

The Role of Histamine H₃ Receptors in Myocardial Function Following Cardiac Ischemic Preconditioning

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ABSTRACT

During a period of low or no blood flow (ischemia) in the heart, mast cells that contain histamine are stimulated to cause an inflammatory response in the heart muscle (myocardial) tissue. Histamine H₁ and H₂ receptors are known to contribute to the injury to the heart that follows when blood flow is restored (ischemia reperfusion injury) after a heart attack. Histamine H₃ receptors, on the other hand, are known to protect the heart from injury after a heart attack. Ischemic preconditioning is another mechanism that attenuates ischemia reperfusion injury. In this case, a short decrease in blood flow preceding the longer damaging episode of ischemia reduces its damaging effects. The purpose of this experiment was to test the hypothesis that H₃ receptor stimulation is one of the mechanisms responsible for the positive effects of ischemic preconditioning. I used two experimental groups and one control group of five rats each. The experimental groups tested the effects of R- α -methyl histamine (RHA), an H₃ receptor agonist, and Thioperamide (Thiop), an H₃ receptor antagonist. The control group had no substituted chemicals. The results of the Thioperamide treated hearts supported our hypothesis that H₃ receptor stimulation is necessary for effective preconditioning. However, the results of the RHA treated hearts caused a decrease in myocardial function over the course of the experiment, which suggests that H₃ receptor stimulation has no effect on the protective mechanisms of preconditioning. The reasoning behind this may be related to the effects of the H₃ receptor agonist on vascular smooth muscle function and thus coronary flow. More research is required to further decipher the results.

INTRODUCTION

Cardiovascular disease is considered the number one killer in America. More than two in every five Americans die of cardiovascular disease and at least 250,000 people die of heart attacks every year (Medi-Smart, 2004). Of those suffering with heart disease, 52.2% are men and 47.8% are women (UNISYS, 2005). Myocardial infarction (a heart attack that destroys heart muscle tissue) caused by ischemia/reperfusion injury is one of the major causes of cardiovascular mortality (Singh and Saini, 2003). This is because myocardial tissue is non-regenerative and when the muscle is damaged, the heart has less strength to pump nutrients and oxygen into circulation (Mason and Davis, 1987)

Following acute myocardial infarction (AMI), the ventricle begins healing. Within the first hours or days after AMI, however, infarct expansion can occur, due in part to inflammation. Reperfusion is one of the most effective means of reducing tissue damage (Masini et al., 1990). However, according to Samarkandi and Mansour, as a result of ischemia, expression of certain pro-inflammatory gene products and bioactive agents are promoted, while other protective gene products and bioactive agents are repressed. Thus, ischemia causes inflammation that can increase tissue vulnerability to further injury upon reperfusion (Samarkandi and Mansour, 2004).

Mast cells (cells distributed throughout the body's connective tissues that are critical to the inflammatory response) are found in the arteriolar network of the heart (Davani et al., 2002) and release their contents into the tissues when injured. Mast cells contain histamine, which is a key factor of the initiation of inflammation within the immune system. There are three subtypes of histamine receptors, H₁, H₂, and H₃ (Yamamoto et al., 2004). The histamine H₁ and H₂ receptors are known to contribute to ischemia reperfusion induced myocardial injury by increasing the white blood cell (leukocyte) adhesion to platelets and endothelial cells, and thus increasing the likelihood of blood clot and further blood vessel obstruction (Singh and Saini, 2003). On the other hand, histamine H₃ receptors (H₃Rs) have been shown to attenuate the damage of ischemia/reperfusion injury by regulating the amount of norepinephrine released in hearts (Yamamoto et al., 2004). This is important because high levels of norepinephrine released during ischemic stress in a heart are known to contribute to ischemia reperfusion injury and myocardial damage (Yamamoto et al., 2004; Mackins and Levi, 2000). This damage may occur because the norepinephrine increases the metabolic demands of the myocardium at a time when delivery of oxygen and nutrients is limited. Thus, H₃ receptor stimulation and the following decrease in norepinephrine release during ischemia could protect the heart by preventing such increases in metabolic demand. Since the overall effects of histamine in an organism depend on how much of the histamine is released (Valen et al., 1995) and on a balance between the distribution of these receptors, it is important to understand the relative contribution of H₃ receptors in this mix.

One way for the body to reduce the effects of ischemia reperfusion injury is by ischemic preconditioning. This is described as short episodes of ischemia that "condition" the heart to recover more effectively from a longer, damaging period of ischemia. As stated in a recent study, "overall, preconditioning signifies an adaptive endogenous response to short sub lethal episodes of ischemia leading to contradictory protection against subsequent lethal ischemia" (Samarkandi and Mansour, 2004). Some studies assert that before a large episode of ischemia, these short preconditioning periods will degranulate cardiac mast cells and consequently allow the cytotoxic products that they release to be washed away when reperfusion occurs (Singh and Saini, 2003). As a result, the heart will be relieved of some toxic material before a more intense episode of ischemia takes place, thus helping the heart recover faster and more efficiently. Others suggest that preconditioning preserves the availability of ATP by decreasing calcium overload, therefore preventing the activation of energy consuming Na⁺-H⁺ and Na⁺-Ca⁺ exchange (Singh and Saini, 2003).

Consequently, the mechanisms that have been proposed to explain ischemic preconditioning (washout of cytotoxic products or metabolic preservation) are similar to those that have been proposed to explain the effects of histamine on ischemia reperfusion injury: histamine acts as a cytotoxic product when it stimulates H₁ and H₂ receptors and it prevents norepinephrine induced increases in metabolic demands when it stimulates H₃ receptors. This experiment attempts to determine if histamine H₃ receptor stimulation is a possible mechanism of the cardio-protective effects of preconditioning. In other words, we tried to prove that preconditioning could cause the release of histamine that acts on H₃ receptors early in the injury process before it is washed away, thereby interfering with the

norepinephrine release and the metabolic effects that occur later during ischemia reperfusion injury.

METHODS

Rats were chosen in this experiment with no regard to gender. This is because no significant difference was found between gender and cardiac response to ischemia reperfusion injury (Kang et al., 1999). Rats were obtained from Bassett Healthcare, Taconic Farms, and from Dr. Kinho Chan (Hartwick Psychology Professor). Each rat was given a 12 hour light/dark cycle with an unlimited supply of food and water. After at least three days of acclimation, the rats were anesthetized to a surgical level with a pentobarbital sodium injection (50 mg/kg supplemented as necessary) as approved by Hartwick's Institutional Animal Care and Use Committee (IACUC). The injection was also supplemented with the anticoagulant heparin (100 units) in order to prevent any blood clots from forming before the initiation of the experiment. The thoracic cavity was then opened and the heart was quickly excised and placed in a beaker of chilled Krebs Henseleit buffer to arrest the heart for a short period to prevent initial injury. The heart was cannulated in a Langendorff perfusion apparatus and perfused through the aorta with a modified Krebs-Henseleit buffer solution (in mM; NaCl 117.0, KCl 4.7, CaCl₂ 3.0, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 23.7, glucose 11.5). The perfusate was continuously bubbled throughout the experiment with a gas mixture of 95% O₂/5% CO₂ in order to maintain adequate levels of oxygen and pH. A heater was placed in the buffer to keep the temperature constant at 37 degrees Celsius. After cannulation, the heart was carefully cleared of fat and connective tissue to allow access to the left ventricle. A saline filled balloon made from expanded polyethylene tubing was then inserted into the left ventricle through the left atrium of the hearts and connected to a pressure transducer in order to measure heart rate, force of contraction (LVDP), and rate of contraction (RLVDP). The hearts were allowed to stabilize in the perfusion buffer for 30 minutes before starting the experimental measurements. When the stabilization period was complete, the hearts were subjected to 3 minutes of preconditioning ischemia followed by 10 minutes of reperfusion. They were then subjected to 7 minutes of total ischemia by stopping the flow of the buffer, and 60 minutes of reperfusion by restarting the buffer flow (Kang et al., 1999). The amount of fluid leaving the heart (coronary effluent) was collected every 5 minutes during both the stabilization and reperfusion periods to measure coronary flow (Koyama et al., 2003). Measurements from the balloon pressure transducer were taken every 5 minutes during the stabilization period and reperfusion and more often during preconditioning and ischemia to compensate for quicker decreases in heart activity. These measurements were used to calculate the heart rate, force of contraction, and rate of contraction.

One control group and two experimental groups (n=5) were tested. One experimental group was perfused with an H₃ receptor agonist, R- α -methyl histamine (RHA; 1 μ M), and the other experimental group was perfused with an H₃ receptor antagonist, Thioperamide (Thiop; 1 μ M). All concentrations were taken from Yamamoto et al. (2004). The final group consisted of the untreated control rats. The H₃ agonist and antagonist were dissolved into the corresponding perfusate buffer 10 minutes into the initial stabilization stage and allowed to flow through the heart through preconditioning

reperfusion. The treatment buffer was replaced with an untreated Krebs-Henseleit buffer during ischemia.

A total of 26 measurements were taken throughout the experiment to obtain accurate conclusions. Four were taken during the stabilization period, 2 were taken during preconditioning reperfusion, and 13 were taken during ischemic reperfusion, all at 5 minute intervals. During preconditioning, 3 measurements were taken at 1 minute intervals. During ischemia 4 measurements were taken at 2 minute intervals. The last 3 measurements of the stabilization period were averaged. Each following measurement afterwards was converted into a percentile of that mean.

Heart rate was calculated by using the measurements from the balloon pressure through the pressure transducer. The number of beats examined (n=5) were divided by the time period from the first to the last beat. That number was then multiplied by 60 in order to convert the measurement to beats per minute. Coronary flow, the amount of fluid entering the heart, was measured by collecting the coronary effluent, the amount of fluid coming out of the heart. These measurements were taken every 2 minutes to achieve a more accurate reading. Those results were then divided by 2 in order to look at milliliters per minute. Rate of contraction (RLVDP) was also measured by looking at the graph produced from the balloon pressure. In this case, the slope of the beat was calculated to obtain a measurement in mmHg per second. The final measurement attained was force of contraction (LVDP). This was again measured by looking at the recorded readings of balloon pressure. Here, the height of each beat was calculated to obtain this measurement.

We applied a selection criteria for the rat hearts stating if they were unable to recover from ischemia, then they were not used in analysis. As stated above, the four graphs compared coronary flow, heart rate, force of contraction, and rate of contraction between control and experimental hearts over time (from preconditioning through ischemia to reperfusion). Two-way analysis of variance (ANOVA) with repeated measures was used to compare myocardial performance among treatment groups over the course of the experiment, during preconditioning recovery, and during ischemic reperfusion. Measurements were considered statistically significant only if P values were less than 0.05.

RESULTS

As stated by the selection criteria, we were able to use 15 out of 35 rats to perform our analysis. Each experimental group contained 5 rats each. The average weight of the rats in the control group was 458.3g, while the average weight of the rats with RHA treated hearts was 349.9g, and those with Thiop treated hearts was 355.5g. This difference in weight was a result of obtaining the rats from different sources and time periods. The last 3 measurements taken during the stabilization period of each experiment for each measurement were averaged. For the control experiment, the average HR during stabilization was 178.248 beats/min, CF was 9.2994 mL/min, RLVDP was 498.97 mmHg/sec, and LVDP was 37.58 mmHg. For the RHA treated hearts, the average HR was 203.284 beats/min, CF was 7.2494 mL/min, RLVDP was 944.318 mmHg/sec, and LVDP was 51.79 mmHg. Finally, for the Thiop treated hearts, the average HR was 250.41 beats/min, CF was 8.8648 mL/min, RLVDP was 826.1776 mmHg/sec, and LVDP was 54.782 mmHg.

Throughout the entire experiment, the heart rate between the treatments showed no statistically significant differences, with a P value of 0.157 (Figure 1a). Looking closer at this information, it was also deduced that the heart rate had no significant differences during preconditioning reperfusion (P=0.331) or ischemic reperfusion (P=0.157) between the treatments (Figure 1b,c).

Coronary flow measurements over the entire experiment illustrated that there was an overall decrease in coronary flow from the beginning of ischemic reperfusion to the end. However, no statistically significant differences (P=0.406) were found between the treatment groups (Figure 2a). Nonetheless, treatment effects did appear upon closer examination. While there were no differences between Thiop and control hearts during either reperfusion periods, significant differences were found between RHA treated hearts and both the control (P=0.025) and Thioperamide (P=0.001) treated hearts (Figure 2b) during preconditioning reperfusion. The RHA treated hearts had much lower coronary flow measurements than either of the two other treatments. This was not the case for the ischemic reperfusion period, where no significant differences (P=0.345) were found among any of the treatments (Figure 2c).

Overall, there were no statistically significant differences (P=0.318) in rate of contraction (RLVDP) between treatments throughout the entire experiment (Figure 3a). However, looking specifically at the preconditioning reperfusion measurements, a significant difference was found between RHA and Thiop treated hearts (P=0.044) (Figure 3b). The RHA treated hearts also had the lowest overall RLVDP compared to the other treatments. Although, it appeared that the slope of the RLVDP curve during ischemic reperfusion demonstrated a steeper decline than the other treatments, there were still no significant differences over time (Figure 3c; P=0.318).

Statistically significant differences were found (P=0.039) in force of contraction (LVDP) throughout the entire experiment between treatments (Figure 4a). Although no significant differences were found between treatments (P=0.317), specifically during preconditioning reperfusion (Figure 4b), the RHA treated hearts did seem to cause a slight decrease in heart function compared to the other treatments. On the other hand, there were significant differences found between the control heart and both the RHA treated hearts (P=0.017) and Thiop treated hearts (P=0.017) during ischemic reperfusion (Figure 4c). Although the results were not the same, the ischemia reperfusion section of the graph resembles that of rate of contraction. By looking at Figures 3c and 4c, the RHA treated hearts unusually displayed a rapid increase in LVDP right after ischemia followed by a rapid decrease in LVDP afterwards. This can also be compared to the other treated groups. For both LVDP and RLVDP, Thiop treated hearts had constant low measurements, while control hearts had higher constant measurements, which is what we expected.

DISCUSSION

The purpose of this study was to evaluate the role of H₃ receptors on the effects of ischemic preconditioning on myocardial cell function. Our results from the Thiop LVDP measurements support the notion that H₃ receptor stimulation is necessary for effective preconditioning. However, results from the RHA experiment complicated this interpretation.

Both experimental treatments had no effect on heart rate (Figure 1). Heart rate measurements reflect the activity of the electrochemical signals in the heart. The sinoatrial (SA) node, a small mass of cells in the right atrium of the heart, acts as the hearts pacemaker and determines the heart rate by passing electrochemical signals through the heart. Thus, the measurement of heart rate does not tell us how the myocardial cells function, and acts as a type of “control”. This allowed us to conclude that there were no significant differences between treatments and that the electrochemical signals to the heart, and thus the SA node, had no negative effects on the heart and were unaffected by the treatments. Therefore, any differences that we saw in other measurements could not be attributed to differences at the level of the SA node.

The coronary flow measurement worked as a “control” graph as well. Instead of looking at the myocardial function of the cells, this measurement reflects how the blood vessels supply blood to the heart. Over the entire experiment, we were unable to resolve differences between treatments. However, by looking specifically at preconditioning reperfusion, we discovered that RHA had a negative effect on coronary flow (Figure 2). This tells us that the RHA treatment caused a constriction of the blood vessels, preventing adequate amounts of blood to reach the heart during preconditioning reperfusion. This in turn could cause damaging effects by limiting the amount of nutrients and oxygen the heart receives. Thus, the function of the myocardial cells in RHA treated hearts could be impaired simply because their blood supply was compromised rather than because H₃ receptor stimulation had a damaging effect on metabolic activity of the myocardial cells. The Thiop treated hearts also showed a decrease in coronary flow. However, since this is what we expected and there were no significant differences between Thiop and the other treatments, we could conclude that any subsequent effects on those hearts were caused by direct damage to the myocardium.

Rate and force of contraction both looked specifically at the effects of preconditioning reperfusion and ischemic reperfusion on the function of the myocardial cells. There were no differences in RLVDP among treatments over the course of the whole experiment (Figure 3a). Although not significant, the preconditioning reperfusion graph (Figure 3b) does portray that the hearts treated with RHA had relatively constant low RLVDP measurements, whereas the other treated hearts showed an increase in RLVDP. This decrease in RHA treated hearts over the course of the experiment could be explained by the damage done to the blood vessels during preconditioning reperfusion when the drug was present. On the other hand, both RHA and Thiop did appear to slightly decrease heart function at the very end of the ischemia reperfusion period (Figure 3c). This observation is constant with the notion that Thiop, the H₃ receptor antagonist, blocks the ability of histamine to have a positive effect on the heart. However, we expected that the converse would also be true, and that the RHA treated hearts would increase heart function, since this treatment was supposed to increase the amount of histamine H₃ receptor stimulation in the area of injury. It is possible that this RHA effect was caused by the reduction in coronary flow. Damage caused by inadequate perfusion during preconditioning reperfusion could have potentially obscured any positive effects of the H₃ receptor agonist. However, this requires further explanation for a thorough confirmation.

As mentioned earlier, the force of contraction (LVDP) was another measurement to help determine the function of the myocardial cells. The LVDP did show a significant

difference between treated groups over the course of the experiment (Figure 4a). Thiop and RHA treated hearts both significantly decreased the LVDP compared to the control hearts. Closer examination demonstrated that there were no significant differences between treatments during preconditioning reperfusion while drugs were in the perfusate (Figure 4b). This means that the drugs were having no direct effect on myocardial performance during preconditioning reperfusion, and subsequent differences must be because of interferences with the consequences of preconditioning, such as blood vessel obstruction. However, there may be a trend suggesting that the RHA treated hearts had slightly lower LVDP while it was slightly higher in the other treatment groups.

Most important is that significant differences were found during the ischemia reperfusion period with RHA and Thiop definitely decreasing the LVDP. We believe that the RHA effects could have resulted from the damage done to the blood vessels during preconditioning reperfusion carrying over to the next reperfusion period. The cause of the rapid increase in LVDP in RHA treated hearts directly following ischemia is unknown. However, instances of this have been observed in other research. Valen et al. (1995) found that adding a high concentration of histamine (1mM) caused an initial increase, followed by a large decrease in LVDP. This resulted in the histamine treated rats having the lowest value for LVDP at the end of reperfusion. Opposed to this, adding a low concentration of histamine (0.1 μ M) had absolutely no effect on LVDP. This is not exactly the same as our observation, since Valen et al. (1995) only tested the effects of histamine on ischemia reperfusion injury and not on preconditioning, however, it is consistent with our findings and merits further investigation of the role of histamine in rat hearts.

Overall, the results of the Thioperamide treated hearts, specifically on LVDP, supported our hypothesis that H₃ receptor stimulation is necessary for effective preconditioning. The lack of blood vessel obstruction from Thiop treated hearts also portrayed that the subsequent injury from ischemia reperfusion was completely due to damage of the myocardial cells. The purpose of an H₃ receptor antagonist is to block the H₃ receptors from binding to histamine and causing a preventative effect. The consequent lack of defense following ischemia/reperfusion injury will then allow the cytotoxic products to have an enhanced negative effect on the myocardium. This decrease in myocardial function is an effective indicator of the involvement of H₃ receptors in preconditioning. However, the results of the RHA treated hearts confused these conclusions. Since RHA is an H₃ receptor agonist, its purpose should be to increase the amount of histamine specific to H₃ receptors in the heart, therefore increasing the positive effects of H₃ receptor stimulation. Conversely, the decrease in myocardial function in the RHA treated hearts over the course of the experiment demonstrates that H₃ receptor stimulation has no effect on the protective mechanisms of preconditioning. The reasoning behind this decrease is currently unknown, although it is thought that the damage done to the blood vessels during preconditioning could have been one of the major causes.

Since the RHA treated hearts acted opposite of our expectations, I believe that more research into this drug should be completed in order to better understand its mechanism of action. Although there have been studies that support the use of RHA, other experiments concluded that RHA does not have a protective effect after ischemia reperfusion injury. Imamura et al. (1994), who examined the effects of RHA and Thiop

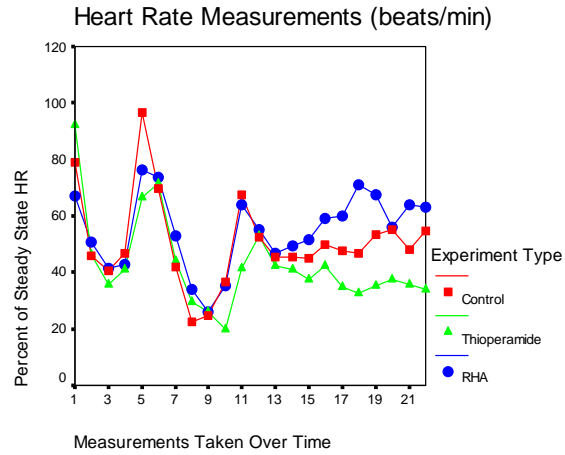
more in depth at the molecular level, demonstrated that RHA was unable to modify NE overflow at reperfusion. This being the case, the NE would still be available to increase metabolic demands upon the heart, resulting in injury. However, in agreement with our results, Imamura et al. (1994) did find that hearts treated with Thiop doubled the overflow of NE during reperfusion, thus causing more injury.

The complicated set-up of this experiment could have also contributed to the confusing results. First of all, as opposed to other experiments, the rats we used in this experiment came from multiple different sources, did not weigh the same, and were not all the same age. This could have caused a problem because older or heavier rats may experience higher metabolic demands than younger, thinner rats. The administration of the drug was also imprecise. It may have taken longer than we expected for the drug to be perfused through the tubing and into the heart, therefore, the heart was not exposed to treatment as long as desired. This could also have caused a problem when we switched the perfusate from the drug treated buffer into the untreated buffer. The drug could have been left over in the tubing and perfused into the heart when it was not intended, thus exposing the heart to the treatment when it was not supposed to be available. Lastly, due to the lack of time, every heart that survived the period of ischemia was considered acceptable to use upon analysis, whereas other experiments created a standard range for which the hearts were considered acceptable. For example, while we did not take into consideration the values before hand, Valen et al. (1995) only analyzed the results from hearts with an LVDP between 60-160 mmHg, CF between 8-15 mL/min, and HR between 240-340 beats/min at the end of stabilization.

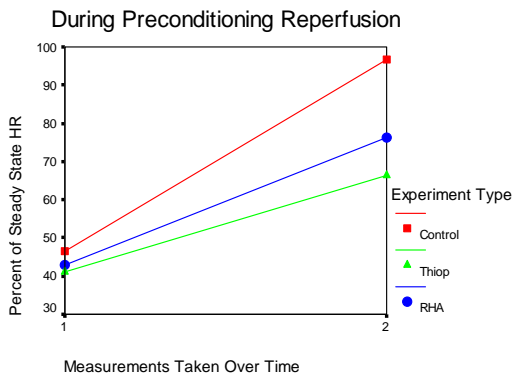
Although the decrease in myocardial function by RHA revealed from our experiment can not yet be explained, a possible reason why the drug did not have a protective effect on heart tissue could be that all of the histamine H₃ receptors were saturated with the endogenous release of histamine by mast cells. In order to test this prediction, it is possible to pharmacologically prevent the degranulation of mast cells and supplement an H₃ agonist. This would allow someone to specifically look at the effects of a treatment as opposed to having the treatment compete with the body's own defense mechanisms. Another experiment that could be performed in order to enhance the credibility of the current one under study could be to administer an H₁ and H₂ antagonist as well as an H₃ agonist. Since stimulation of H₁ and H₂ receptors both cause harmful effects, blocking them would allow someone to completely rule out the effects of cytotoxic materials, thus permitting the observation of strictly preventative treatments.

The therapeutic use of natural mechanisms like preconditioning in cardiac surgery could have great value in the future. For example, preconditioning could be used before aortic cross-clamping in open heart surgery to enhance the current methods of myocardial protection. However, since an ischemic preconditioning stimulus might also cause complications, it is significantly important to identify the mechanisms for this phenomenon in order to use it most effectively (Perrault and Menasché, 1997). If histamine H₃ receptors are involved, there may be pharmacological pretreatment with fewer risks that could help prepare patients for open heart surgery. There are many controversial views on the effectiveness of histamine H₃ receptor stimulation with and without preconditioning, but it is definitely a very important concept and should be examined to the fullest until a true mechanism for preconditioning and use for H₃ receptors has been discovered.

a.



b. Heart Rate Measurements (beats/min)



c.

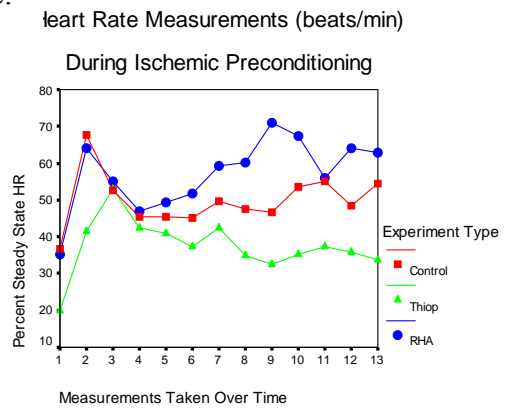


Figure 1: The effect of Control (n=5), RHA (n=5), and Thiop (n=5) treated rat hearts on Heart Rate (HR) a.) Over the entire experiment (P=0.157), and more closely at b.) During preconditioning reperfusion (P=0.331), and c.) During ischemic reperfusion (P=0.157).

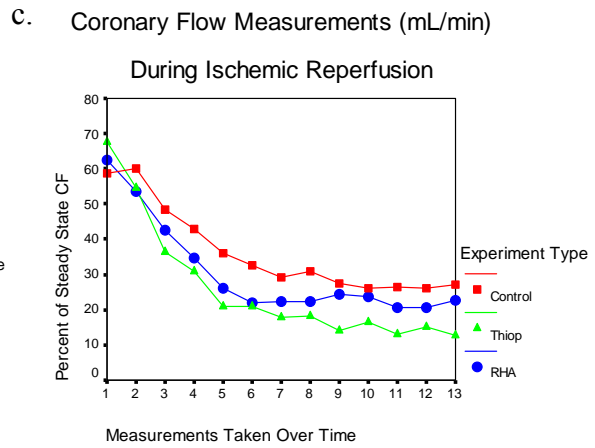
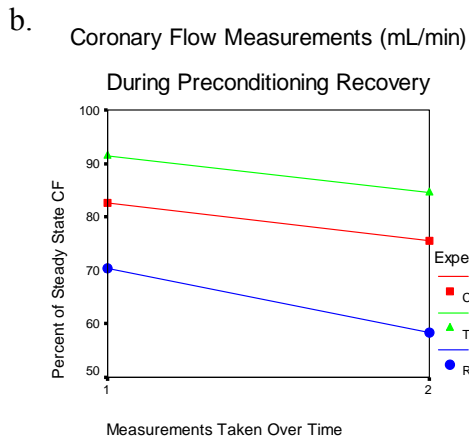
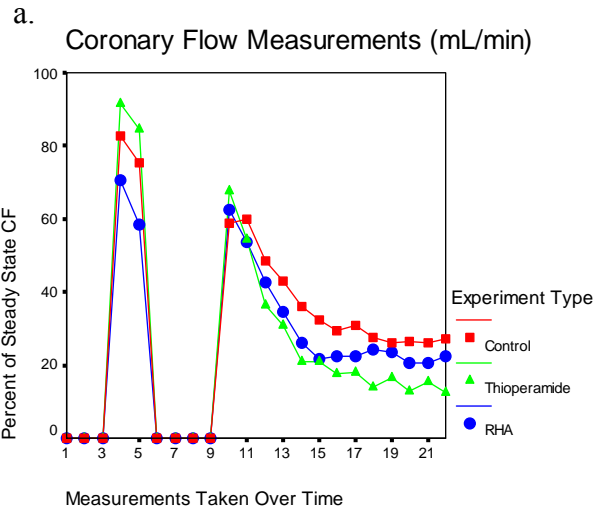


Figure 2: The effects of Control (n=5), RHA (n=5), and Thiop (n=5) treated rat heats on Coronary Flow (CF) a.) Over the entire experiment (P=0.406), and more specifically b.) During preconditioning reperfusion (P=0.005) and c.) During ischemia reperfusion (P=0.345).

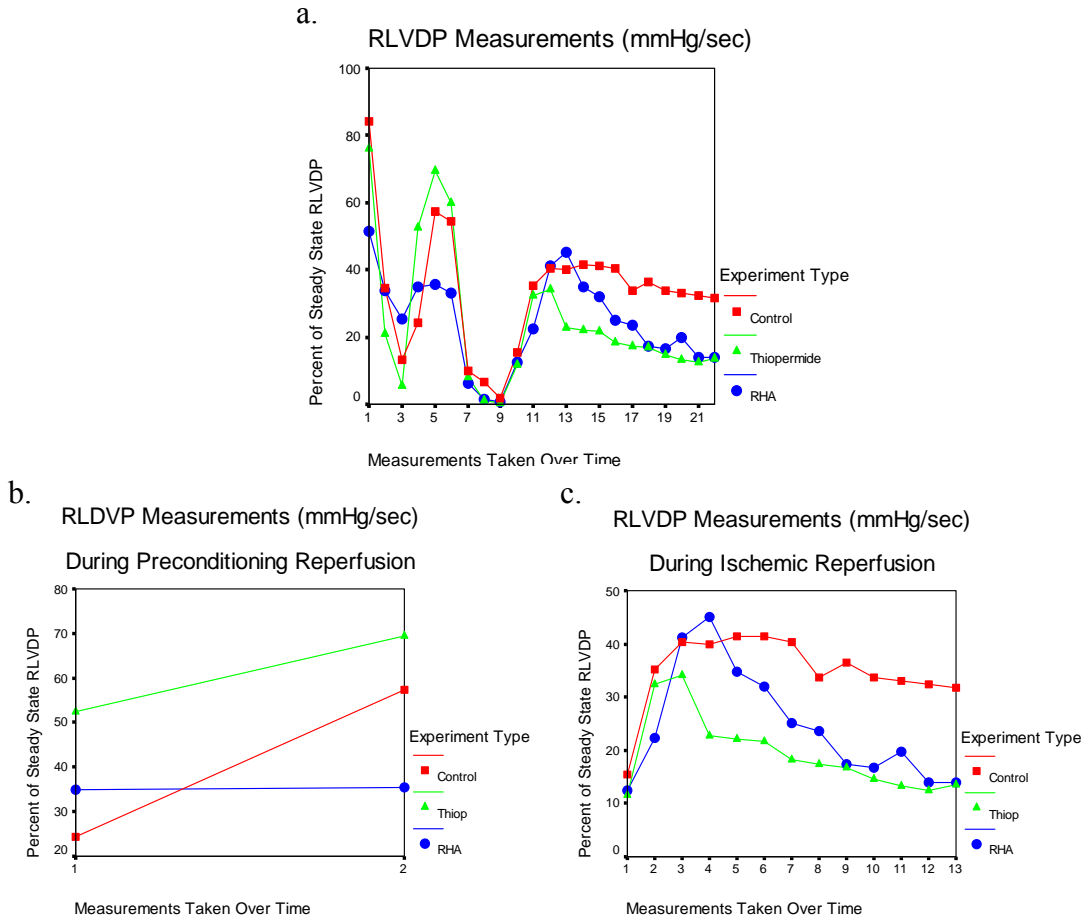


Figure 3: The effects of Control (n=5), RHA (n=5), and Thiop (n=5) treated rat hearts on Rate of Contraction (RLVDP – Rate of Left Ventricular Developed Pressure) a.) Over the entire experiment (P=0.318), and more specifically b.) During preconditioning reperfusion (P=0.100) and c.) During ischemia reperfusion (P=0.182).

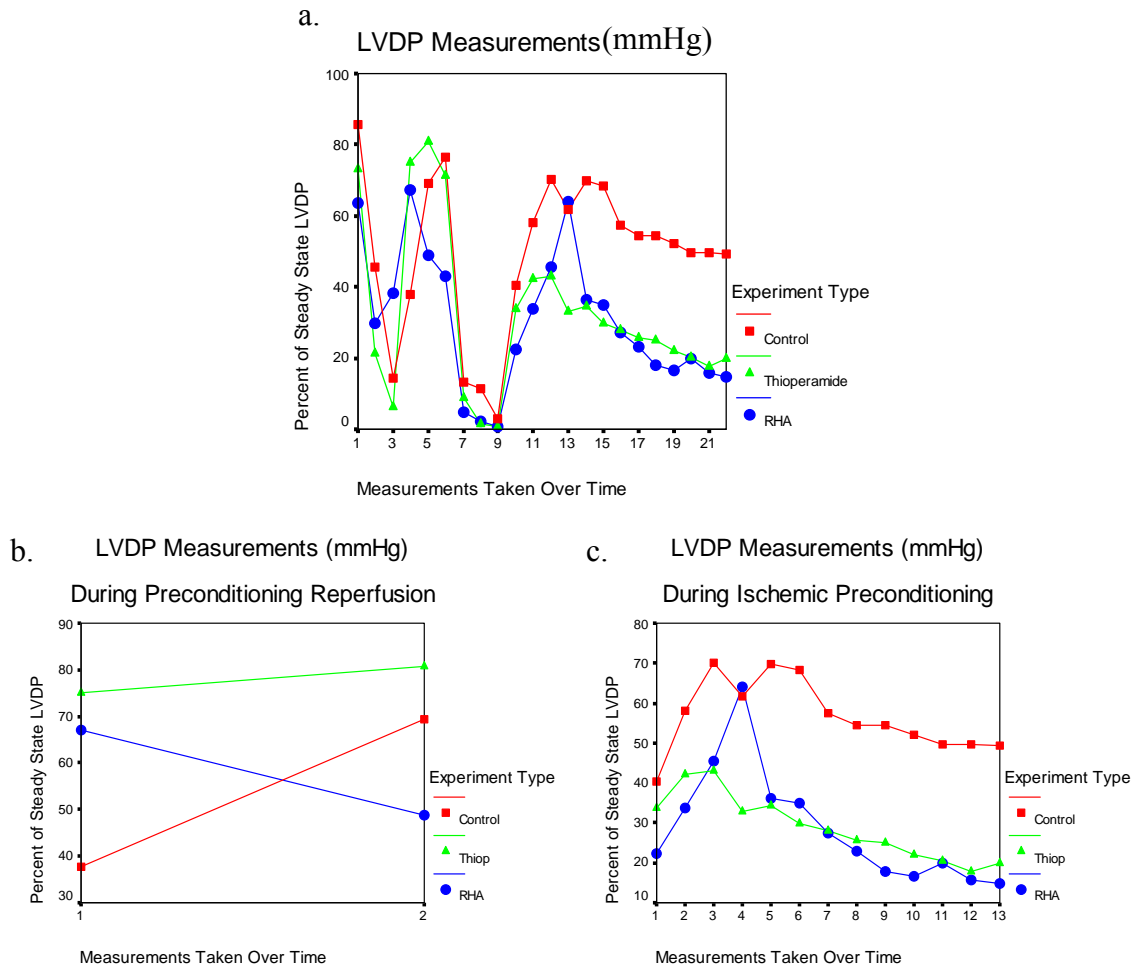


Figure 4: The effects of Control (n=5), RHA (n=5), and Thiop (n=5) treated rat hearts on Force of Contraction (LVDP-Left Ventricular Developed Pressure) a.) Over the entire experiment (P=0.039), and more specifically b.) During preconditioning reperfusion (P=0.317) and c.) During ischemia reperfusion (P=0.025).

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