CHARACTERIZING THE POTENTIAL FOR NEUTROPHILS TO MEDIATE THE
INFLAMMATION RESPONSE ASSOCIATED WITH CARDIAC ARREST AND RESUSCITATION

By

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A Thesis Submitted to the Honors College
In Partial Fulfillment of the Bachelors Degree
With Honors In

Physiology

THE UNIVERSITY OF ARIZONA

MAY 2013

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DEDICATION

This Honors Thesis is dedicated to everyone who has supported me in earning my degrees in Physiology and Biology during the past four years. I would like to give special thanks to my parents for instilling in me the value of hard work and a passion for learning. I would also like to extend a special thanks to my thesis advisor, Dr. Paul McDonagh, for mentoring me and giving me the opportunity to perform research in his laboratory.
ABSTRACT

Post-Resuscitation Injury (PRI) may contribute to poor recovery after cardiac arrest and resuscitation, and this complication may be due to a dramatic inflammatory response suspected to occur in the early hours following successful resuscitation. The purpose of this study was to evaluate whether blood neutrophils, a known source of oxidants under other acute inflammatory conditions, mediate an inflammatory response soon after resuscitation. Using a laboratory model of cardiac arrest and resuscitation (A/R), adult Sprague Dawley rats were anesthetized and subjected to a cardiac arrest and resuscitation protocol. During the experiments, blood samples were taken at specific time points to monitor blood glucose and mean arterial blood pressure. Sham experiments were performed as a control. Tissues from animals receiving no surgical procedures were also obtained as a further control. After two hours of resuscitation, heart, lung, liver, and brain tissues were collected and quick-frozen. Later, samples were thawed, homogenized, and analyzed for neutrophil-specific myeloperoxidase (MPO) accumulation using the Hycult Biotech ELISA kit. MPO accumulation is used as an indicator of neutrophil sequestration in the organ. We found a marked increase in tissue MPO in the lung, indicative of neutrophil accumulation. There was a statistically significant difference (t-Test) between sham vs. resuscitation groups for both hepatic and cardiac tissues. These findings indicate that neutrophils do sequester in the heart and liver in the early hours following successful resuscitation. Because of their potential for oxidative injury, neutrophils likely contribute to the inflammatory response and cardiac stunning observed in the early hours of resuscitation. Efforts to limit
neutrophil-mediated microvascular and oxidative injury may reduce PRI and improve recovery.
STATEMENT OF PURPOSE

The purpose of this study was to determine if neutrophil-mediated inflammation may play a significant role in Post-Resuscitation Injury following cardiac arrest. In patients that have been successfully resuscitated, high mortality and poor recovery may be due in part to an inflammation response that occurs during cardiac arrest and resuscitation. To cause significant tissue injury, neutrophils must first sequester in the organ and then release toxic oxidants. Determining if neutrophils accumulate in various tissues will allow for further characterization of their potential role in the inflammatory response. This finding could lead to further studies of the mechanisms underlying the role of inflammation in Post-Resuscitation Injury, and ultimately lead to improved treatment of patients in the early hours of resuscitation potentially leading to longer-term outcomes.
INTRODUCTION

In the United States, approximately 300,000 cardiac arrests occur each year.¹ While efforts to improve resuscitation are constantly being explored, patient survival from cardiac arrest to discharge from the hospital for both in-hospital and out-of-hospital cardiac arrest is still very low. Survival from in-hospital cardiac arrest occurs in only about 19% of patients, while survival from out-of-hospital arrest is even lower at about 9%.² These striking statistics suggest that an additional injury occurs following apparently "successful" resuscitation.

When the heart suddenly beats abnormally or stops beating completely, a patient is said to be in a state of cardiac arrest. Cardiac arrest often results from arrhythmias, or irregular heartbeats, which are caused by malfunctions with the heart’s electrical system.³ Usually, the cardiac conduction system stimulates contraction of the atria and then the ventricles in a coordinated fashion.⁴ Ventricular fibrillation is the most serious form of cardiac arrhythmia and occurs when the lower chambers of the heart contract in a swift, disorganized pattern.³ These arrhythmias render the heart inefficient to pump blood to the rest of the body. Decreased blood pressure results in a reduction of blood flow to the body’s tissues depriving them of oxygen and nutrients vital for cell function. The term ischemia is used to describe this period of reduced reperfusion.

Cardiac arrest can cause serious damage to vital organs such as the brain within just a few minutes. Cardiopulmonary Resuscitation (CPR) is performed in an attempt to restore proper cardiopulmonary function and blood flow to vital organs. CPR guidelines for the resuscitation procedure were recently revised in 2010 to reflect the relative importance of
administering proper chest compressions and defibrillations in restoring cardiac function.\textsuperscript{5} The most beneficial outcome occurs when the rescuer provides “hard and fast” continuous chest compressions and appropriate, early defibrillation for cardiac arrhythmias.\textsuperscript{5} Drugs are also administered during cardiac arrest in order to restore proper cardiopulmonary function. Vasopressors, like epinephrine and vasopressin, and antiarrhythmics, like amiodarone and lidocaine, are the most commonly used drugs.\textsuperscript{6} Epinephrine is used to increase contractility of the heart and raise blood pressure, and vasopressin is used to increase blood flow.\textsuperscript{6} Lidocaine and amiodarone are used in treatment of cardiac arrest via ventricular fibrillation. However, amiodarone is now the drug of choice because it has fewer side effects, increases coronary blood supply and vasodilation, and decreases systemic vascular resistance.\textsuperscript{6} Drugs are used in conjunction with chest compressions in almost all cases to restore cardiac contraction and proper blood flow. Successful resuscitation is defined as when the mean arterial blood pressure reaches 50 mmHg or higher and return of spontaneous circulation (ROSC) has been achieved.

It has long been known that the extent of myocardial injury is directly correlated with the amount of time spent in ischemia, and therefore practices have been set to return blood circulation to the body as quickly as possible. However, a complication of the return of circulation occurs and is known as ischemia-reperfusion (I/R) injury.\textsuperscript{7} We suspect that some of the mechanisms responsible for I/R injury are also involved in PRI. As evidenced through research and by the low discharge rate of patients from the hospital, there is an additional injury that occurs upon successful resuscitation. The etiology of this PRI is largely unknown.
Many studies, especially those concerning inflammatory responses and their effects on patient recovery, have been performed in order to characterize the PRI that occurs after cardiac arrest. One particular study was conducted in order to determine the presence and concentration of inflammatory cytokines and correlation to the survival status of cardiac arrest patients. In this study, patients with higher concentrations of cytokines like interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) also had worse critical status following resuscitation and overall a less favorable prognoses. Because these cytokines are known to regulate neutrophil trafficking and activation, they may induce neutrophil sequestration during resuscitation. However, to our knowledge no study has been conducted to determine if neutrophils accumulate in key organs such as the brain, heart, and lung during the first few hours after successful resuscitation from cardiac arrest.

For many years, scientists have known that neutrophils sequester in the myocardium during reperfusion following cardiac arrest and resuscitation, likely contributing to an inflammatory process. Originally, neutrophils were believed to serve only a beneficial, protective function, but studies in animal models indicate that neutrophils may also actually cause injury to the myocardium. In fact, reducing the number of neutrophils or inhibiting their function has been shown to exert a cardioprotective effect during reperfusion, indicating that they cause deleterious effects. Although the underlying mechanisms are unknown, we hypothesize that neutrophils may contribute to PRI by compromising (plugging) the microvasculature and by releasing reactive oxygen species.

Neutrophils are an essential part of the body’s immune system. They are the most abundant white blood cells in the body and are the first cells to respond to inflammatory
stimuli. Neutrophils are drawn to sites of inflammation by chemotaxis from cytokines expressed by the endothelium and other cells like macrophages. Once neutrophils are drawn to the site of reperfusion, they become activated to produce cytokines and express adhesion proteins, causing adhesion to the endothelium. As neutrophils accumulate and become more viscous, they cannot pass through microvessels and plug the capillaries and venules. Thus, perfusion to the tissues may decrease, and ultimately stop if the vessels become completely occluded. Injury to the tissues may occur upon exposure to proteolytic enzymes and oxidation products secreted by the activated neutrophils. These enzymes may then break down structural matrix proteins and contribute to increased microvascular permeability.

Neutrophils also contribute to host defense by trapping invading microorganisms and releasing cytotoxic elements. Myeloperoxidase (MPO), an enzyme specific to neutrophils, catalyses the formation of hypochlorous acid (HOCl) from chloride and hydrogen peroxide. HOCl is the prime mediator in oxidative killing. Extended exposure to these cytotoxic elements may be detrimental to the host, and thus neutrophils may also be linked to autoimmunity. Activated neutrophils also produce oxygen radicals, which can be defined as any molecule having unpaired electrons. Neutrophils contain the membrane-bound enzyme complex, NADPH oxidase, which is a major generator of reactive oxygen species during reperfusion. When the enzyme is activated it produces the superoxide anion (O₂⁻), a precursor to reactive oxygen species. In normal tissue, O₂⁻ is produced at low levels, converted to the less cytotoxic hydrogen peroxide (H₂O₂), and then converted to water by the catalase or glutathione peroxidase systems. During reperfusion, however, O₂⁻ production is markedly increased and is more likely to be converted to the hydroxyl radical (OH⁻). In
addition, nitric oxide production is greatly increased during ischemia and reacts with $\text{O}_2^-$ to produce $\text{ONOO}^-$, which through protonation forms peroxynitrous acid (ONOOH).\textsuperscript{15} ONOOH then rapidly degrades to nitrogen dioxide and $\text{OH}^-$\textsuperscript{15} These radical molecules are very reactive and cause cellular injury through interactions with lipids, proteins, and nucleic acids.\textsuperscript{16}
METHODOLOGY

Using an established laboratory model of Arrest/Resuscitation (A/R)\textsuperscript{17, 18}, adult Sprague Dawley rats were anesthetized and subjected to a cardiac arrest and resuscitation protocol. Sham experiments in which the animals were anesthetized, intubated, and catheterized, but the hearts were not arrested, were conducted as a control. Tissue samples of heart, lung, liver, and brain were obtained at the end of the two hour resuscitation period. Non-surgery tissue samples were also obtained as a further control. All tissue samples were homogenized and analyzed with the Rat Myeloperoxidase (MPO) ELISA Kit (Hycult Biotech).\textsuperscript{19}

\textit{Arrest/Resuscitation (A/R) Protocol}

Sprague Dawley Rats were weighed and anesthetized with an injection of Nebutal (50mg/kg). The animals were intubated with a PE-200 catheter and placed onto a ventilator (65 strokes/minute at 2.5CC/stroke for a 500 gram animal). A rectal thermometer (Yellow Springs) was inserted to monitor body temperature throughout the experiment. Surgery was performed to insert a PE-50 femoral arterial catheter to obtain blood samples, administer drugs, and monitor blood pressure throughout the study. Surgery was then performed to insert a PE-190 jugular catheter into the jugular artery and advance it into the right ventricle. A J-wire was threaded through the jugular catheter until it contacted the base of the right ventricle. Once the animal was stabilized, “PRE” measurements were obtained for arterial blood pressure, heart rate, body temperature, and arterial blood gases. Cardiac arrest was induced by electrical stimulation for a
continuous period of 3 minutes. The respirator was stopped as soon as arrest was established. Stimulation was removed and the animal continued to arrest for another minute. After 4 minutes of cardiac arrest, the ventilator was turned back on and an injection of epinephrine (20μg/kg) was administered through the femoral catheter. CPR was immediately initiated at a rate of 200 compressions/minute and continued for a period of 2 minutes. After 2 minutes of CPR, animals were defibrillated with using the CodeMaster XL+ machine (5J for animals weighing less than 500 grams, 7J for animals heavier than 500 grams). If return of spontaneous circulation (ROSC) was not achieved, another dose of epinephrine was administered, CPR and defibrillation repeated, and more ephinephrine administered. Three to 5 attempts were made to resuscitate each animal. When the mean arterial blood reached approximately 50 mmHg, ROSC was achieved. Upon ROSC, 1.5 cc of 8.5% sodium bicarbonate was administered to the animal slowly (over a period of about 2 minutes). Blood samples were obtained at the 15, 30, 60, and 120 minutes following successful ROSC. After 120 minutes, heart, lung, liver, and brain tissues were harvested, frozen in liquid nitrogen, and stored at -80°C until used for MPO determination.

*Sham Protocol*

Sprague Dawley Rats were weighed and anesthetized with an injection of nebutal (50mg/kg) and by chambering in isofluorane gas. The animals were intubated with a PE-200 tube and placed onto a ventilator (65 strokes/minute at 2.5CC/stroke for a 500 gram animal). A rectal thermometer was inserted to monitor body temperature throughout the experiment. Surgery was performed to insert a PE-50 femoral arterial catheter to obtain blood samples, administer medication, and monitor blood pressure throughout the study.
Surgery was then performed to insert a PE-190 jugular catheter into the jugular artery and advance it into the right ventricle. A J-wire was threaded through the jugular catheter until it contacted the base of the right ventricle. “PRE” measurements were obtained for blood pressure, mean arterial pressure (MAP), heart rate, and temperature. Blood samples were obtained at the 15, 30, 60, and 120 minute marks following successful ROSC. Heart, lung, liver, and brain tissue was harvested, frozen in liquid nitrogen, and stored at -80°C until used for MPO determination.

Non-Surgery Protocol

Sprague Dawley Rats were weighed and anesthetized with an injection of nebutal (50mg/kg) and by chambering in isofluorane gas. The rats were cervically dislocated and blood plasma was harvested. Heart, lung, liver, and brain tissues were also removed. Tissue samples were frozen immediately in liquid nitrogen and stored at -80°C until used for MPO determination.

Tissue Preparation

Tissue samples were prepared for analysis according to the Hycult Biotech ELISA kit product manual. About 50 mg of each tissue was weighed into a conical tube. Each tube was labeled with the type of tissue and the date of the experiment. Lysis buffer was added 200 μL per 10 mg of sample. Prepared samples were stored on ice. Each sample was homogenized for 2 minutes using the Polytron Model (level 5) and returned to ice. The machine was cleaned between each sample using PBS and ethanol. The Beckman Coulter Allegra 6R Centrifuge was used to centrifuge each sample (1500xg at 4°C for 15 min) in
order to avoid contamination with cellular debris. Each sample was then pipetted into a color-coded tube marked with the type of tissue and the date of the experiment. The samples were centrifuged again using the Eppendorf Mini Spin. Each sample was then pipetted into a color-coded tube marked with the type of tissue and the date of the experiment. The samples were bagged, frozen in liquid nitrogen, and stored at -80°C until use.

**ELISA Protocol**

The samples were prepped and analyzed according to the Hycult Biotech ELISA kit product manual. Standards were prepared and transferred 100 μL in duplicate into appropriate plate wells. Samples were diluted appropriately based on intervention and tissue type and transferred 100 μL in duplicate into appropriate plate wells. A sandwich type ELISA was performed and the plate was analyzed using a SpectraMax M2 plate reader (Molecular Devices). The results were recorded and analyzed using SoftMax Pro software (Version 5.0).

All data, including notes and printouts from each experiment, was kept in a laboratory notebook. Blood glucose and MAP data from the experiments, as well as the results from the ELISAs were then uploaded into a Microsoft Excel Spreadsheet on the laboratory computer. Statistical analysis was performed using Prism 6 software (GraphPad). Analysis of variance (ANOVA) was performed on all tissues. All results are presented as Mean±SEM, and P<0.05 is considered statistically significant.
RESULTS

A total of 20 animals are utilized in this study. Of those animals, 4 were non-surgery models, 5 were sham models, and 11 were resuscitation (A/R) models. Figure 1 summarizes the MPO findings for all of the groups and all of the tissues. The greatest MPO concentration was found in the lung, regardless of intervention. The next greatest MPO accumulation was found in the liver, followed by the heart, and then the brain. There appeared to be a consistent increase in MPO accumulation between the non-surgery tissue and sham for most tissue types. In addition, the heart, lung, and liver tissues appear to demonstrate a marked increase in neutrophil accumulation in the A/R group compared to the non-surgery control group, indicating organ-specific trends of neutrophil sequestration following cardiac arrest and resuscitation.

Referring to Figure 2, in cardiac tissue, a significant difference was found between non-surgery vs. resuscitation groups. Although the ANOVA was significant, Post Hoc testing did not indicate significant differences between non-surgery and sham or sham and resuscitation groups. *However, using a t-Test (P<0.05) significance was found between sham and resuscitation groups.

Referring to Figure 3, in pulmonary tissue, a significant difference was found between non-surgery and resuscitation groups. Significant differences were not found between non-surgery and sham or sham and resuscitation groups.

Referring to Figure 4, in hepatic tissue, a significant difference was found between non-surgery and resuscitation groups. Although the ANOVA was significant, Post Hoc testing did not indicate significant differences between non-surgery and sham or sham and
resuscitation groups. *However, using a t-Test (P<0.05), significance was found between sham and resuscitation groups.

Referring to Figure 5, in cerebral tissue, significant differences were not found among any of the tissues, non-surgery and sham, non-surgery and resuscitation, or sham and resuscitation groups.

Referring to Table 1, the initial blood glucose levels for the sham and experimental groups were quite similar at about 93 mg/dL. The blood glucose levels of the sham group increased slightly, but not significantly throughout the two hour period. In contrast, a sharp increase in blood glucose was noted for the experimental group at the 15 minute time point following arrest and resuscitation. The blood glucose of the experimental group, while remaining very high, appeared to descend to normal throughout the rest of the two hour period. The arterial blood pressure measurements are summarized in Table 2. The initial MAP of both sham and resuscitation groups was approximately 130 mmHg. The anesthetic and artificial respiration appear to cause a modest hypertensive effect. Although decreasing towards the end of the experiment, the MAP of the sham group remained relatively steady throughout two hour period. In contrast, the MAP of the resuscitation group showed a sharp decline at 15 minutes following arrest and resuscitation. The MAP then slowly rose at the 30 minute mark and then decreased again towards the end of the experiment.
The purpose of this study was to utilize a small animal model of cardiac arrest and resuscitation to investigate if neutrophils accumulate in tissues in the early hours following successful resuscitation. MPO was used as a marker of neutrophil sequestration in the organs evaluated. MPO accumulation appears to increase in sham tissues as compared to non-surgery, indicating that a modest inflammatory response may be occurring due to surgery and anesthesia alone. Furthermore, we found a marked increase in MPO accumulation between almost all non-surgery and resuscitation tissues, which suggests that a neutrophil-mediated inflammatory response is indeed occurring in the body following cardiac arrest and resuscitation. The differences in the amount of neutrophil sequestration among the tissue types, indicates an organ-specific trend of neutrophil accumulation during the early hours following successful resuscitation. Furthermore, it was surprising to find incredibly high numbers (upwards of 2.5 million ng/g in some cases) for MPO accumulation in pulmonary tissues. This marked sequestration of MPO in the lungs, even in the non-surgery model, may be due to the innate immunity of the organ. Respiration exposes the lungs to a variety of airborne pathogens, so an efficient host defense system is necessary to maintain proper health. This defense system includes a reserve of MPO-containing phagocytes like macrophages and neutrophils, which would give the lungs the ability to mount a quick immune response against the constant flux of invading pathogens. Furthermore, the significant difference between non-surgery vs. resuscitation groups in pulmonary tissues indicates that there may be some kind of acute inflammatory response occurring in the lungs during cardiac arrest and resuscitation. This
response may be largely due to surgery, however, as a significant difference was not found between sham vs. resuscitation models. At this point, it is difficult to elucidate the importance of neutrophils in contributing to the pulmonary dysfunction associated with PRI and in mediating an inflammatory response in the lungs during cardiac arrest and resuscitation.

We were also somewhat surprised to find no significant differences in MPO accumulation between any of the models for cerebral tissue. These findings suggest that neutrophils do not play a significant role in the acute inflammatory response in the brain following cardiac arrest and resuscitation. A significant difference in MPO accumulation was found between sham vs. resuscitation models in both hepatic tissue and cardiac tissue (t-Test, P<0.05). This finding could indicate that neutrophils may play a significant role in PRI and contribute to hepatic and cardiac dysfunction following cardiac arrest and resuscitation.

Because glucose is released under stressful situations, blood glucose levels can serve as an indicator of the stress response that is occurring during cardiac arrest and resuscitation. The relatively steady glucose levels and small increase toward the end of the experiment in the sham group indicate that a low-level stress response may be occurring after a few hours of deep anesthesia and artificial respiration. Increased blood glucose throughout the experiment in the resuscitation group likely indicates that a much larger stress response occurred. The MAP is a measure of the systemic perfusion pressure and a good indicator of overall cardiovascular function. A decline in the MAP will cause a reduction of blood reaching the organs, which can result in inadequate oxygen and nutrients being delivered to the tissues. Because the MAP of the resuscitation group rose
from about 80 mmHg 15 minutes after resuscitation to values greater than 90 mmHg, the animals are believed to have achieved decent recovery. These MAP values and overall animal recovery are comparable to the Arrest/Resuscitation study of Studer, et al.\textsuperscript{18}

While a few studies of hemorrhage and resuscitation exist to characterize neutrophil sequestration and Post-Resuscitation Injury in the heart, there is not a substantial body of work characterizing the effects of neutrophil accumulation in other organs of the body. In a similar study performed by Zakaria et al. to evaluate neutrophil infiltration in a model of hemorrhagic shock and resuscitation, an increase in tissue MPO was found to occur in an organ-specific and time-dependent manner.\textsuperscript{21} Specifically, MPO sequestration was much higher in the lung than in the liver in both sham and experimental groups.\textsuperscript{21} Our study supports these findings and further characterizes this distinction by evaluating basal levels of neutrophil accumulation with a non-surgery model, showing approximately a 10-fold difference in neutrophil accumulation between lung (~500,000 ng/g) and liver (~50,000 ng/g) tissues, as measured with tissue MPO. Another study by Schott et al. attempted to improve the neurologic outcome in dogs after cardiac arrest and resuscitation by using and immune antiserum for neutrophil-depletion.\textsuperscript{22} Their study failed to improve neurologic outcomes and prolong survival after successful resuscitation, and it was concluded that neutrophils might not contribute to central nervous system dysfunction.\textsuperscript{22} Our study provides support and explanation for these findings in that no significant increase in neutrophil accumulation was found to occur in either our sham or experimental groups after cardiac arrest and resuscitation.

Our study appears to be one of the first to compare neutrophil accumulation across multiple organs and provide evidence that a neutrophil-mediated inflammatory response is
occurring in the liver, as well as the heart, in the early hours following successful resuscitation from cardiac arrest. In the future, we hope to characterize cytokine levels in the blood plasma of animals that have undergone cardiac arrest and resuscitation in order to provide a more complete picture of whether neutrophil accumulation and activity play a role in acute inflammatory responses that may contribute to PRI. Further studies aimed at limiting neutrophil accumulation would also be useful to determine the relative importance of neutrophils in the overall poor recovery observed in the first two days after successful resuscitation. These laboratory studies could pave the way for future clinical studies aimed to limit neutrophil-mediated oxidative injury and lead to changes in the management of patients following successful resuscitation.
Figure 1: MPO accumulation in heart, lung, liver, and brain tissues for non-surgery, sham, and resuscitation (A/R) animals.
Figure 2: MPO accumulation in cardiac tissue for non-surgery, sham, and resuscitation (A/R) models. *Significance between sham and resuscitation (A/R) models was found.

Figure 3: MPO accumulation in pulmonary tissue for non-surgery, sham, and resuscitation (A/R) models.
Figure 4: MPO accumulation in hepatic tissue for non-surgery, sham, and resuscitation (A/R) models.

Figure 5: MPO accumulation in pulmonary tissue for non-surgery, sham, and resuscitation (A/R) models.
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<th>Blood Glucose (mg/dL)</th>
<th>PRE</th>
<th>R15</th>
<th>R30</th>
<th>R60</th>
<th>R120</th>
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<td>Sham</td>
<td>92.6 ± 7.02</td>
<td>94.8 ± 10.38</td>
<td>97.0 ± 9.77</td>
<td>106.0 ± 6.12</td>
<td>107.8 ± 12.8</td>
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<td>Resuscitation</td>
<td>94.3 ± 6.58</td>
<td>239.4 ± 14.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>229.0 ± 16.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>226.2 ± 25.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>193.6 ± 24.15&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> ≤ 0.05, <sup>b</sup> ≤ 0.01, <sup>c</sup> ≤ 0.001 vs. Sham

Table 1: Blood glucose levels for sham and resuscitation experiments for time points previous (PRE) to arrest and at time points 15 minutes, 30 minutes, 60 minutes, and 120 minutes following resuscitation.

<table>
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<th>Mean Arterial Pressure (mmHg)</th>
<th>PRE</th>
<th>R15</th>
<th>R30</th>
<th>R60</th>
<th>R120</th>
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<tr>
<td>Sham</td>
<td>128.4 ± 7.42</td>
<td>128.8 ± 2.76</td>
<td>120.5 ± 5.45</td>
<td>124.8 ± 4.54</td>
<td>107.2 ± 15.52</td>
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<tr>
<td>Resuscitation</td>
<td>130.1 ± 5.44</td>
<td>80.1 ± 6.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.5 ± 7.67</td>
<td>92.8 ± 4.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.5 ± 9.78</td>
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<sup>a</sup> ≤ 0.05, <sup>b</sup> ≤ 0.01, <sup>c</sup> ≤ 0.001 vs. Sham

Table 2: Mean arterial blood pressure (MAP) for sham and resuscitation experiments for time points previous (PRE) to arrest and at time points 15 minutes, 30 minutes, 60 minutes, and 120 minutes following resuscitation.
REFERENCES


19. HK105 RAT MPO ELISA KIT PRODUCT INFORMATION & MANUAL.

