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PLAB #4: Frog Heart Muscle

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Abstract:

Cardiac muscle contraction differs from skeletal muscle contraction in a few behaviors essential to proper function of the heart. Additionally, not all cardiac muscle is the same. Although frog myocardium differs from mammalian myocardium in its ionic basis of activation, it is a useful starting point in studying myocardial behavior. Different control mechanisms exist to alter the rate, power of contraction and stroke volume of the heart. Using an intact frog ventricle muscle and a force transducer, this lab explores the chronotropic and inotropic effects of isoproterenol, the inhibitory effects of high extracellular potassium and the effects of increasing stimulus frequency on frog cardiac ventricular muscle.

Introduction:

Cardiac function is arguably one of the most imperative bodily functions, for without proper behavior of the heart most animals (humans included) cannot survive. Studying the heart can be difficult because of its location and protection inside of the chest cavity. However properties of cardiac tissue that allow it to beat in situ for several hours, facilitate the study of basic cardiac functions in prepared hearts isolated from pithed animals. In the lab outlined here, the isolated organ comes from a frog. There are several differences between frog and mammalian hearts. The frog heart contains two atria connected to a single ventricle, as opposed to most mammals which have two ventricles each tied to an atrium. Additionally the myocardium of the frog heart is composed of spongy (or trabeculated) muscle whereas mammalian myocardium is compact. In mammals the two circulations of blood (oxygenated and deoxygenated) are kept separate by running through different chambers, but in a frog heart the two pools share the ventricle. In frogs the sinus venosus receives deoxygenated blood from the veins (coming from most of the organs) and empties that blood into the right atrium. Oxygenated blood comes from the lungs and enters the left atrium. Both atria dump their blood into the large single ventricle which pumps it out through a single artery called truncus arteriosus. Fortunately, the ventricle is structured in such a way that prevents serious mixing of oxygenated and deoxygenated blood. Oxygenated blood goes out to the brain and most tissues while deoxygenated blood flows to the lungs and skin through which frogs exchange gases.

The basic cardiac property studied in this lab is the strength dependence of cardiac contraction on the degree of stretch of the ventricular muscle. Starling's Law of The Heart refers to this relationship, summarized as follows: Before capping at some maximum, the greater the end diastolic ventricle stretch, the greater the strength of the subsequent contraction & stroke volume. Left ventricular end diastolic pressure and volume can both be increased by increasing the venous return to the left ventricle. This increase will increase ventricular preload. Such a behavior means stroke volume can be modulated to match venous return as well be maintained should there be a rise in aortic pressure. To increase the strength of contraction at any degree of stretch, the body circulates epinephrine.

Gaining insight into the properties of myocardial tissue is imperative in diagnosing causes of heart arrhythmias, prescribing drug therapies or treatments for abnormal heart conditions as well as defining tolerances for mechanical assist devices or artificial hearts. The human body is a dynamic system, and each organ must in turn modify its behavior to suit different conditions or circumstances. Understanding the methods used to modulate heart rate, power of contraction and volume ejected are

all necessary in the design of a replacement organ. Given that the supply of donor hearts is well below the demand for transplants, artificial intervention is the logical solution.

Methods & Materials:

A ring stand, micro-positioner and force transducer along with a bridge pod, Power-Lab and Lab-Chart software were used to measure force of the cardiac muscle. The force transducer was plugged into the Bridge Pod and the Bridge Pod was plugged into Input 1. The Lab-Chart software was configured to read the Force with a range to 5mV, and the signal was zeros by turning the knob on the front of the Bridge Pod. The equipment was calibrated using the equation Force (N) = 0.001mass(g) * 9.80665.

A frog was pithed and secured ventral side up on a dissecting tray. Using scissors and tweezers, the surface of the chest and abdomen was opened laterally and longitudinally. Tissue and the sternum were cut away to expose the thoracic cavity. The pericardium was cut away to expose the heart. A thread with a small barbless hook was used to attach the apex of the ventricle to the force transducer. Careful attention was paid not to pierce the chambers of the heart. The line was pulled taught so there was no slack in the thread.

Once the frog heart was hooked up, baseline heart rate was examined. With a slack thread, the Bridge Pod was zeroed. The micro-positioner was adjusted so that a medium amount of stretch of the heart was visible. A test-setup recording was made. Room temperature Ringer's solution applied routinely with a Pasteu pipette helped keep the frog heart moist.

The length-tension relationships of cardiac muscle were analyzed by stretching the ventricle in increments of 0.5mm and allowing the muscle to reach steady state, recording its contractions for around 30 seconds. This process was repeated until the maximum contractile force stopped increasing.

The same length-tension procedure was repeated for the muscle while it was routinely doused in isoproterenol, and then again while the muscle was routinely wetted with High KCL Ringer's solution, rinsing with normal Ringer's in between.

Following that, the ventricle was dissected from the frog and pinned to the dissection tray by its anterior end. An attempt was made to supply stimuli (of varying frequencies) to the muscle, however the ventricle seemed to be unresponsive. It was concluded that the ventricle was either too tired or completely dead. No further procedures could be conducted on the ventricle.

Results:

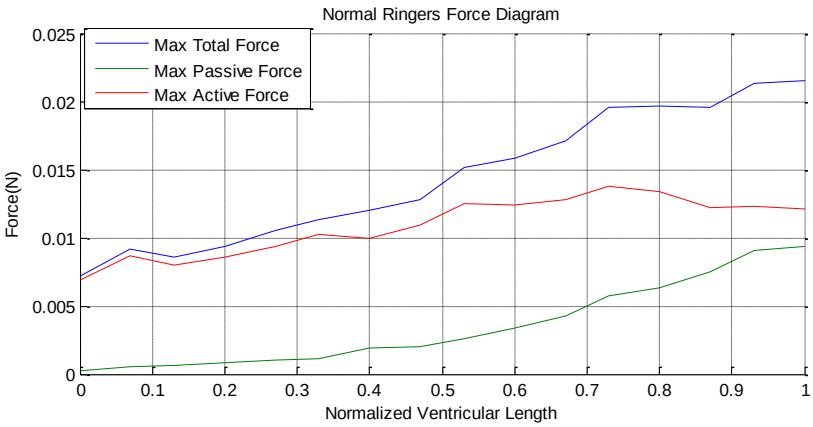
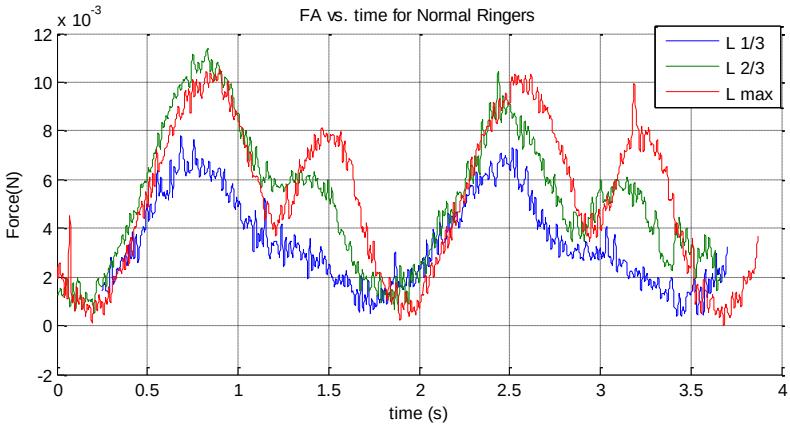
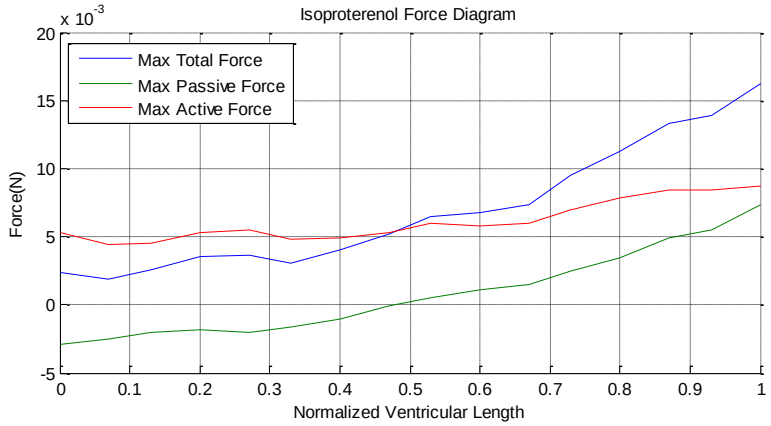


Figure 1: Normal Ringer's Solution Force Diagrams over normalized ventricular length and time

For the normal Ringer's solution, the unmodified heart demonstrated expected behavior. That is to say that as relative length increased, amplitude of contraction increased, up to a certain point where active force leveled off. All graphs of active force over time, including the one for normal Ringer's, demonstrated an unexpected behavior with two humps per contraction. This is most likely due to the variable movement of the middle of the heart while contraction both pulled down on the transducer and up on the connection of the heart to the chest cavity. (Figure 1)





For the isoproterenol soaked muscle trial, the expected behavior was the same trend as the normal Ringer's trial but with increased rates and peaks (above those of normal Ringer's) for each length. (Figure 2)

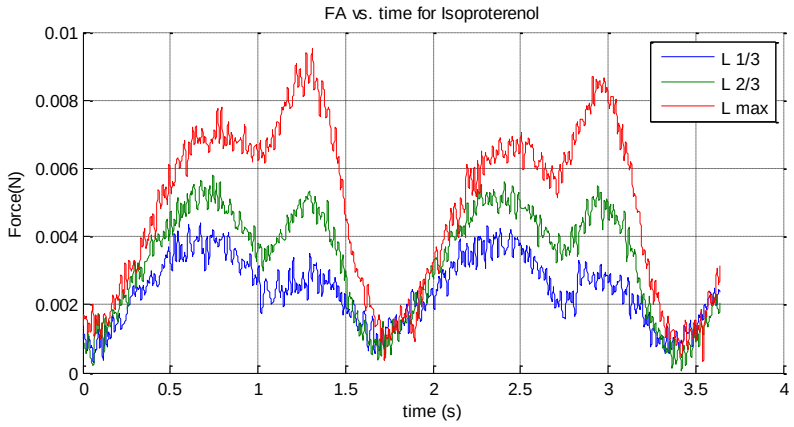


Figure 2: Isoproterenol Solution Force Diagrams over normalized ventricular length and time

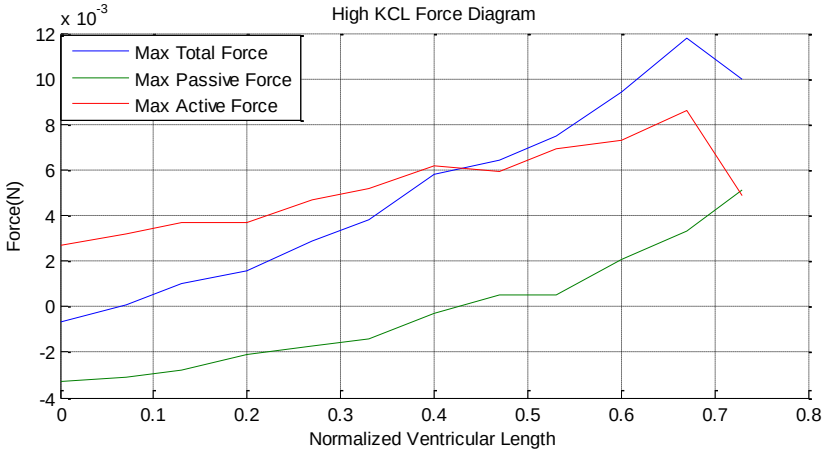
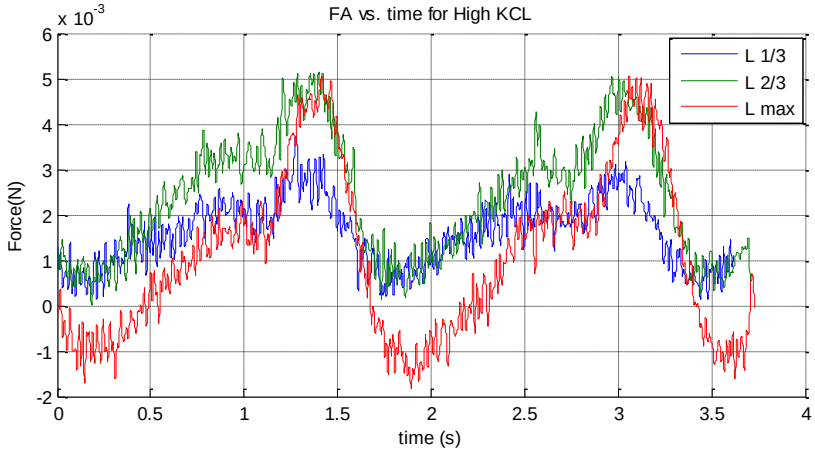


Figure 3: High KCL Solution Force Diagrams over normalized ventricular length and time



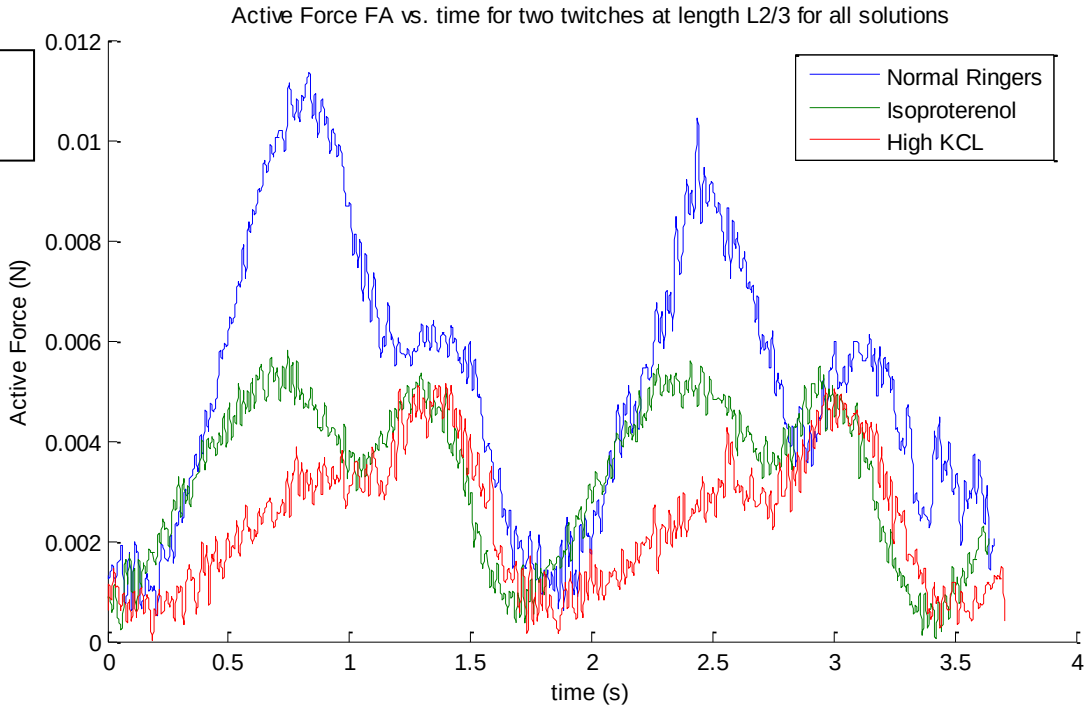
For the high extracellular KCL trial, it was expected that the rates would be decreased and the peaks would be lower than normal Ringer's. (Figure 3)

Table 1 shows the active force developed and relaxation rates for the drop from 75% to 25% max active force for each length and solution combination. These results were not as expected.

Solution	Normal Ringers			Isoproterenol			High KCL		
Length	1/3L	2/3L	Lmax	1/3L	2/3L	Lmax	1/3L	2/3L	Lmax
Active Force Developed	0.0039	0.0057	0.0053	0.0022	0.0029	0.0047	0.0019	0.0026	0.0025
Relaxation Rate	0.0055	0.0093	0.0072	0.0031	0.0046	0.0212	0.0063	0.0153	0.0189

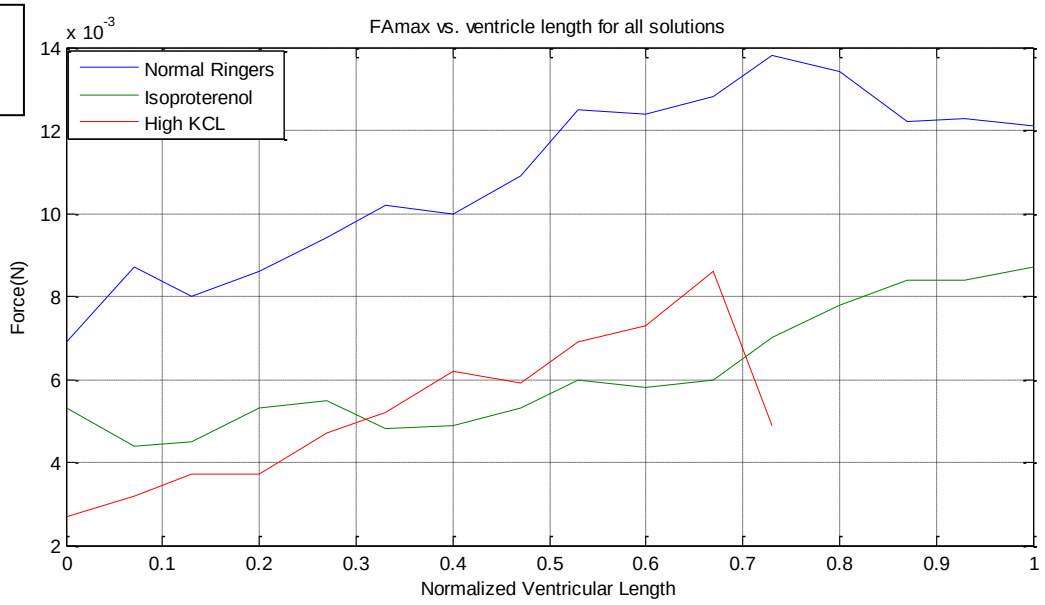
Table 1: Active Force Developed and Relaxation Rates for different lengths and solutions

Figure 4: Active Force vs. Time for all Solutions graphed at length L2/3



The graph of active force vs. time with all solutions compared (Figure 4) shows behavior deviating from the expected. It was expected that the Isoproterenol would yield the highest peaks as well as the biggest rates of development and relaxation; however normal Ringer's held both maximums. High KCL trial is somewhat expected, with the lowest rate of development and the lowest maximum active force.

Figure 5: Active Force vs. Ventricle Length graphed for all Solutions



Once again, data for the maximum active force vs. ventricle length for all solution was expected to demonstrate a different behavior where all solutions were linear increasing, but with isoproterenol on

top, normal Ringer's in the middle and high KCL on bottom. Instead the results demonstrate Normal Ringer's above both isoproterenol and High KCL, with High KCL and Isoproterenol intersecting. (Figure 5)

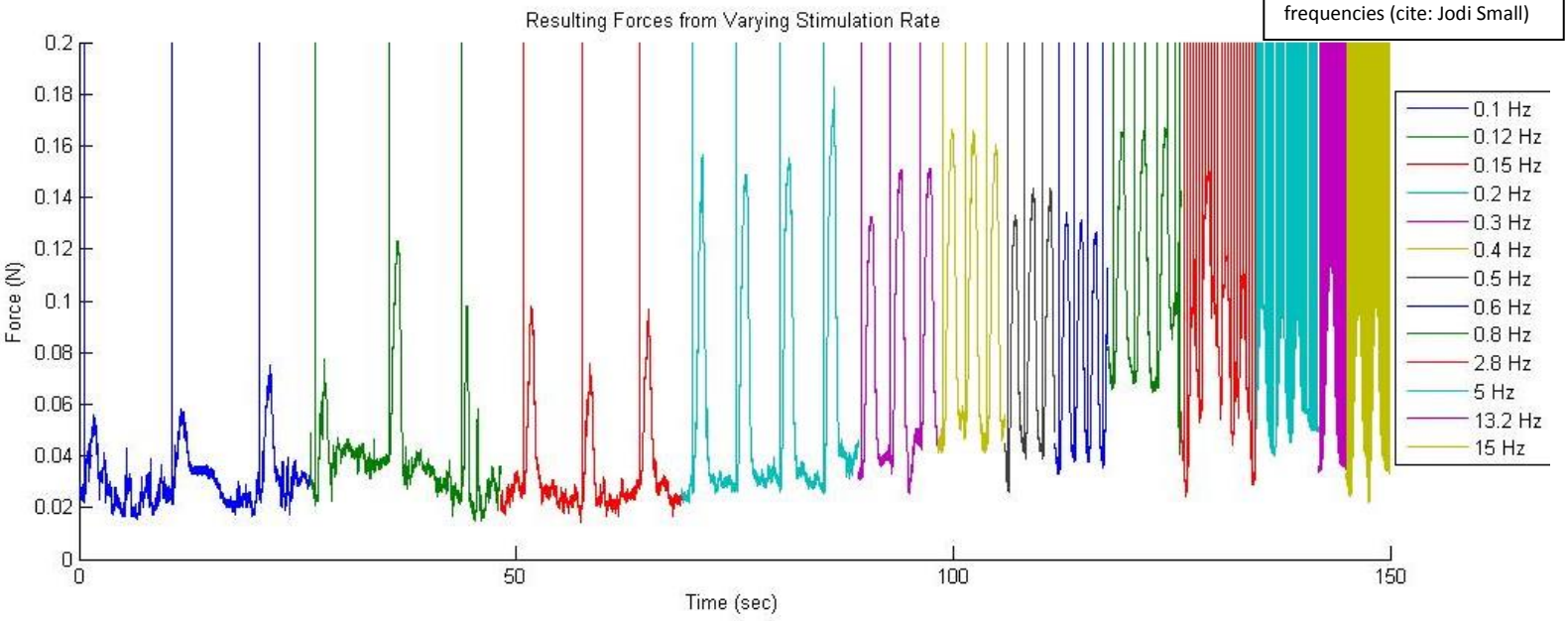


Figure 6: Forces resulting from various stimulation frequencies (cite: Jodi Small)

Stimulus Frequency (Hz)	Peak Force Generated (N)
0.1	0.0749
0.12	0.1229
0.15	0.0981
0.2	0.1827
0.3	0.1506
0.4	0.1656
0.5	0.1435
0.6	0.1656
0.8	0.1501
2.8	0.0976
5	0.1003
13.2	0.1156
15	0.1034

Table 2: Peak Force Generated at various stimulus frequencies

Because the heart used was incapable of continuing for the stimulation experiment, data was borrowed from another group. (Karthik Krishnan, Matt Everett, Paras Vora)

Figure 6 and Table 2 show how, as stimulus frequency increases, peak force generated by the frog heart muscle increased up to a point before decreasing.

Similarly, the premature beat experiment was not possible with a dead heart. Data supplied by a course administrator (Patricia Widder) was used for premature beat results. One can see how in the normal beat, with onset around 1 second, there is no beat for the rest of the 4 second graph. However for the premature beat, with onset slightly before 1 second, a premature beat is onset 2 seconds into the recording. (Figure 7)

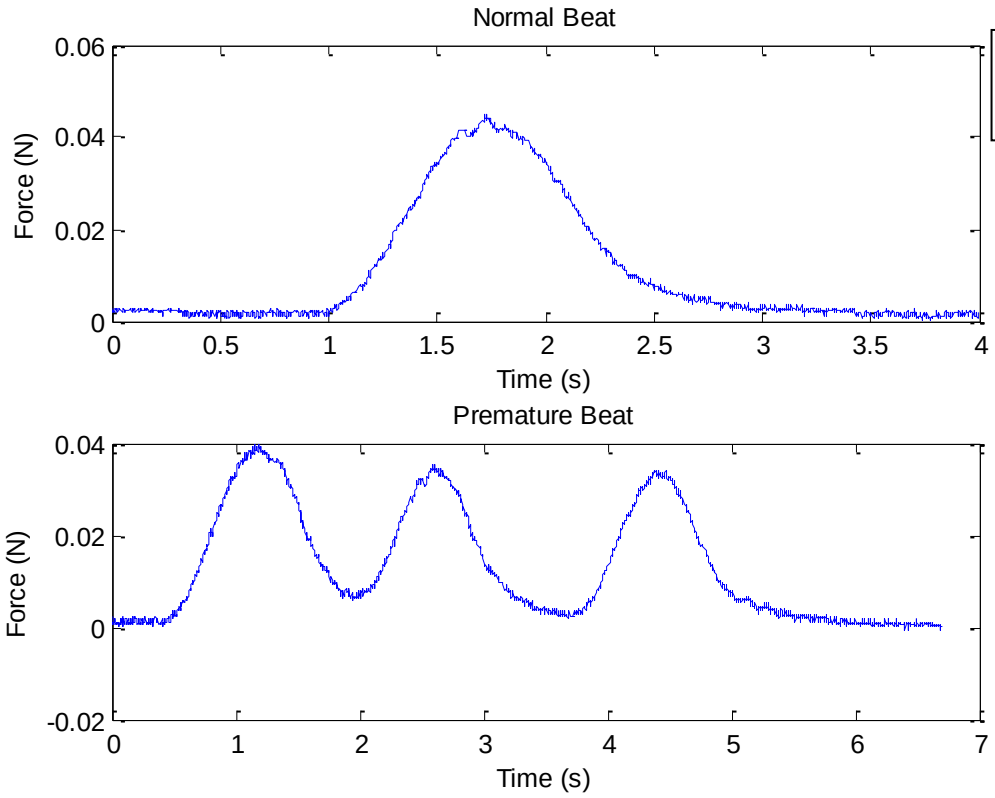


Figure 7: Recording demonstrating normal beat and premature beat

Table 3: Individual Force Values and development/relaxation rates for normal, premature and post premature beats

	FP (N)	FAmax (N)	FTmax (N)	Development Rate (N/s)	Relaxation Rate (N/s)
Normal Beat	0.000698	0.044192	0.044890	0.080600	0.064000
Premature Beat	0.005796	0.029464	0.035260	0.064600	0.048300
Beat Following Premature	0.002964	0.031156	0.034120	0.061300	0.054500

Table 3 shows the individual force values, as well as the development and relaxation rates for a normal, a premature and a post premature beat.

Discussion:

Cardiac muscle contraction is a complex process. The source of the contractile force is not singular. There are passive and active elements that contribute to the total developed force. This can make defining the length tension relationship of cardiac difficult. Titin, a protein responsible for the passive elasticity of muscle, acts like a spring, generating passive contractile force that increases with increasing length or stretch of the myocardium. However, beyond a certain length of stretch, cross bridge formation decreases with increasing length and active tension decreases. For the active contractile force, calcium sensitivity of cardiac myofibrils plays a role. The sensitivity increases with muscle length, as does the amount of calcium supplied to myofibrils especially during systole. Change in calcium sensitivity is arguably the most responsible player in rapid change of tension in response to changes in length. Although the mechanism hasn't yet been identified, the most likely cause for the change in calcium sensitivity is variance in the affinity of troponin for calcium. (References 1 & 2)

Regulation or modulation of cardiac muscle contraction is arguably even more complex. There are many points of inhibition, retardation, acceleration and other forms of modulation. In this lab only a few were explored. The first was isoproterenol, a beta-adrenergic agonist similar to adrenaline. Isoproterenol induces chronotropic (increase heart rate) and inotropic (increase in force of contraction and stroke volume) effects. It activates all beta adrenergic receptors, stimulating the sympathetic pathways and increasing contractile force along with the peak rate of force development df/dt. It also increases the peak rate of force relaxation. Cardiac output and stoke volume can increase in response to isoproterenol. (References 3 & 4)

Another, often unwanted or pathological control mechanism is excess extracellular potassium. In this lab excess extracellular potassium was simulated with High molar KCL Ringer's solution applied to the muscle. This mirrored the basic effects of hyperkalemia. (Reference 5)

Resting membrane potential is maintained mainly by a concentration gradient of potassium. An increase in extracellular potassium levels result in the depolarization of the membrane due to increase in the equilibrium potential of potassium and therefore an increased resting membrane potential. The increase can occasionally open voltage gated sodium channels but tends to increase the inactivation of sodium channels. This migration (depolarization) of the membrane potential inactivates sodium channels, opens potassium channels and the cell is stuck in refractory period without the ability to conduct action potentials and contract. Impaired cardiac conduction can lead to ventricular fibrillation.

Unexplored in this lab, but relevant is another control mechanism called 2,3 Butanedione monoxime (BDM). BDM is a low-affinity, non competitive inhibitor of skeletal muscle myosin. It inhibits ATPases associated with skeletal muscle myosin-II and V. It is not however a general inhibitor of myosin ATPase. BDM can increase the equilibrium constant for ATP hydrolysis and inhibit the rate of phosphate release, stabilizing an intermediate in the conversion process. The stabilization of an intermediate inhibits the myosin ATPase rate and therefore decreases produced force of muscle contraction by inhibiting the ATP dependent cross bridge formation. (References 6 & 7)

There are properties intrinsic to heart muscle that both determine its behavior and also protect it and the human body from damage. For example, heart muscle has a longer refractory period than skeletal muscle. By the time refractory period is up for heart muscle, the muscle is already relaxed. It therefore cannot be tetanized. It is imperative that cardiac muscle maintain a proper refractory period without the ability to tetanize because the heart needs to beat rhythmically, and it cannot beat above a certain rate or its functionality begins to fall off and pumping becomes inefficient or fails completely.

Rate Treppe, a method of auto-regulation by which myocardial contraction increases with increasing heart rate (ie: stimulus frequency) was displayed in the frog heart data shown in the results, as all cardiac tissue should. Cardiac tissue should be able to increase contractility by increasing heart rate. However this works up to a certain point at which the higher rates cannot stimulate more or at more defined contractions. The data's initial adherence to Rate Treppe can be explained by the sodium calcium exchanger having less time to remove Calcium that entered the cytosol because of the decreased diastole period associated with chronotropic effects.

Increasing stimulus rate has other effects on mammalian cardiac tissue as well. As the time period between the normal systole and the extrasystole shortens, the degree of potentiation (strength of contraction) increases. Causes of this extrastyolic potentiation could include the enhancement of sympathetic nerve activity. (References 8 & 9)

Mammalian cardiac muscle should show postextrasystolic potentiation and a weaker premature beat. Frog cardiac muscle should not show these. One reason for this is that intracellular bound calcium may not play as big of a role in frog cardiac muscle. Frog muscle may have sparse sarcoplasmic reticulum and absent T-tubules in some tissue.

If heart contractility is truly independent of preload and after load, then indexing it should not require normalizing by some load. Indexing by dP/dt and by dP/dt/P are not equivalent. One is normalizing, the other is not. If we consider P to represent some form of loading, then using dP/dt/P would be normalizing each result by that loading, which is counter the point that heart contractility is independent of pre or afterload. Therefore ideally, one would use the non-normalized version dP/dt. However it is not unanimously agreed that heart contractility is 100% independent of loading, nor are measurements usually accurate for varying conditions. Most often compensation or normalization factors can be beneficial in producing relatively useful results.

A stress over strain curve for the cardiac muscle could have been created fairly simply provided some additional measurements had been made. Supposing the cross sectional area of the ventricle could be obtained (and assuming that is stayed constant during contraction), and using Lmin to be the reference length, then strain could be determined by changes in length over Lmin, while stress could be determined by contractile force divided by cross sectional area.

Observing frog myocardium in comparison to mammalian myocardium yields very interesting discussion. Developed tension in frog myocardium seems to depend only on duration and amplitude of a depolarization stimulus. Unlike skeletal muscle, it is independent of contractile history. There are differences in the method of ionic control for contraction when comparing mammalian and frog cardiac muscle. Although Ca^{2+} activates contraction in both, the method of entrance is different. In frogs, activator calcium does not come in full force from internal stores, to nearly the same degree as it does in mammalian cardiac muscle. Instead it seems to come from extracellular spaces, activating muscle contraction after entering through slowly inactivating channels. The strength of contraction therefore depends not only on amplitude and duration of stimulus, but also on $[Ca]_{ext}$. Extended contractions of frog ventricle could be caused by very slow to inactivate calcium channels that permit the entrance of extracellular calcium that triggers and sustains such contraction. The lack of circulating stores of internal calcium in frog myocardium explains the lack of the mammalian like post-extrasystole potentiation. By mechanisms mentioned previously, decreases in $[K]_{ext}$ should lead to increases in tension, and increases should lead to decreases in tension. (Reference 10)

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11. Quantitative Physiology Plab Manual

Discussants:

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