The Effects of Epinephrine, Methacholine, Adenosine-5'-diphosphate, and Calcium on Intestinal Motility

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Introduction:

The mouth is one of the most important sites of communication for the human race. It gives individuals the ability to speak and create noises, however, it is also the starting point of another very important mechanism necessary for homeostatic functioning of the body: the gastrointestinal system. The digestive system transfers nutrients, water, and electrolytes from the external environment to the internal environment, all the while maintaining homeostasis by optimizing the conditions for digestion and absorption from what is physically ingested. The intestine has various ways of regulating its motility via extrinsic or intrinsic nervous systems (Sherwood, 2013). The purpose of this lab was to examine the basic response of an intestinal segment *in vitro* to adrenergic, cholinergic, and purinergic substances, using epinephrine, methacholine, and adenosine-5'-diphosphate (ADP), respectively. The specific interest was to isolate how the substances affected the strength and rate of the smooth muscle contractions of the intestinal segment, and by doing so, illustrate how those same substances affected the smooth muscle contractions of the gastrointestinal system within the body. Additionally, the lab also examined whether extracellular calcium plays a role in smooth muscle contraction.

Humans eat because food is crucial to the generation of adenosine triphosphate (ATP). However, just ingesting food does nothing for the body and its cells; the food must be chemically and mechanically broken down into simple molecules that can be absorbed from the digestive tract and into the blood for distribution to cells around the body (Sherwood, 2013). As previously mentioned, the digestive tract begins at the site of the mouth. The teeth chew and mechanically break down the food, while the sublingual, parotid, and submandibular salivary glands start producing saliva to lubricate and chemically break down the food before it continues on its way (Fruhwald et al., 2002). The tongue and muscles in the throat assist the bolus, or partially

digested food, past the pharynx and down the esophagus. The esophagus engages in peristaltic contractions, propelling the bolus forward by contracting the muscle region behind the mass, and relaxing the muscle region ahead of the mass simultaneously, allowing for less resistance and easier movement. The lower esophageal sphincter is a muscular valve at the bottom of the esophagus that gauges pressure from the bolus and opens to allow entry to the stomach (Sherwood, 2013). The stomach has a thick lining of powerful smooth muscle that continues to break the bolus into smaller pieces. The stomach contains gastric juice, and releases enzymes to break down the bolus even further, until it forms a pasty substance called chyme. From the stomach, the chyme is pushed into the small intestine (Furness et al., 2010). Digestive juices are released by the liver, pancreas, and gallbladder to assist in complete digestion of the chyme upon entry into the small intestine. Segmentation, which is stationary mixing of the chyme, occurs in the small intestine, as do migrating motility waves. These migrating motility complexes are waves of electrical stimulation that sweep through the intestines during periods of fasting. The waves are powerful, and serve to move all leftover chyme into the large intestine for inevitable evacuation; these waves can be heard when the stomach rumbles out of perceived hunger. All nutrients, most electrolytes, and a significant amount of water are absorbed in the small intestine (Sherwood, 2013). After the absorption of nutrients, electrolytes, and water in the small intestine, only waste products remain. The waste enters the large intestine, and moves through the colon by means of peristalsis. Haustral contractions, which are slow, powerful, segmented contractions, occur in the colon as well. The final bit of water and salt is absorbed from the chyme, converting the waste into feces. The feces travel by way of mass movements into a holding chamber called the rectum about twice a day, and eventually exit the body through the anus (Sherwood, 2013).

The digestive system serves to digest, propel, absorb, and excrete as needed through the process previously described. In order to do this well, the digestive system requires a great deal of motility. This motility is well regulated and occurs in response to appropriate stimuli, whether that be chemicals, or stretching of the intestinal lumen due to increased contents. Gastrointestinal smooth muscle is involuntarily controlled, however the muscles can be influenced greatly by the physical and psychological state of the individual.

The digestive system smooth muscle contractions can be controlled by the endocrine system with hormonal secretions like gastrin, or by the extrinsic and intrinsic nervous systems located within the body. Extrinsically, the parasympathetic system acts through cholinergic receptors while the sympathetic system responds to adrenergic receptors. Intrinsically, the regulation occurs from within the gut wall itself, through the myenteric plexus and submucosal plexus (Sherwood, 2013).

The purpose of this lab was to subject an intestinal segment *in vitro* to adrenergic, cholinergic, and purinergic substances to see how the frequency and amplitude of the smooth muscle contractions would be affected. Extracellular calcium also plays a role in smooth muscle contraction, and the lab investigates in which ways it does so. To examine whether adrenergic substances had an impact on gut activity, the intestinal segment was submerged in solution containing epinephrine. The hypothesis was that the epinephrine would activate the sympathetic nervous system, resulting in decline of tension and motility. To examine whether cholinergic substances had an impact on gut activity, the intestinal segment was submerged in solution containing methacholine. The hypothesis was that the methacholine would activate the parasympathetic nervous system, resulting in an increase of baseline intestinal tension and contraction. To examine whether purinergic substances had an impact on gut activity, the

intestinal segment was submerged in solution containing ADP. The hypothesis was that the ADP would activate P2Y receptors, and the G protein-coupled receptors would have a cascading effect, eventually leading to a decrease in contraction and amplitude of the intestinal segment. To examine the role of calcium in smooth muscle contraction, the intestinal segment was submerged in normal Ringer-Tyrode's solution and calcium-free Ringer-Tyrode's solution. The hypothesis was that there would be decreased contraction, motility, and frequency while in the calcium-free solution due to the lack of calcium available for cross bridge cycling.

Methods and Materials:

Specific details of procedures can be found in *NPB 101L Physiology Lab Manual (Bautista & Korber, 2009 pgs 75-82).*

Transducer and Tissue Preparation

The transducer was calibrated in efforts to maintain accuracy of measurements in the following experiments. The circulating water bath within the reaction chambers was at a temperature of 38° Celsius, and all four of the cuvettes were labeled and filled three quarters full with normal Ringer-Tyrode's solution.

A two centimeter intestinal segment from a rabbit was obtained, and continually wet with Ringer-Tyrode's solution as it was prepped for experimental use. About three millimeters from the end of the intestinal segment a suture needle was used to thread a string through and create a small loop about half a centimeter in diameter. This process was repeated at the other end of the intestinal segment. A third piece of string was threaded through the second loop created, but was left untied for the time being. The first loop created was attached to the long wire hook hanging from the transducer. The intestinal segment was stretched upwards, until when pinched it had the consistency and feel of the fleshy part of the hand below the thumb. As soon as the desired

consistency was reached, the loose, untied piece of string was attached to the top of the transducer; the tie point. The intestinal segment was not floppy, nor too stretched. The intestinal segment on the transducer was lowered into the first reaction cuvette until it was completely submerged. The air bubble valve was set to deliver two bubbles per second continually for every reaction cuvette being utilized throughout the following experiments.

Experiment and Data Collection

Normal Gut Activity: Initial Set Up

The BioPac software computer program was opened, calibrated, and autoscaled appropriately for optimum results - this process was repeated for each following experiment. The program was left open until normal gut activity was seen and recorded, however if the intestinal segment had not performed as it should have, a new intestinal segment would have to have been prepared and used for accurate results.

Normal Gut Activity

The initial software prep and calibration process from previously listed methods was repeated. The motility pattern of the intestinal segment was observed and recorded for ten minutes. Baseline tension, frequency, and amplitude values were assessed using BioPac software. Baseline tension was calculated by averaging the minimum force of ten waves using the "Min" tool in BioPac. Frequency was assessed by taking the average of ten wave cycles within the baseline activity using the "Freq" tool in BioPac. Amplitude values were assessed by utilizing the "p-p" function in BioPac, and taking the average of ten wave cycles. Standard deviations were also calculated for each set of values. Percent-change relative to the baseline activity was calculated by subtracting the baseline value from the new value, and then dividing

by the baseline value. This calculation was relevant to seeing the actual physical impact of substances on the intestinal segment.

Gut Activity in the Presence of Epinephrine

The intestinal segment was moved into the second reaction cuvette. The computer program was opened, calibrated, and autoscaled, and five minutes of baseline gut activity was recorded. After five minutes, three drops of epinephrine were added to the cuvette. Results were recorded for ten more minutes. Baseline tension, frequency, and amplitude were assessed in the same manner as described previously, however, it was done once through with wave cycle values that existed prior to addition of the drug, and once through with wave cycles that existed after addition of the drug.

Gut Activity in the Presence of a Cholinergic Agent

The intestinal segment was rinsed thoroughly with Ringer-Tyrode's solution before being moved into the third reaction cuvette. Gut activity was monitored to confirm that the intestinal segment was contracting to its baseline potential, and once it was, five minutes of baseline activity was recorded. After five minutes, one drop of methacholine was added to the cuvette. Two minutes after that, three drops of methacholine were added. Results were recorded for ten more minutes. Baseline tension, frequency, and amplitude were assessed in the same manner as previously described. Values were assessed for baseline waves, waves after the initial addition of drugs, and values after the final addition of drugs.

Gut Activity in the Presence of a Purinergic Agent

The intestinal segment was rinsed thoroughly with Ringer-Tyrode's solution once again before being moved into the fourth reaction cuvette. Gut activity was equilibrated, to ensure the activity was normal before proceeding with another experiment. Baseline activity was recorded

for five minutes. After five minutes, ten drops of adenosine-5'-diphosphate (ADP) were added to the cuvette. Results were recorded for ten minutes after the last drop was added. Baseline tension, frequency, and amplitude were assessed in the same manner as previously described.

Gut Activity in Calcium-free Ringer-Tyrode's Solution

Gut segment was rinsed as in the previous exercise. The first reaction cuvette was cleaned thoroughly and filled with normal Ringer-Tyrode's solution, and the intestinal segment was put into this cuvette and allowed to equilibrate. Meanwhile, the second reaction cuvette was emptied, and rinsed with calcium-free solution, and subsequently filled with calcium-free Ringer-Tyrode's solution. Baseline activity of the intestinal segment was recorded for five minutes in the normal Ringer-Tyrode's solution. After five minutes, the segment was lifted, blotted, and moved into the second cuvette prepped with calcium-free Ringer-Tyrode's solution. Results were recorded for five minutes. After five minutes, the segment was lifted, blotted, and moved back into the first reaction cuvette and the response was recorded for an additional five minutes upon submersion. Baseline tension, frequency, and amplitude were assessed in the same manner as previously described.

Results:

Experiment and Data Collection

Normal Gut Activity: Initial Set Up

Despite the copious amount of physical handling of the intestinal segment during the tissue preparation, normal gut activity was seen immediately upon stimulation and start up of the computer program.

Normal Gut Activity

Normal gut activity was initially recorded to provide a baseline (Table 1) of what smooth muscle contraction in the intestinal segment looked like in normal Ringer-Tyrode's solution. The baseline gut activity was recorded for the sole purpose of comparing gut activity after experimental substances had been added, or for later comparison with the calcium-free Ringer-Tyrode's solution. The intestinal segment performed as expected under the controlled conditions, showing no obvious deviations from what baseline gut activity should look like. Baseline tension, frequency and amplitude were reasonably stable across the recording (Figure 1).

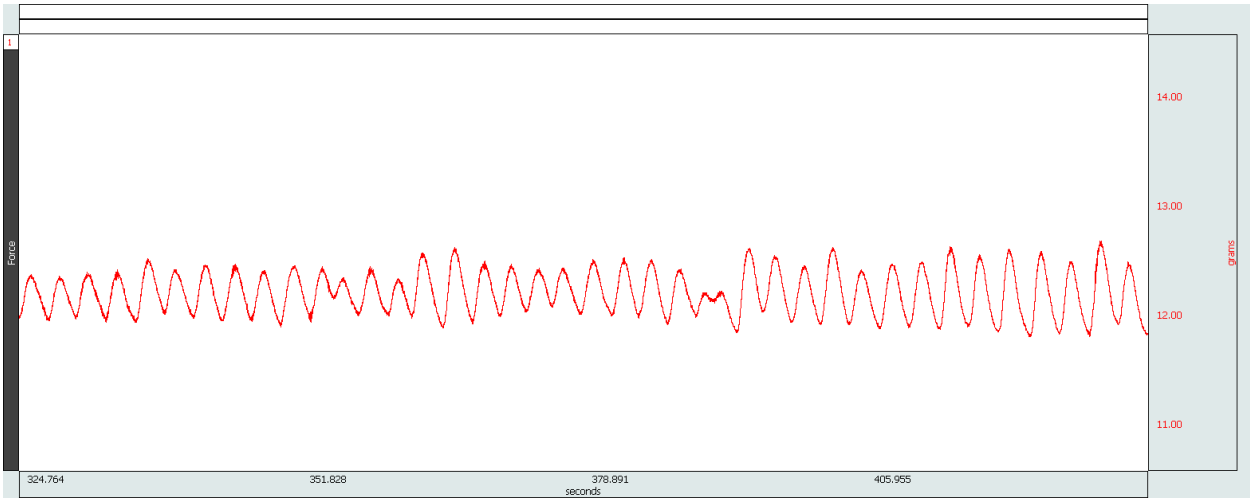

Figure 1. *Gastrointestinal smooth muscle contraction in the presence of normal Ringer-Tyrode's solution. Excerpt of baseline normal gut activity. Waves were similar amplitude and frequency throughout the ten minute baseline recording period.*

Table 1. *Average baseline tension, frequency, and amplitude of intestinal segment's smooth muscle contraction in normal Ringer-Tyrode's solution.*

Baseline Tension (g)	Frequency (Hz)	Amplitude (g)
11.86 ± .092	0.3372 ± .019	.8923 ± .143

Gut Activity in the Presence of Epinephrine

This experiment illustrated the impact of adrenergic substances on the digestive system. Average baseline tension prior to addition of epinephrine was 8.02 g with a standard deviation of .031 g. As soon as the intestinal segment was subjected to the epinephrine, the average baseline tension immediately dropped to 7.39 g with a standard deviation of .08 g (Figure 2). Baseline tension of the intestinal segment decreased by 7.9% after epinephrine was added to the reaction cuvette.

Average frequency of the smooth muscle contractions prior to addition of the epinephrine was .337 Hz with a standard deviation of .025 Hz. When the intestinal segment was subjected to the epinephrine, the average frequency increased minutely to .369 Hz with a standard deviation of .027 Hz (Figure 3). Average frequency of the smooth muscle contraction increased by 9.8%.

Average amplitude of the smooth muscle contractions prior to addition of the epinephrine was 1.402 g with a standard deviation of .261 g. When the intestinal segment was subjected to the epinephrine, the average amplitude decreased to .331 g with a standard deviation of .073 g (Figure 4). Average amplitude of the smooth muscle contractions decreased by 76.4%.

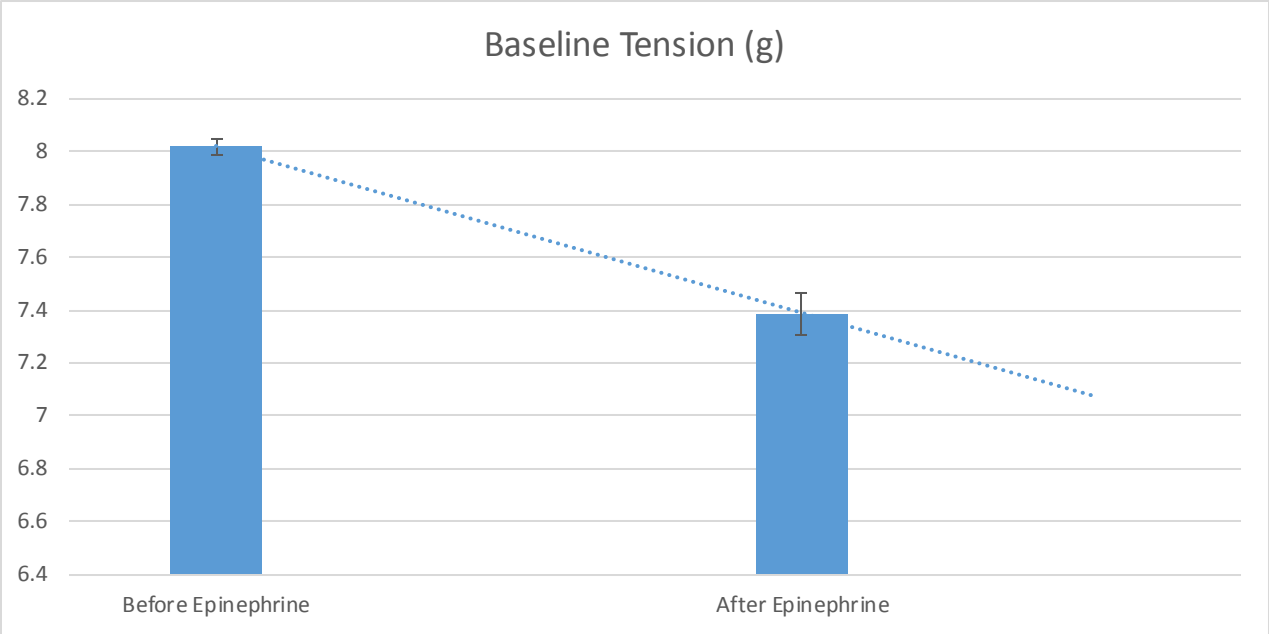

Figure 2. *Baseline tension (g) prior to the addition of epinephrine and after the addition of epinephrine. Data represents the mean baseline tension of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for post-substance activity. Baseline tension decreased by 7.9% after epinephrine was added.*

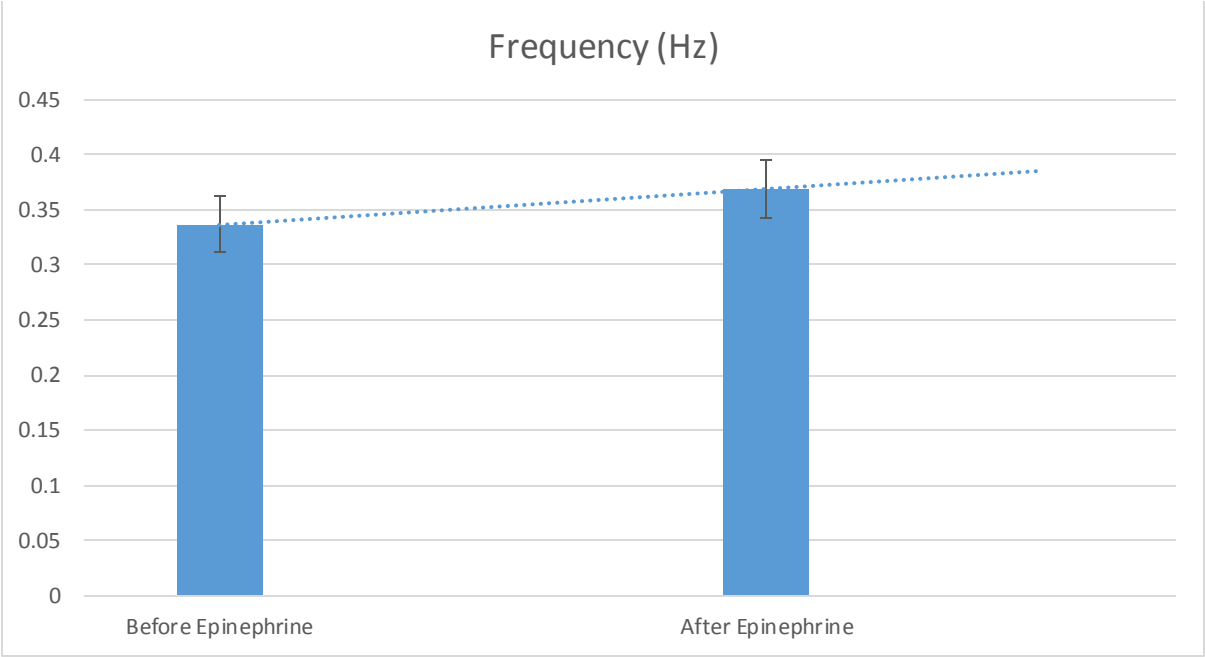

Figure 3. *Frequency (Hz) prior to the addition of epinephrine and after the addition of epinephrine. Data represents the mean frequency of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for post-substance activity. Frequency increased by 9.8% when epinephrine was added.*

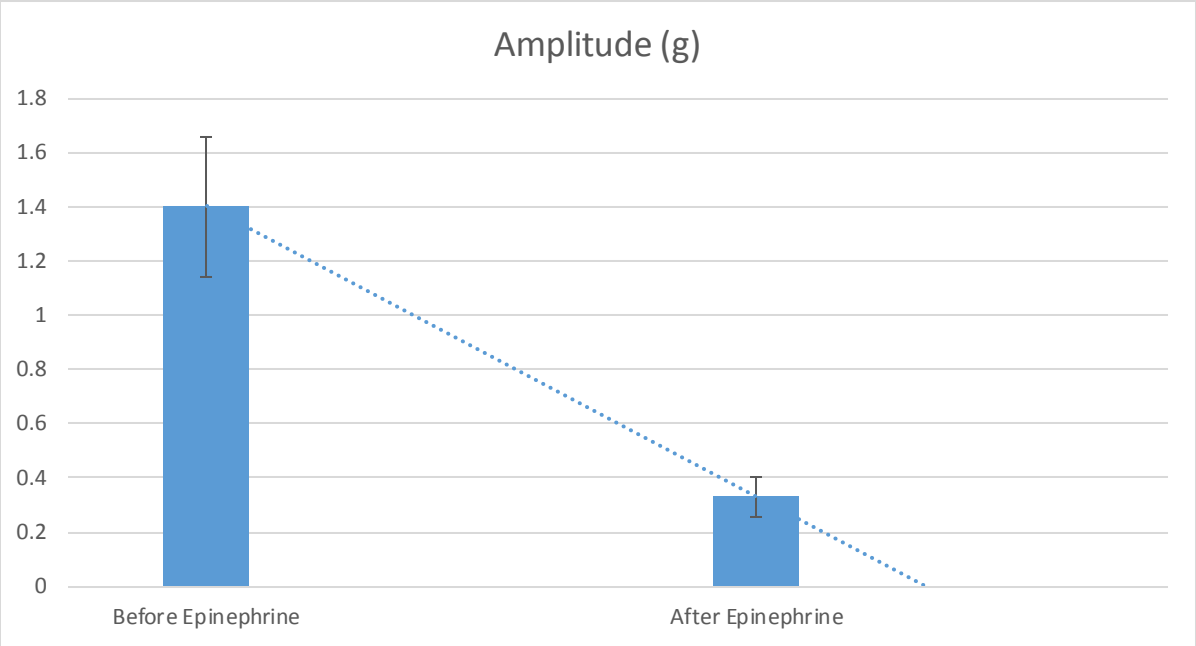

Figure 4. *Amplitude (g) prior to the addition of epinephrine and after the addition of epinephrine. Data represents the mean amplitude of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for post-substance activity. Amplitude decreased by 76.4%.*

Gut Activity in the Presence of a Cholinergic Agent

This experiment illustrated the impact of cholinergic substances on the digestive system via methacholine. Average baseline tension prior to the first drop of methacholine was 7.65 g with a standard deviation of .114 g. After the first drop of methacholine, baseline tension increased to 10.65 g with a standard deviation of .164 g. After three additional drops of methacholine, baseline tension of the intestinal segment was 10.52 g with a standard deviation of .093 g (Figure 5). The percent change from the control baseline tension to the baseline tension after the addition of all of the methacholine drops was 37.5%.

Average frequency of the smooth muscle contractions prior to the first drop of methacholine was .337 Hz with a standard deviation of .034 Hz. After the first drop of methacholine, average frequency stood at .324 Hz with a standard deviation of .069 Hz. After three additional drops of methacholine, frequency of the contractions was .34 Hz with a standard

deviation of .024 Hz (Figure 6). The percent change from the control frequency to the frequency after the addition of all of the methacholine drops was .9%.

Average amplitude of the smooth muscle contractions prior to the first drop of methacholine was 2.59 g with a standard deviation of .349 g. After the first drop of methacholine, average amplitude of contraction was .404 g with a standard deviation of .159 g. After three additional drops of methacholine, amplitude of the contractions was .937 g with a standard deviation of .167 g (Figure 7). The percent change from the control amplitude to the amplitude after the addition of all of the methacholine drops was -63.8%.

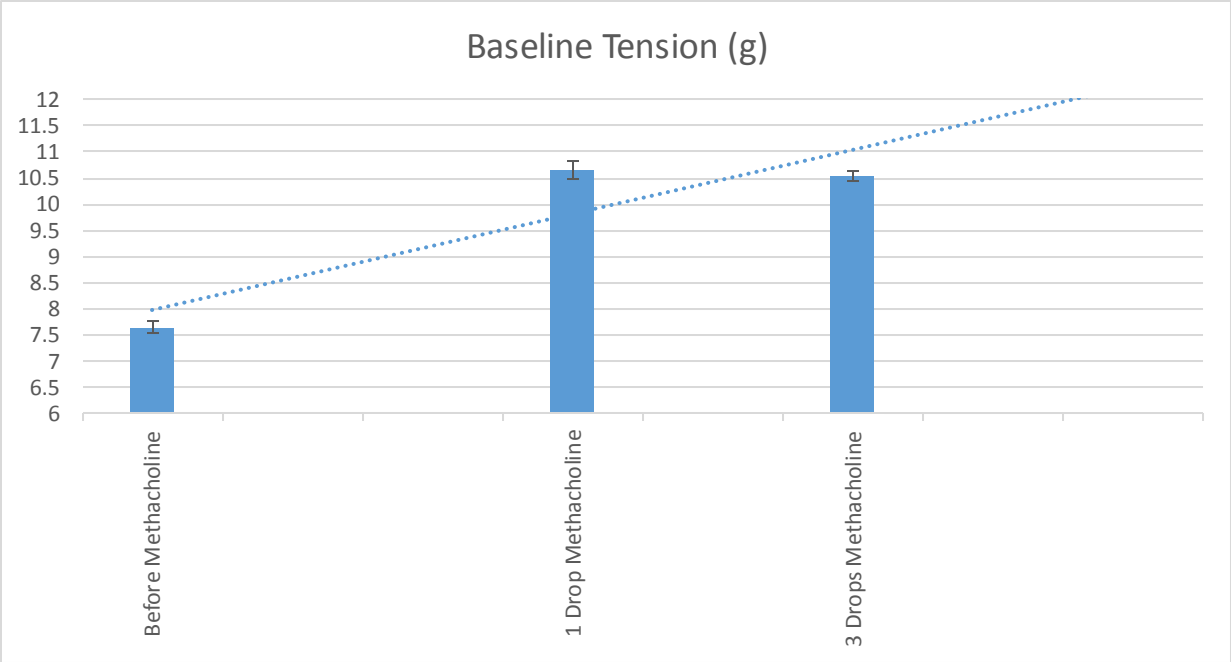

Figure 5. *Baseline tension (g) prior to the addition of one drop of methacholine, after one drop, and after three drops of methacholine. Data represents the mean baseline tension of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Baseline tension increased by 37.5% overall after methacholine was added.*

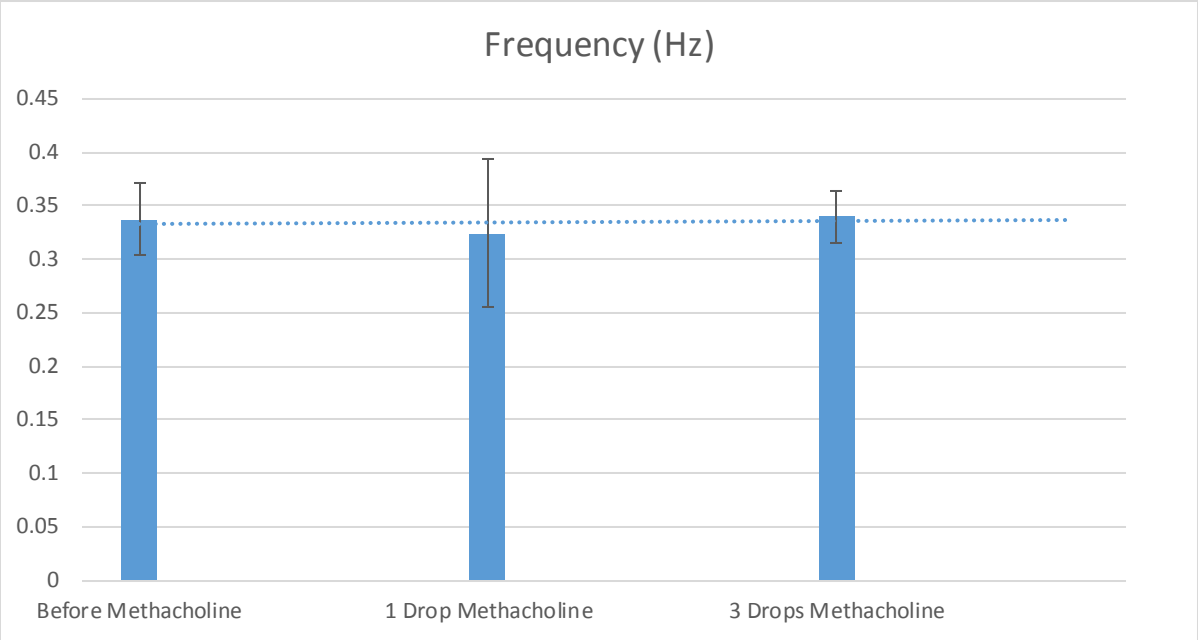

Figure 6. *Frequency (Hz) prior to the addition of one drop of methacholine, after one drop, and after three drops of methacholine. Data represents the mean frequency of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Frequency increased by .9% overall after methacholine was added.*

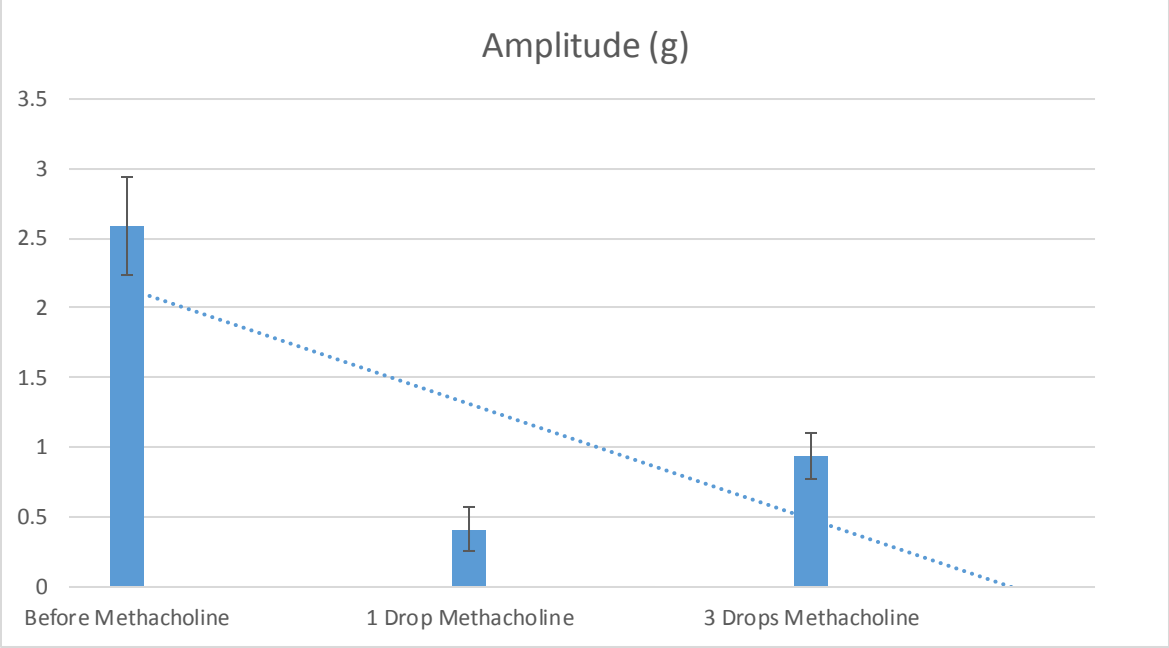

Figure 7. *Amplitude (g) prior to the addition of one drop of methacholine, after one drop, and after three drops of methacholine. Data represents the mean amplitude of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Amplitude decreased by 63.8% overall after methacholine was added.*

Gut Activity in the Presence of a Purinergic Agent

This experiment illustrated the impact of purinergic agents on the digestive system via adenosine-5'-diphosphate (ADP). Average baseline tension prior to the addition of ADP was 11.92 g with a standard deviation of .197 g. As soon as the intestinal segment was subjected to the ADP, the average baseline tension dropped to 11.86 g with a standard deviation of .108 g (Figure 8). Baseline tension of the intestinal segment decreased by .5% after ADP was added to the reaction cuvette.

Average frequency of the smooth muscle contractions prior to addition of the ADP was .36 Hz with a standard deviation of .045 Hz. When the intestinal segment was subjected to the ADP, the average frequency remained at .36 Hz with a standard deviation of .04 Hz (Figure 9). Average frequency of the smooth muscle contraction showed no percent change.

Average amplitude of the smooth muscle contractions prior to addition of the ADP was .641 g with a standard deviation of .136 g. When the intestinal segment was subjected to the ADP, the average amplitude decreased to .589 g with a standard deviation of .211 g (Figure 10). Average amplitude of the smooth muscle contractions decreased by 8.2%.

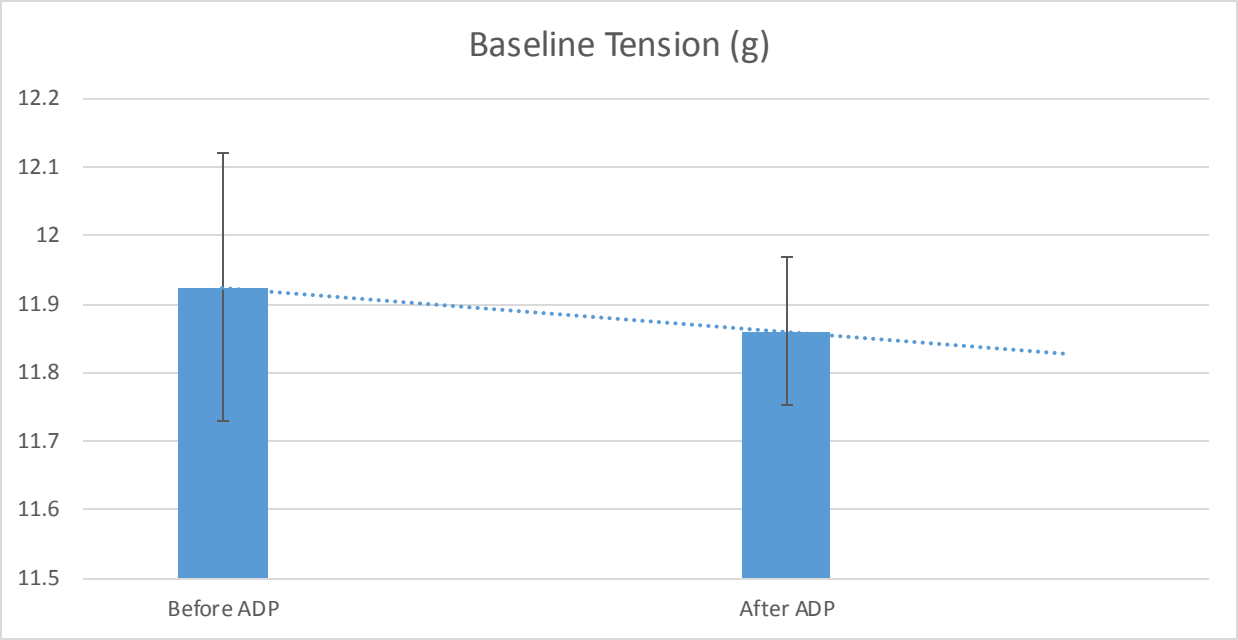

Figure 8. *Baseline tension (g) prior to the addition of ADP and after the addition of ADP. Data represents the mean baseline tension of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Baseline tension decreased by .5% overall after ADP was added.*

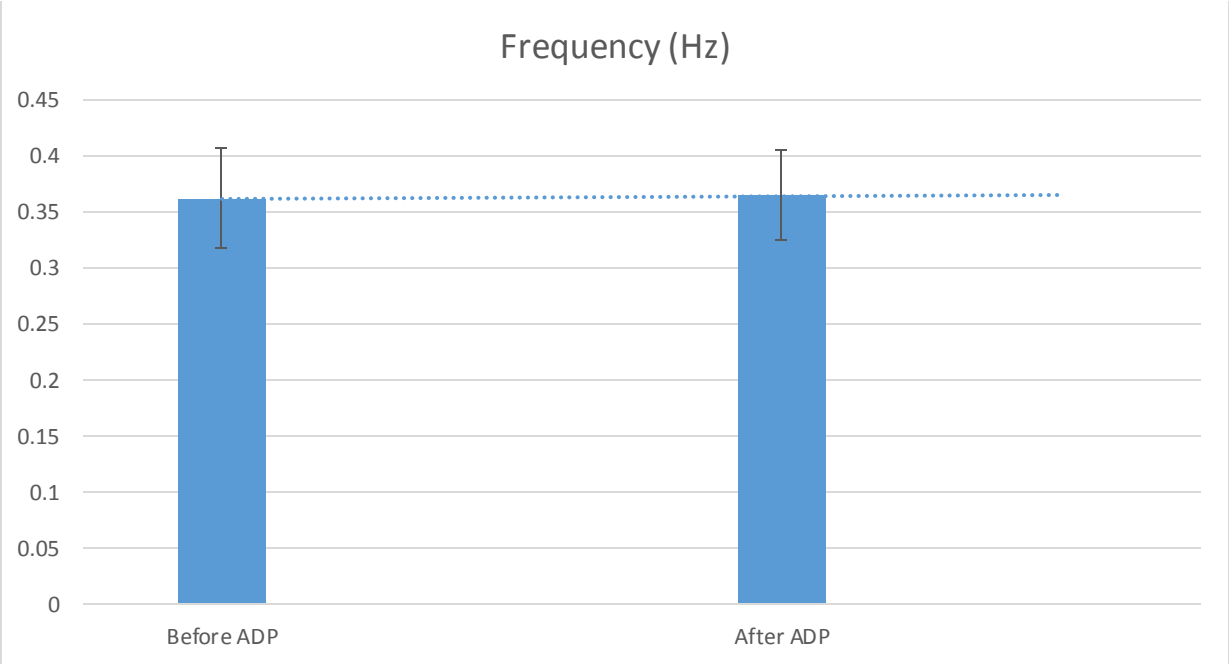

Figure 9. *Frequency (Hz) prior to the addition of ADP and after the addition of ADP. Data represents the mean frequency of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Frequency remained constant after ADP was added.*

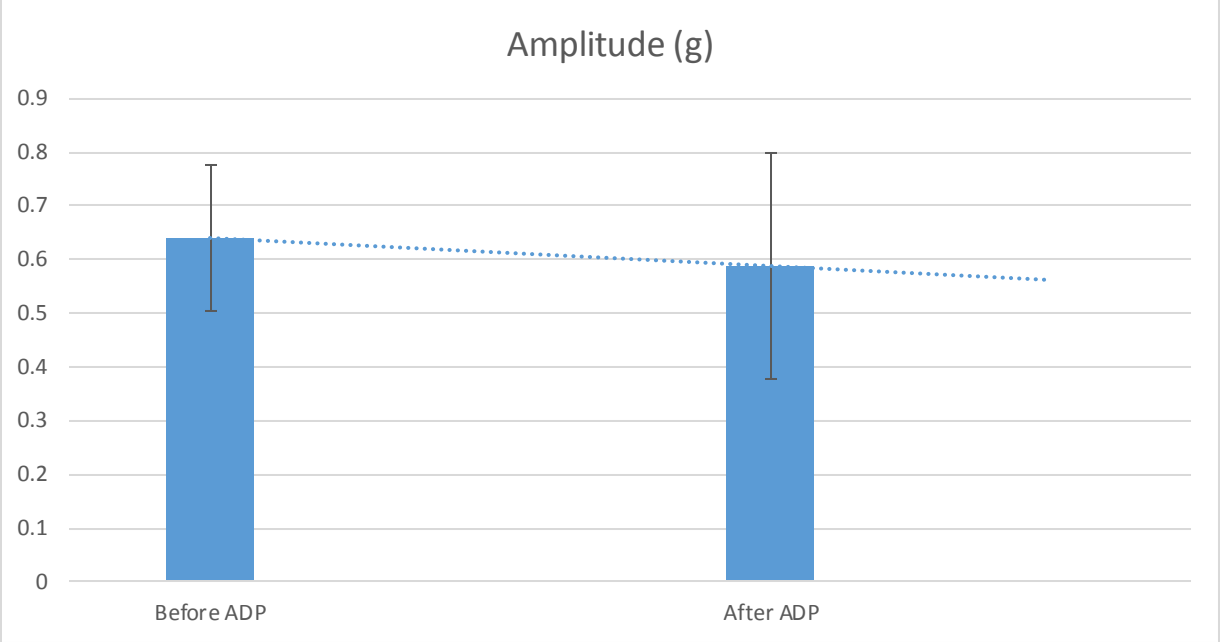

Figure 10. *Amplitude (g) prior to the addition of ADP and after the addition of ADP. Data represents the mean amplitude of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-substance activity. Amplitude decreased by 8.2% after ADP was added.*

Gut Activity in Calcium-free Ringer-Tyrode's Solution

This experiment examined whether extracellular calcium plays a role in smooth muscle contraction. Average baseline tension in the normal Ringer-Tyrode's solution was 9.94 g with a standard deviation of .108 g. Upon shifting the intestinal segment to a cuvette filled with calcium-free solution, the baseline tension dropped to 6.92 g with a standard deviation of .119 g. As the intestinal segment was placed back in the normal solution with calcium, the average baseline tension returned to a higher value of 10.75 g with a standard deviation of .081 g (Figure 11). Shifting from the initial baseline tension in the normal solution to the baseline tension in the calcium-free solution, there was a -30.4% change in values.

Average frequency in the normal Ringer-Tyrode's solution was .344 Hz with a standard deviation of .015 Hz. Upon shifting the intestinal segment to a cuvette filled with calcium-free

solution, the frequency dropped to .239 Hz with a standard deviation of .022 Hz. As the intestinal segment was placed back in the normal solution with calcium, the average frequency returned to a higher value of .348 Hz with a standard deviation of .027 Hz (Figure 12). Shifting from the initial frequency of muscle contraction in the normal solution to the frequency in the calcium-free solution, there was a -30.3% change in values.

Average amplitude of muscle contraction in the normal Ringer-Tyrode's solution was 1.29 g with a standard deviation of .346 g. Upon shifting the intestinal segment to a cuvette filled with calcium-free solution, the amplitude dropped to .241 g with a standard deviation of .043 g. As the intestinal segment was placed back in the normal solution with calcium, the average amplitude returned to a higher value of .583 g with a standard deviation of .114 g (Figure 13). Shifting from the initial amplitude of muscle contraction in the normal solution to the amplitude in the calcium-free solution, there was a -81.4% change in values. The changes in baseline tension, frequency and amplitude happened immediately upon submersion of the intestinal segment into calcium-free Ringers solution.

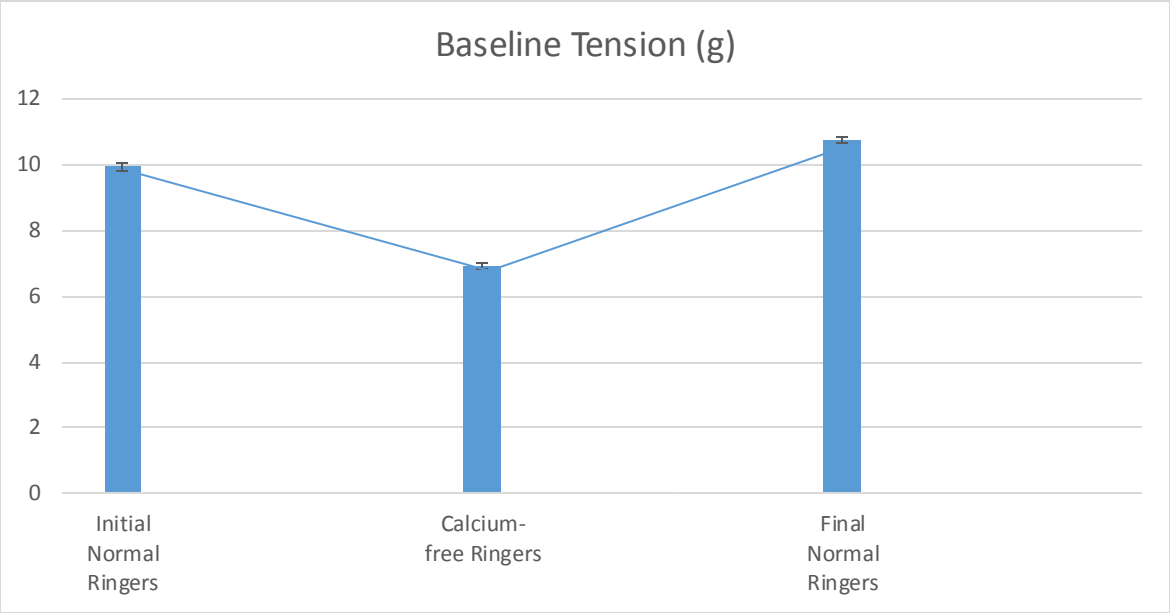

Figure 11. *Baseline tension (g), starting from the control of normal Ringers solution to calcium-free Ringers, and back to control. Data represents the mean baseline tension of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-control activity. Baseline tension decreased by 30.4% due to lack of extracellular calcium.*

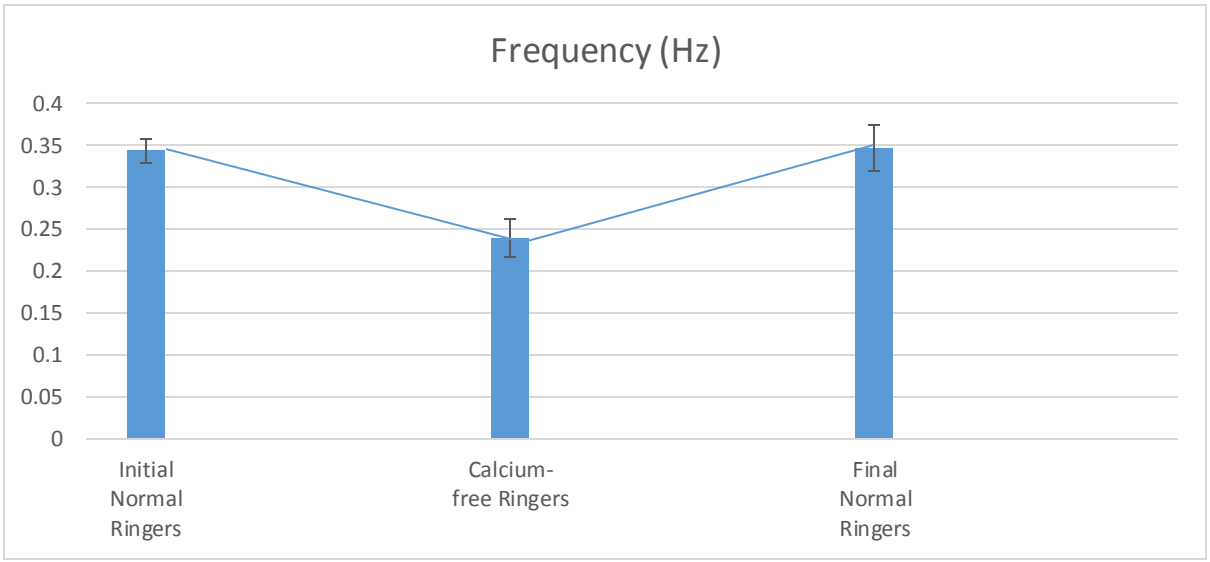

Figure 12. *Frequency (Hz), starting from the control of normal Ringers solution to calcium-free Ringers, and back to control. Data represents the mean frequency of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-control activity. Frequency decreased by 30.3% due to lack of extracellular calcium.*

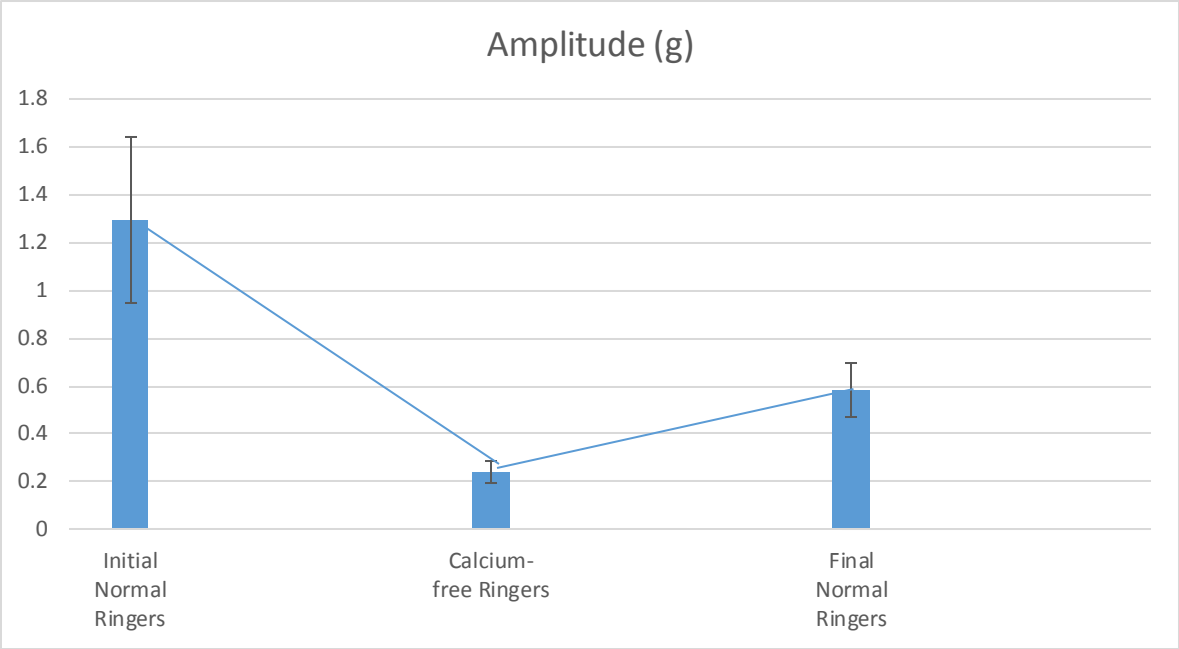

Figure 13. *Amplitude (g), starting from the control of normal Ringers solution to calcium-free Ringers, and back to control. Data represents the mean amplitude of ten intestinal waveforms within the first five minutes of recorded activity for baseline activity, as well as for all post-control activity. Amplitude of smooth muscle contractions decreased by 81.4% due to lack of extracellular calcium.*

Discussion:

The gastrointestinal system is one of the most important systems of the body, and it is special not only because it is responsible for breaking up nutrients and energy into attainable and digestible pieces, but because it acts subconsciously; constantly regulating homeostasis (Sherwood, 2013). This study has major implications because it allows for some insight on what can be affecting smooth muscle contraction strength, and the rates of those contractions. Additionally, knowing the true role of extracellular calcium in smooth muscle contraction is incredibly important; perhaps advances can be made to come up with a substitute substance for those with calcium deficiencies and deficits. Scientific knowledge, medical reasons, and personal interest all stand to benefit from discovery of the gastrointestinal system. The major finding of this lab was that the smooth muscle contractions of the intestine are quite variable depending on

their externalizing or internalizing factors, and that contractions of smooth muscle are very inefficient and ineffective in terms of amplitude and frequency with a lack of calcium.

When examining the impacts of adrenergic substances on gastrointestinal response via epinephrine, the hypothesis was that the epinephrine would activate the sympathetic nervous system, resulting in a decline of tension and motility in the intestinal segment. The results supported the hypothesis. Epinephrine mimics sympathetic activity to the enteric plexus located within the smooth muscle walls of the digestive tract (Sherwood, 2013). The hormone initiates a cascade of events beginning by stimulating a beta receptor which activates a G-protein. Levels of adenylyl cyclase increase, leading to an increase of the generation of cyclic AMP (cAMP) from ATP, and these increases activate protein kinase (PKA). PKA stimulates the eventual phosphorylation of myosin light chain kinase (MLCK), which decreases the affinity of cAMP to MLCK, and in turn, decreases the ability of MLCK to phosphorylate anything at all (Sherwood, 2013). Consequently, the baseline tension and motility of the smooth muscle decreases (Figure 2, Figure 4). The basal electrical rhythms were not inhibited or changed, and so the frequency should not have differed from the control rate, although in Figure 3 there is a slight increase in contractile frequency. This could be due to computer software mishaps or human error in regards to the randomization of picking waveforms to assess. In a study conducted by Furness et al. regarding the adrenergic nerves present in the enteric plexus and their role inhibiting gastrointestinal movements, results were similar to this lab. Furness et al. found that adrenergic substances inhibit motility and contraction and tension through the release of noradrenaline and the stimulation of the sympathetic nervous system via innervations of the intestinal tract (Furness et al., 2010). In a similar series of studies conducted by Fruhwald et. al, it was demonstrated that catecholamines also exert direct effects on intestinal motility via epinephrine subjection.

Fruhwald et al. carried out a very similar experiment on an isolated guinea pig small bowel, and reached the same conclusion; epinephrine inhibits peristalsis in the intestinal tract, leading to less baseline tension, and less force of contraction (Fruhwald et al., 2002).

When examining the impacts of cholinergic substances on gastrointestinal response via methacholine, the hypothesis was that the methacholine would activate the parasympathetic system, or act very similarly, resulting in an increase of baseline intestinal tension and contraction. The results supported the hypothesis, except for when considering the amplitude factor (Figure 7). Methacholine essentially mimics acetylcholine being released by the vagus nerve, which is a part of the parasympathetic pathway that innervates the enteric nervous system within the smooth muscle layers of the intestinal tract (Sherwood, 2013). The methacholine acts on muscarinic receptors, which decreases G-protein activity; this is the opposite effect of epinephrine. Levels of adenylyl cyclase fall, and the generation of cAMP from ATP decreases as well. This decrease leads to a fall in PKA activity, which results in a decline in phosphorylation of MLCK by PKA. The decrease in phosphorylation of MLCK increases the affinity of calcium-CaM for MLCK, increasing the ability of MLCK to phosphorylate MLC. These shifts increased the activation of longitudinal and circular smooth muscles within the intestinal segment (Sherwood, 2013). As a result, baseline tension increased (Figure 5), and since there was no change in basal electrical rhythms, the frequency remain constant before and after addition of the methacholine (Figure 6). The drops of methacholine were distributed and added at two different times so that the reaction and physical changes could take place and the results would be very apparent to the experimenter. Amplitude theoretically should have increased according to the hypothesis presented and the cascading pathway described, however amplitude of the waveforms explicated a decrease (Figure 7) after addition of the methacholine. These differences in expected

and actual results could be due to computer software mishaps or human error in regards to not waiting long enough for differences to accumulate and occur before assessing waveforms of contractile amplitude. The lab study was also limited to a very small intestinal segment that had already undergone stimulation of different substances, and this might have affected this portion of the lab's results (Figure 7).

When examining the impact of purinergic substances on intestinal motility via the addition of ADP, the hypothesis was that the ADP would activate P2Y receptors, and the G protein-coupled receptors would have a cascading effect, eventually leading to a decrease in contraction and amplitude of the intestinal segment. The results supported this hypothesis (Figure 8, Figure 10). Physiologically, ADP mimics ATP released from sympathetic neurons innervating the enteric plexus (Sherwood, 2013). ADP stimulates P2Y receptors, which activates a Gq protein as well as a phospholipase A2 enzyme. Through a cascading series of reactions, there is an eventual influx of calcium, which increases the activity of phosphokinase C. This substance phosphorylates MLCK at sites other than where the MLCK is active, which decreases the phosphorylation of MLC by MLCK, and finally, leads to a decrease in contraction and amplitude. Basal electrical rhythms remain unchanged by this activity within the intestinal wall, and so frequency was not impacted (Figure 9). In a study regarding the antipropulsive effects of morphine and whether it was a G-protein-mediated mechanism by Parolaro et al., morphine was administered and intestinal motility was subsequently monitored in rats. Morphine acted much like ADP and epinephrine in the current lab, inhibiting intestinal motility and abolishing the migrating motility complexes completely. Morphine proved to be an antagonizing force, much like ADP and epinephrine on the intestinal motility in this study (Parolaro et al., 1990). ADP and epinephrine were the only two substances that should have decreased just amplitude and baseline

tension, while not impacting frequency. Methacholine, ideally, should have increased baseline tension as well as amplitude of the contractile waves, which the results provided did not show (Figure 7).

When examining the effects of extracellular calcium on smooth muscle contraction, the hypothesis was that there would be decreased contraction, motility, and frequency while in the presence of calcium-free solution due to the lack of calcium available for cross bridge cycling. The results supported this hypothesis. Intestinal smooth muscle is constantly emitting a basal electrical rhythm originating from the interstitial cells of Cajal, which are the pacemaker cells of the small intestine. They undergo slow waves of tonic contraction until a threshold is reached, allowing an action potential to propagate and generate occasional phasic contractions. Smooth muscle contractions occur when an action potential activates calcium voltage gated channels, and an influx of cytosolic calcium arises (Sherwood, 2013). This influx leads to calcium induced calcium release, which binds to calmodulin. The calcium-calmodulin complex binds and activates myosin light chain kinase, which phosphorylates, and allows the myosin cross bridge to bind to the actin filament and allow muscle contraction to begin, carrying on much like it does in skeletal muscle (Fruhwald et al., 2002). Without the presence of calcium, there is no way for the intestinal segment smooth muscle to reach any kind of potential. Figures 11, 12, and 13 illustrate the importance of calcium for smooth muscle contraction. Without calcium, cross bridge cycling can simply not occur, and the muscles cannot contract. In a study by Atta et al., a regular solution utilized for baseline tension was replaced by a calcium free solution as well, and the shift to calcium free solution completely abolished the contractile responses of the smooth muscle tissue (Atta et al., 2004). These results were exactly the same as the results reached in this lab, although the purpose of Atta et al.'s experiment regarded the effect of Egyptian plant extracts on

antidiarrhoeal activity. Atta et al.'s study had a more medical basis, however the results they reached with what affected contractility of the smooth muscle within the intestinal tract were the same (Atta et al., 2004). The basal electrical rhythms cannot reach their pacemaking potential without calcium, and so frequency drastically decreases with a lack of calcium (Figure 12). Without contraction of smooth muscles, digestion, absorption, propulsion, and excretion cannot take place. This would lead to an incredible disharmonious state for the body, and inevitable death. The results of this portion of the lab exemplify the importance of calcium in the body, and how it quite literally allows smooth muscle to contract in efforts to maintain homeostasis.

In conclusion, this lab explicated the basic responses of gastrointestinal smooth muscle contraction to adrenergic, cholinergic, and purinergic substances, as well as examining the role of extracellular calcium in smooth muscle contraction. Overall, when presented with adrenergic substances, like epinephrine, the frequency of the contractions should remain constant due to a normalized basal electrical rhythm, however the amplitude and baseline tension of the contractions should decrease. In the presence of cholinergic substances, like methacholine, frequency should remain constant, while amplitude and baseline tension of the contractions should increase. Purinergic substances, like ADP, should act much like methacholine in terms of responses, and lead to decreases in contraction and amplitude of the smooth muscle. Lack of calcium leads to a total abolishment of baseline tension, frequency, and amplitude of the smooth muscle contraction because cross bridge cycling can't occur without calcium. The lab proved that the digestive system is sensitive to all kinds of moderation and mediation and regulating pathways from all parts of the body. Functionality and efficiency is incredibly important in the gastrointestinal tract, and the ability to respond appropriately to the body's stimuli allows for the maintenance of homeostasis.

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