first part, the vagus nerve was isolated that thread was looped through. The nerve was connected with the hook electrode leads for stimulation. The electrical and mechanical activities were recorded with no stimulation. The second part, extrasystolic contractions were produced by directly stimulating the ventricle at late diastole, early diastole, again at late diastole at threshold and double threshold which need to be determined. bradycardia, cardiac arrest, vagal escape were elicited by stimulating the vagus nerve. Because the frog was not dead, Ringer solution has to be applied through its skin in order to keep the body ventilated because frogs breathe through their skin. The frog's legs need to be massaged throughout the experiment in order to increase the blood flow returning to the heart. The last part, four drops of epinephrine was directly injected to the heart in order to observe the effect of sympathetic regulation. The heart rate and ventricle force were recorded. Data is recorded during all the experiments by BioPac software. For determination of force of ventricular contraction, the “P-to-P” option was selected. For determination of time intervals, the “delta T” function was used. Heart rate per minute was determined by BPM function.

Result:***Electrical and Mechanical Activity of the Heart***

In the first part of experiment, the force transducer was used to measure the mechanical and electrical heart activity by generating tension on the frog heart. The forces of contractions of atria and ventricles were analyzed by using “P-P” tool. The heart rate was analyzed using BPM tool of the biopac program. The atrial contraction was found to be 0.18 g, and the ventricular contraction was found to be 0.63g, which was stronger force than atrial contraction. The heart rate was found to be 55 BPM with an latency period of 0.25 seconds. The latency period was specifically reflects time difference between a electrical stimulus and its response generated.

Table 1. The Electrical and Mechanical Activity of the Frog Heart in span of two minutes It shows the atrial and ventricular contractions ,heart rate, and latency period recorded in a double-pithed frog’s heart.

Atrial Contraction (g)	Ventricular Contraction (g)	Heart Rate (BPM)	Latency Period (s)
0.18	0.63	55	0.25

Extrasystolic contractions:

In this part of experiment, contractile forces produced by extrasystole were measured during late diastole and early diastole by applying different stimulus intensity. The threshold voltage was the minimum stimulus intensity needed to initiate an extrasystolic contraction. The

tenison produced by ventricles before extrasystole was 0.40g .At threshold (3.5 V),the force of initial extrasystolic contraction during the late diastole was 0.64g, After the extrasystolic contraction, the next ventricle force was 0.42g.Then the stimulus voltage was doubled at 7V,and applied at late diastole to initiate an extrasystolic contraction. The tenison produced by ventricles before extrasystole was 0.37 .The force of extrasystolic contraction during the late diastole was 0.59g, After the extrasystolic contraction, the next ventricle force was 0.36g.Finally, an extrasystolic contraction was produced during early systole by setting a threshold voltage of 5 V as the intensity. The tenison produced by ventricles before extrasystole was 0.33 .The force of extrasystolic contraction during the late diastole was 0.36g, After the extrasystolic contraction, the next ventricle force was 0.33g.From the data, the contraction during late diastole and diastole after stimulus was reamin the same with the contraction before the stimulus. When voltage was increased to double the threshold, there was no obvious difference observed in data comparied to the tension at threshold stimulus. Also, the threshold voltage required to elicit an extrasystolic contraction during early diastole was grater than the voltage at late diastole

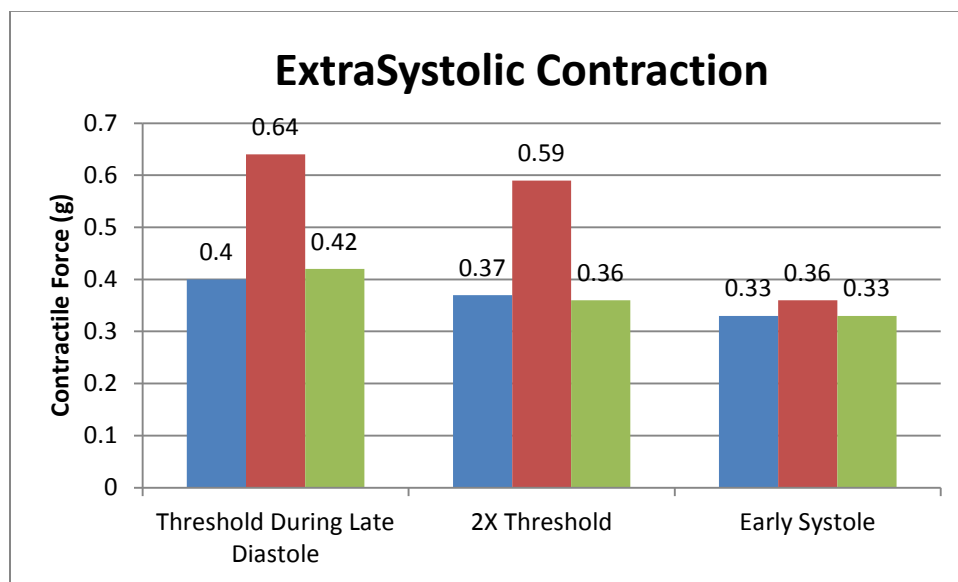


Figure 1. shows the tension caused due to extrasystolic contractions for threshold voltage (3.5V) during late diastole, double the threshold voltage (7V) for late diastole and at threshold voltage (5v) for early systole. The contractions after extrasystole are seen to be higher than the contractions before stimulus was applied. Also, a negligible difference is seen between the contractions when the stimulus is increased by two times the threshold. The force of contractions after stimulus for early systole is seen to be slightly smaller than the force of contraction after stimulus for late diastole.

Extrasystolic Contractions: Compensatory Pause

When an extrasystolic contraction was produced during late diastole, the compensatory pause was found to be 2.1seconds. When increased the voltage to double threshold, the compensatory pause was found to be 2.05 seconds. When an extrasystolic contraction was produced during early diastole, the compensatory pause was found to be 2.50 sec which was greater than the other two values.

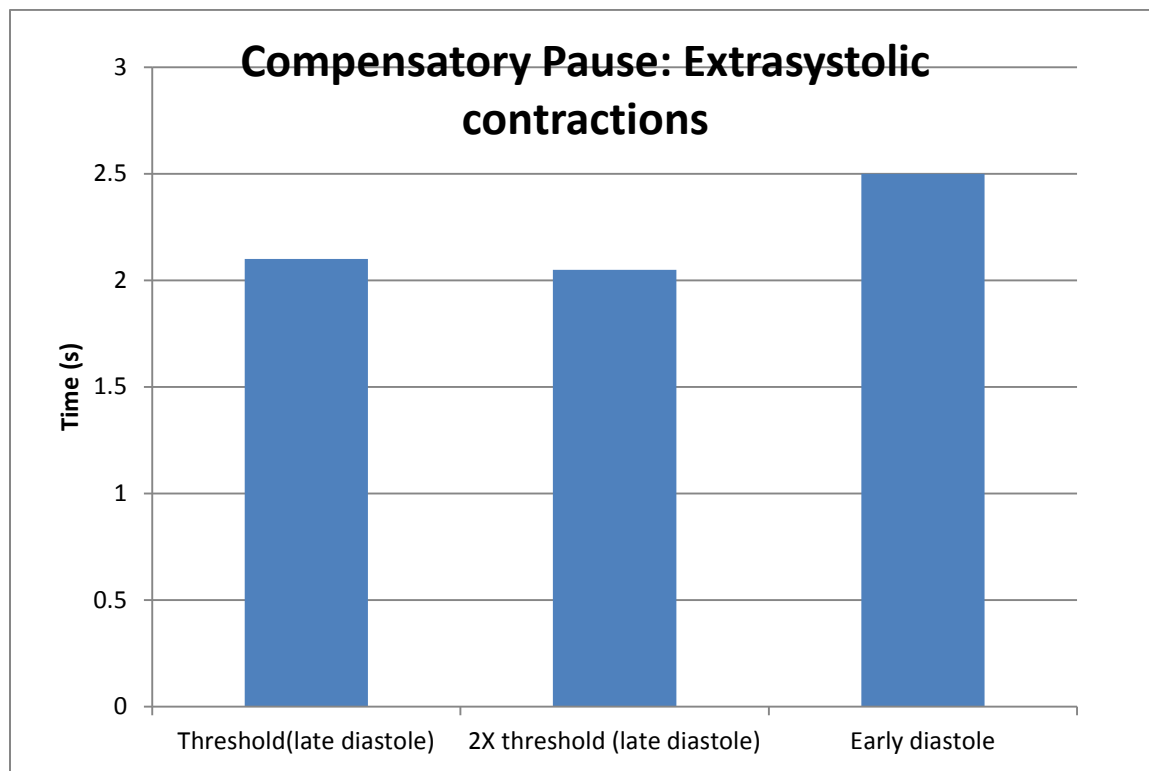


Figure 2. shows the different compensatory pauses time value after extrasystolic contractions were produce during different times of diastole. From the graph, the compensatory pause didn't change much as doubled the threshold voltage . The time was found to be around 2.1 seconds. But the compensatory pause increased to 2.5 seconds when an extrasystolic contraction was produced during early diastole.

Vagal Stimulation: Bradycardia, cardiac arrest and vagal escape

The vagus nerve was directly stimulated in order to study the impact of parasympatic system on heart activity. Prior to vagal stimulation, the heart rate and tension were found to be to be 46 BPM and 1.36 g respectively. Bradycardia was observed where the heart rate and tension decreased to 40 BPM and 0.31g respectively when the voltage increased to 1.42 V. When further increased the voltage to 2.5V, cardiac arrest was observed that the heart rate and the contraction were 0. And then, the heart rate started and tension went up again, where vagal escape occurred. However, the tension and intensity were found to be 40.1BPM and 0.78g respectively which were still lower than normal

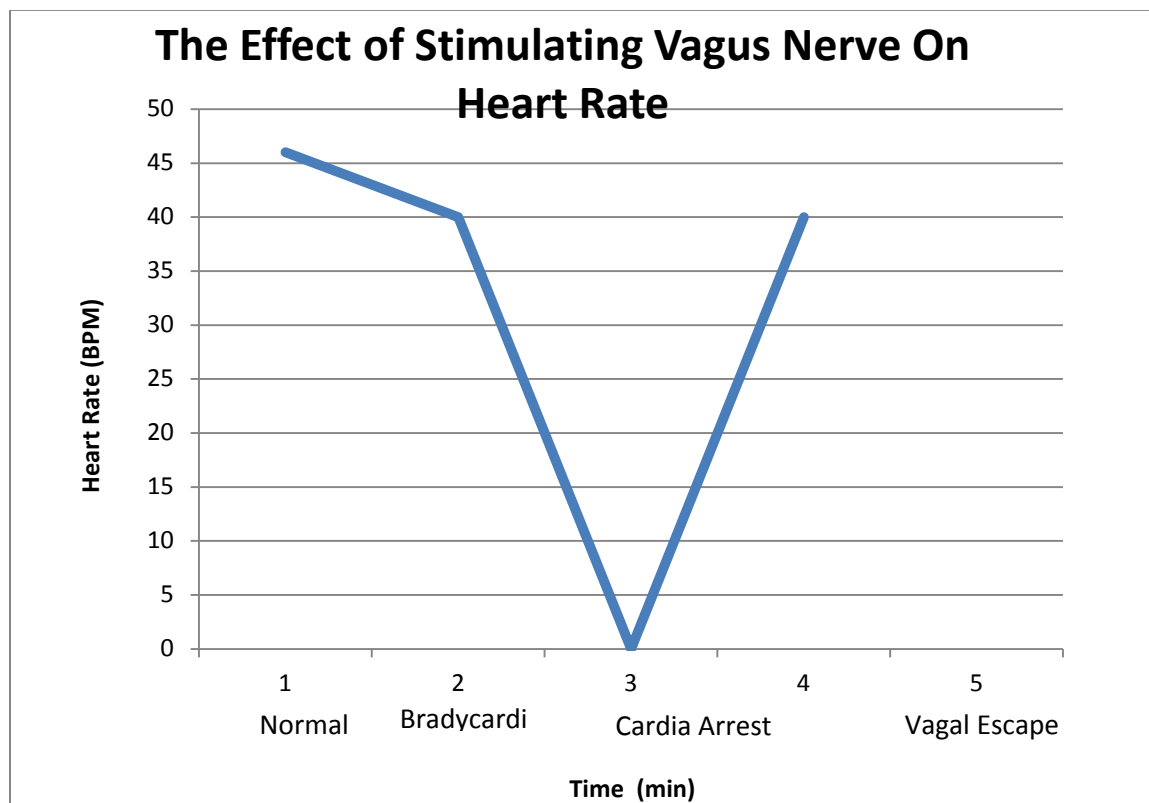


Figure 3. demonstrate the heart rate change when stimulating the vagaus nerve. From, as the intensity was increased, the heart rate and tension were seen to decrease. At 1.42V, bradycardia was observed and at 2.5V, the heart stopped beating altogether. This stimulus intensity was kept up for a while, the heart rate started to increase slowly, until it got to 40.1BPM.

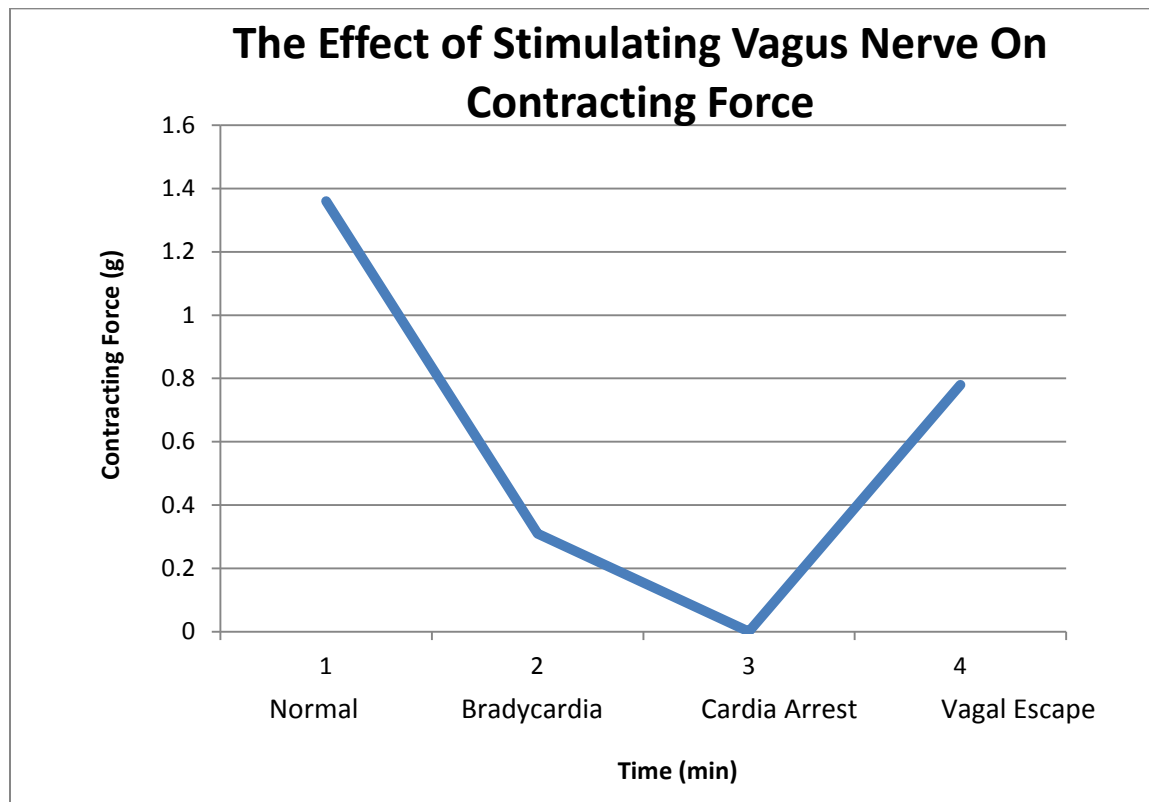


Figure 4. demonstrate the results of stimulation of the vagus nerve oncontracting force. Based on the table, as the intensity was increased, the heart rate and tension were seen to decrease. At 1.42V, bradycardia was observed and at 2.5V, the heart stopped beating altogether. This stimulus intensity was kept up for a while, the tension started to increase slowly, until it reach to 0.78g.

Effects of Epinephrine on heart rate and contractility

In the last part of the experiment, epinephrine were added to the ventricle of the frog in order to learn the effects of sympathetic innervation on heart rate and contractility. Due to computer technical problems, the data were lost before save. So the data in this part were borrowed from other group. They recorded the heart activities for time period of 5 minutes which was shorter

than the instruction of the lab manual. As epinephrine was applied to the ventricle, the heart rate and force went up rapidly. Heart rate increased from 31.34 BPM to about 38.8 BPM, after this time, the heart rate began to decrease again and stay constant at 38 BPM, and at the last min, heart rate dropped rapidly to 27.83 BPM. The tension went from 1.92 to about 2.97g. After this time, the tension began to decrease again, and stayed relatively constant at about 2.23g.

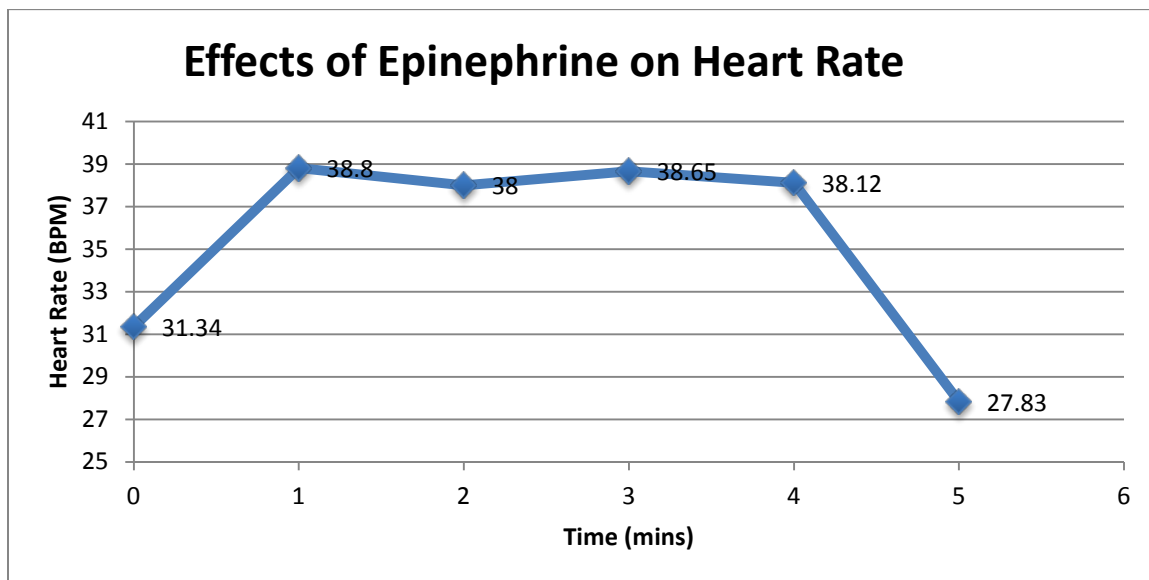


Figure 5. demonstrates the effects of adding epinephrine to the ventricle of a double-pithed bullfrog. Heart rate increases from 31.34 BPM to around 38 BPM, where it starts decreasing again.

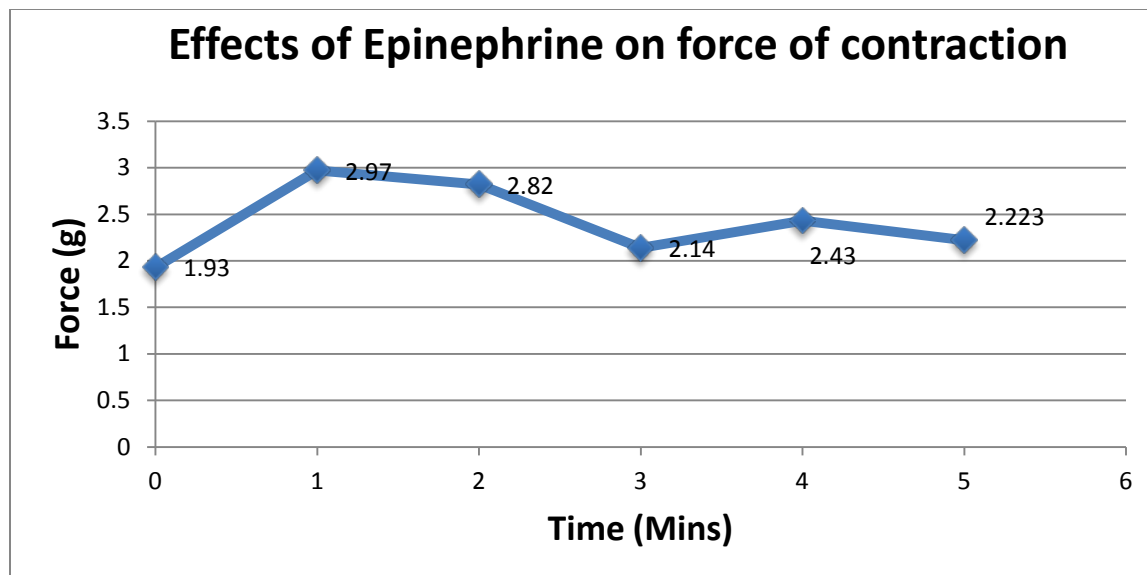


Figure 6. demonstrate the change in contractile force when epinephrine is being added on the ventricle of the frog. The contractile force shows an increasing trend, going from 1.93g to about 2.97g. At this point, the tension decreases to 2.223g.

Discussion:

Electrical and Mechanical Activity of the Heart

The heart is considered one of the most vital organs in organisms. The heart , act as a pump that send blood ,deliver essential nutrients, hormones, oxygen, and help gets rid of waste products for every organ throughout of the body . Without the function of heart, none of living cells could survive. Therefore, the knowledge of the electrical and mechanical process behind heart contraction and heart rate should be learned in order to understand the importance of the heart. In the first part of the experiment, the electrical and mechanical activity of the SA node which is one type of pacemaker cells was measured. Pacemaker cells are autorhythmic which means they are self-excitabile and able to spontaneously depolarize as a result of a slow continuous influx of sodium through Na^+ channels. Unlike skeletal muscle cell, the depolarization is initiated mostly by Ca^{2+} influx instead of Na^+ to reach the threshold , and there is no plateau phase occurred. The repolarization phase of the membrane occurs due to

efflux of K^+ through K^+ channel. Then the action potentials fired by pacemaker cells travel through gap junctions that allows electrical signal to spread to neighbor cells to cause simultaneous contractions in the right and left atrium. Action potential initiated by SA node travel slower to bundle of His and Purkinje fibers to cause entire ventricle contracts after contraction of atrium. Therefore, the action potential from the autorhythmic cells sets the heart rate and triggers the contractile cells to contract (Sherwood, 2010, pp. 311). In the electrical activity of contractile cells, influx of Ca^{2+} and Na^+ are the force to depolarize the membrane and reach threshold. When the peak of this action potential is reached, the plateau occur due of inactivation Na^+ channel and a constant slow influx of Ca^{2+} . After this, the opening of K^+ channel and inactivation of Ca^{2+} channel allowing repolarization to occur. Those electrical events can be observed during the recording of the mechanical activity of the heart. The mechanical activity of the heart can be explained by systole and diastole portion of cardiac cycle. Table 1 shows that the force of ventricular contraction are larger than atrial contractions. This is because ventricle has to act as the pump to deliver blood out throughout the body, but atria only have to pump blood to the ventricles. In order to produce a contraction, electrical activity has to be carried over to mechanical activity. The time period required for this to happen is called latency (Sherwood, 2010, pp. 311). the latency time recorded was about 0.25 seconds. The mechanical activity lags behind electrical activity because electrical activity is the mechanism that drives mechanical activity to occur, and it has to spread at a very fast speed. However, mechanical activity needs to make sure the heart contract as a whole in order to pump blood efficiently, so speed is not the priority.

Extrasystolic Contractions

There will be a time period called effective refractory period that a second action potential

cannot be fired until the membrane has recovered from the previous action potential after the muscle cells contract. This is due to the prolonged plateau phase of the action potential in contractile muscle. There is another time period called the relative refractory period, where an action potential can occur if there is large enough intensity to stimulate. Those time periods act as protective factors to ensure heart have enough time to contract and relax, and the muscle cannot be re-stimulated until the contraction is almost over. In this part of experiment, the voltage is applied during the late and early diastole. When voltage increased to 3.5 V during late diastole, an extrasystolic contraction was seen, which shows that the cells were in relative refractory period. Frank-Starling law states that increasing diastolic filling produces greater stretch of heart muscle, resulting in a larger stroke volume. Due to the short filling time of the extrasystolic contraction, the extrasystolic contraction tension supposed to be lower than normal contraction tension. However, the graph 1 shows that both extrasystolic contraction during late and early diastole have larger contractile force than normal ventricle contraction forces, which was not consistent with theory. There might be errors involved in this part of experiment that the stimulation may not hit in the right time, and excess calcium and more cross bridge cycling occurring at that point might caused the increased size of contraction. Because early diastole is at the beginning of the relative refractory time period, so it should take more stimulus to elicit a response than during the late diastole. The experimental result was consistent with the theory. The threshold during early diastole recorded (5v) was larger than threshold during late diastole (3v) When the stimulus was increased to twice the intensity of threshold (7v), there was only negligible difference between contraction produced at threshold and contraction produced at double threshold. The data explains the all-or-none theory that the heart will contract as long as the stimuli intensity is above threshold. After that, the voltage doesn't matter in terms of size

of contraction force. This is one of the difference between skeletal muscle and cardiac muscle that an increase in stimulus intensity does cause an increase in the recruitment of motor units in skeletal muscle. The force of contraction of cardiac muscle is depend on the period of compensatory pause caused by refractory period .The compensatory pause, time period between the extrasystolic contraction and the normally elicited contraction, allows the filling phase to be pronged, so increasing the preload volume and cause a stronger force of contraction. This phenomenon could be explained by Frank-Starling Law. Therefore ,based on the theory, it supposed to be a larger ventricle contraction seen when the stimuli was elicited during early diastole.Because the compensatory pause is longer due to the early stimulation,causing a greater response after the extrasystolic contraction. Also based on Frank-Starling law, it has more time to fill more blood into ventricle so that the ventricular muscle will get stretched more, causing a greater contraction.The data collected in the experiment was not consistent with theory ,as seen in Figure 2, the compensatory pause for stimulus during early diastole was longer (2.5 seconds) compared to the compensatory pause for late diastole (2.05 seconds).However, Figure 1 shows that the force of ventricle contraction after stimulus for early diastole was smaller than the late diastole. This is might due to dehydration of the frog skin .Failing to apply Ringer's solution constantly to hydrate the frog skin affects the amount of calcium available for contraction of the heart,and further negatively influences cardiac output of the heart. (Shiels and White, 2008, p. 2010).

Vagal Stimulation

In this part of experiment, the effect of parasympathetic nervous system on heart rate and ventricle contraction were analyzed. As vegaus nerve which is part of parasympathetic system was stimulated, acetylcholine was released through parasympathetic efferent fiber in order to

slow down the pacemaker activities in the SA node by binding to muscarinic Ach receptors to open ligand-gated channels in order to increase the permeability of K^+ in the SA node (Sherwood, 2010, pp. 326). This action is going to decrease the rate of phase 4 depolarization and pacemaker automaticity, resulting in slower heart rate. Figure 3 shown that the parasympathetic system heavily took control over the heart rate activities as the stimulation increased on the vagal nerve by evidence of bradycardia and cardiac arrest observed in the experiment. Figure 4 shows that parasympathetic stimulation caused the decreased heart contractility force by evidence of bradycardia and cardiac arrest observed. The data explained that the activation of acetylcholine receptors from parasympathetic stimulation act to inhibit the opening of L-type Ca^{2+} Channels and reduce the inward current of Ca^{2+} . This action shorten the plateau phase in cardiac muscle and eventually cause a weak contractile force (Rang, Dale, 2007, p 103). As seen in figure 3 and 4, the heart rate and force went back up again after the heart stopped beating, but there was a decrease compared to normal heartbeat and contractility. This is because after the cardiac arrest, the next fastest pacemaker cells take over to initiate the rhythm of the heart which is known as vagal escape, and due to the firing rate of those pacemaker cells is not as fast as SA nodes, the heart rate and contractility are not able to be back to before (Bywater, 1990 p722).

The Effect of Epinephrine

In this part of the experiment, the impact of the sympathetic activity on heart rate and contractility were studied. The sympathetic system initiate flight or fight response to prepare the body to deal with emergency or stressful situation. Epinephrine released from adrenal medulla activated β_1 -adrenergic receptors which increased the rate of depolarization in sa node by opening more Ca^{2+} channels, causing greater influx of Ca^{2+} . Figures 5 and figure 6 demonstrate

the effects of applying epinephrine to the ventricle which was consistent theory. The heart rate and contraction force were seen to increase rapidly after 1min, After that ,the heart rate and contraction force both begins to slow down a little bit and stay constant for 3 mins, and then eventually went down further again. This might be because the effects of epinephrine begin to wear off.

Overall, this experiment helped strengthen the understanding about electrical and mechanical properties of the heart. The pacemaker activity were observed and use it to compare the impact of activating the parasympathetic and sympathetic nervous system on heart. The effects of direct stimulation of the ventricles to produce an extrasystolic contraction at late and early diastole were also studied. These results and in depth look show that the heart is precise machine in the body that has many mechanisms to help regulate and ensure its function to serve the rest of the body.

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