EFFECTS OF RELAXIN ON CARDIAC PERFORMANCE AND CORONARY ARTERY

REACTIVITY IN AGED SPONTANEOUSLY HYPERTENSIVE FEMALE RATS

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EFFECTS OF RELAXIN ON CARDIAC PERFORMANCE AND CORONARY ARTERY

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Thesis

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ABSTRACT

Women have decreased risk of cardiovascular disease compared to men until after menopause when the risk is similar between men and women. Hypertension is prevalent in one out of three adults in the United States. Postmenopausal women have a higher risk of hypertension compared to men of similar age and premenopausal women. Relaxin (RLX) is a vasodilatory hormone that has shown to have cardioprotective effects. To investigate this effect, contractility of the heart, relaxation of the heart, coronary flow, and isolated septal coronary arteries responses to relaxin (RLX) were assessed. I tested the hypotheses that relaxin administration to aged (18 months) spontaneously hypertensive female rats (SHR), improves coronary vascular function and improves cardiac performance. An osmotic pump containing recombinant human relaxin (rhRLX) was placed subcutaneously above the aged female SHR's right shoulder for 5-7 days. Data collected was compared to aged SHR vehicle (VEH) group (control). Left ventricular contractility, left ventricular relaxation, and coronary flow were examined using the Langendorff apparatus. Septal coronary arteries were assessed using a pressure arteriograph. Active and passive diameters of the septal coronary artery were examined over a range of pressures ranging from 10-100 mmHg and percent tone was calculated. Relaxin treated SHR's showed a decreased contractility, relaxation,

coronary flow, and percent tone of septal coronary arteries compared to old SHR

vehicles. In conclusion, relaxin improves cardiac behavior and coronary vascular

function in aged spontaneously hypertensive rats. Decreased contractility,

relaxation, and coronary flow may be beneficial for aged hypertensive hearts

because the heart can work more efficiently. Decreased myogenic response of the

coronary arteries may result in a vasodilatory coronary circulation.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

SHR- spontaneously hypertensive rat

SV- stroke volume

EDV- end diastolic volume

ESV- end systolic volume

CO- cardiac output

HR-heart rate

SVR- systemic vascular resistance

SA- sinoatrial

RLX- relaxin

rhRLX- recombinant human relaxin

VEH- vehicle

ERPF- effective renal plasma flow

EDRF- endothelium derived relaxing factor

GFR- glomular filtration rate

NO- nitric oxide

eNOS- endothelial nitric oxide synthase

MMP-2- matric metalloproteinase 2

cGMP- cyclic guanosine monophosphate

HEPES– hydroxyethyl piperazineethanesulfonic acid

HEPES-PSS – HEPES physiological saline solution

ANOVA- analysis of variance

ACE- angiotensin converting enzyme

CHAPTER I

INTRODUCTION

Coronary artery disease is the leading cause of death in the United States in men and women (3). Coronary disease is caused when the blood vessels to the heart muscle harden and narrow. Atherosclerosis results when plaque builds up in the vessels, which reduces the blood flow to the heart muscle (28). Over time coronary disease will cause the heart muscle to weaken, which will lead to heart failure. In postmenopausal women, the risk of coronary disease is higher compared to premenopausal women.

Women have decreased risk of cardiovascular disease compared to men until after menopause when the risk is similar between men and women. Hypertension is prevalent in one out of three adults in the United States (41). Up until the age of 45, men are more hypertensive than women of the same age. From age 45 to 64, men and women have similar statistics for hypertension, however after 64, there are more women with hypertension than men. There is strong evidence to support that this increase of hypertension in females is due to being postmenopausal, and not having the same levels of hormones in circulation (9).

Relaxin is a 6 KD hormone produced by the corpus luteum in rats as well as humans. It is a protein hormone, which is in high circulation during pregnancy. It also circulates during the luteal phase of the menstrual cycle (34). Relaxin has been investigated as a potential hormone replacement. Recent human studies have shown that there were no side effects or adverse reactions of relaxin given as an intravenous infusion and data suggests it has vasodilatory factors in human pregnancy (40). In spontaneously hypertensive rats, relaxin increased blood flow and decreased systemic vascular resistance (22, 24). Finally Bani-Sacchi and coworkers demonstrated that relaxin is a potent coronary vasodilator in humans (all reviewed in (8)).

The aim of this study was to evaluate the therapeutic potential of relaxin on the coronary arteries in postmenopausal females as well as the contribution of relaxin as a potential hormone replacement therapy on a postmenopausal animal model (aged female spontaneously hypertensive rats) which mimics the human condition. I tested the hypotheses that relaxin administration to aged (18 months) spontaneously hypertensive female rats (SHR) improves coronary vascular function and improves cardiac performance.

CHAPTER II

BACKGROUND OF THE STUDY

Heart Anatomy and Physiology

The mammalian heart has four chambers, a right and left atria as well as a

right and left ventricle. Blood is carried away from the heart through arteries and

back to the heart using veins. The human heart pumps blood carrying oxygen, to all

the organs and muscles throughout the body. Oxygen is essential for everything in

the human body to function. The right atrium fills with deoxygenated blood from

the body. When the tricuspid valve opens, the blood moves from the high pressure

of the right atrium to the low pressure of the right ventricle. The right ventricle then

contracts which ejects the deoxygenated blood through the pulmonary artery to the

lungs to get oxygen and is then returned to the left atrium using the pulmonary vein.

The blood then moves from the left atrium through the mitral valve and into the

muscular left ventricle. When the left ventricle contracts, blood is ejected from the

heart through the aorta to the tissues of the body.

A normal heart functions on a cardiac cycle. The cardiac cycle consists of the

systole and diastole. Systole is the contraction of the atria and ventricles. Diastole is

the relaxation of the atria and ventricles. The pressures and volumes within the

heart change during the cardiac cycle. A healthy, normal human heart beats at an

average rate of 75 beats per minute and each cardiac cycle requires 0.8 seconds
(46). When a ventricle is contracting, an atrium is relaxing, which forces the blood
from high pressures to low pressures. The amount of blood being ejected by each
contraction of the ventricle is called stroke volume. Stroke volume (SV) can be
calculated by taking the end diastolic volume (EDV) minus the end systolic volume
(ESV) [SV=EDV-ESV]. The EDV is the "volume in the ventricle at the end of diastole,"
which is usually around 130 ml in a normal heart (46). The ESV is the "volume in the
ventricle at the end of systole," this is about 60 ml of blood in a normal heart. Stroke
volume is also important for cardiac output.

The body's need for oxygen varies with the level of activity being performed.
The heart's ability to pump out oxygen-carrying blood must also be variable. Body
cells need specific amounts of blood with oxygen each minute to maintain function
and health. The way the body compensates for the different amounts of blood is
through the cardiac output. Cardiac output (CO) is the volume of blood ejected from
the left ventricle (or right ventricle) into the aorta (or pulmonary trunk if coming
from the right ventricle) each minute. Cardiac output equals the stroke volume
multiplied by the heart rate (HR). The heart rate is the number of beats per minute.
Heart rate is controlled by the sympathetic and parasympathetic nerve activity at
the sinoatrial (SA) node. Cardiac output can be measured by a technique called the
Langendorff isolated heart procedure.

Langendorff Background

Oscar Langendorff was the pioneer of the first isolated perfused heart (25).

The isolated heart experiment (or Langendorff technique/procedure) has helped to

understand the physiology of the heart. The Langendorff procedure can examine the

contractile function of the heart, coronary blood flow regulation, cardiac

metabolism, heart rate, electrical activity of the cardiac cycle, and the coronary

resistance (25, 26). This is an *in vitro* experiment, where the heart is removed from

the body and isolated, that way neural or hormonal variables will not occur unlike

when *in vivo* (27). An electrical current is placed on the apex of the heart to maintain

a constant, controlled HR. The sinoatrial node in the heart is normally "crushed," in

order to allow for the bipolar electrical stimulus to maintain a constant HR. The

ascending aorta of the heart is placed onto a needle (cannula) and a perfusate

(blood, Krebs Heinsleit solution, or other physiological buffered solution) is

perfused through the cannula to the heart at a constant pressure (or constant flow),

in a retrograde fashion. This is done because the pressure causes the aortic valve to

close, forcing the perfusate into coronary circulation (26). Like most experiments,

there are plenty of different ways to perform this technique, however this is the

standard means of setting up the Langendorff procedure.

Hypertension and Health Problems

A hypertensive blood pressure is defined as 140 mmHg systolic / 90 mmHg

diastolic or higher (1). The numerator is systolic blood pressure, which is when the

heart is contracting. The denominator is diastolic blood pressure, which is when the

heart is relaxing (not contracting) and filling with blood. In the United States, hypertension is prevalent in one out of three (77.9 million) adults (41). Hypertension is a major risk factor associated with numerous cardiovascular problems such as heart failure, heart attack, stroke, and blood clots (2). Up until the age of 45, men are more hypertensive than women of the same the age. From age 45 to 64, men and women have similar statistics for hypertension, however after age 64, there are more women with hypertension than men. There is strong evidence to show that this increase in hypertension in females is due to the females being postmenopausal (9). Postmenopausal women have lower hormone levels because the ovaries are no longer producing estrogen, as well as other reproductive hormones. Hypertension is also a major contributor to coronary artery disease.

Coronary artery disease is the leading cause of death in the United States in men and women (3). Coronary disease is the most common of all heart disease and caused when the blood vessels to the heart muscle harden and narrow. Atherosclerosis is commonly associated with coronary artery disease. This is when these vessels harden and narrow and allow for plaques to build up in the vessels, which reduces the blood flow to the cardiac muscle. Over time, coronary disease will alter coronary function due to weakening of cardiac muscle, eventually leading to heart failure and arrhythmias. In postmenopausal women, the risk of coronary disease is higher compared to premenopausal women. Hypertension increases the risk of coronary artery disease by increasing oxygen (O_2) demands, vascular injury, over working sympathetic activity, release of vasoactive substances that accelerate the atherosclerosis (4) and endothelial dysfunction (5,6).

In females, the reproductive hormone estrogen is produced by the ovaries and has historically been thought to confer cardio-protection in premenopausal females (2). Its use as a hormone replacement for postmenopausal women remains inconclusive. Estrogen treatment in post-menopausal women increases blood clot formation, as well as increases the risk of cancer, stroke, and heart attack (2). Estrogen is not the only hormone that is no longer being produced after menopause and much work has been done to investigate other hormones that may provide cardio-protection and can be used as a hormone replacement therapy for cardiovascular disease. Relaxin is another hormone that is in circulation, but is not produced after menopause.

Relaxin and Effects

Relaxin is a 6 KD peptide hormone produced by the corpus luteum of the ovaries in rats, as well as humans. It is a protein hormone, which is in high circulation during pregnancy. It also circulates, in smaller concentrations, during the luteal phase of the menstrual cycle. During pregnancy, relaxin causes elongation of the interpubic ligament, softening of the cervix, and relaxation of the uterine smooth muscles (18). In males, relaxin is in circulation as well. It is found in the seminal plasma and has been found to stimulate sperm motility as well as increase sperm penetration into oocytes (16). The relaxin found in the seminal plasma of males, is produced in the prostate (20). Relaxin causes vasodilation in the kidneys as well as in the heart (22,24). Recently, research has shown that during heart failure, relaxin

has higher concentrations in circulation (23), suggesting that relaxin is produced as a vasodilatory response to compensate for the heart failure (23).

Relaxin causes renal vasodilation as well as increases the effective renal plasma flow (ERPF) and the glomular filtration rate (GFR) (22). This concept of relaxin increasing the ERPF and GFR allows for a reduction in blood pressure and vasoconstriction, which causes the kidney to be healthier and perform more effectively. In spontaneously hypertensive rats (SHR), relaxin increases coronary blood flow and reduces platelet aggregation (22). Administering relaxin to hypertensive rats increases CO, decreases systemic vascular resistance (SVR), with no change in the mean arterial pressure (24). In aged hypertensive rats, relaxin can reverse the arterial remodeling and stiffening of arteries (28). Reversing stiffening of arteries allows for these vessels to respond more effectively to changes in pressures. Relaxin has been a potential hormone replacement to treat cardiovascular dysfunction (13). Recent human studies have shown that there were no side effects or adverse reactions of relaxin given as an intravenous infusion and data suggests it has vasodilatory factors in human pregnancy (7). Finally Bani-Sacchi and coworkers demonstrated that relaxin is a potent coronary vasodilator in humans (all reviewed in (8)). The mechanism of how relaxin works remains unclear. To the best of my knowledge, there have been no detrimental effects from relaxin on human studies.

Endothelial Nitric Oxide

Although the mechanism of how relaxin works is unclear, the pathway of the vasodilatory responses of relaxin is known. Conrad's study examined the vasodilatory responses of relaxin and identified the relaxin receptor, RXFP1 (relaxin family peptide 1) (39). Relaxin has an effect on the endothelial layer of the vessel as well as an effect on nitric oxide (NO) production. Relaxin increases the gelatinase activity of matrix metalloproteinase-2 (MMP-2). The increase in MMP-2 causes activation of the endothelial receptors 1-32 and specifically activation of ET_B (10). The activation of ET_B causes activation of endothelial Nitric Oxide Synthase (eNOS). The activation of eNOS causes an increase of nitric oxide production, which causes the vasodilation of a vessel, as well as having increases in renal blood flow, glomular filtration rate, and inhibition of myogenic reactivity (39). Conrad's study developed a pathway similar to Figure 1 to describe the scheme of cellular mechanisms underlying pregnancy and relaxin-induced renal vasodilation (39).
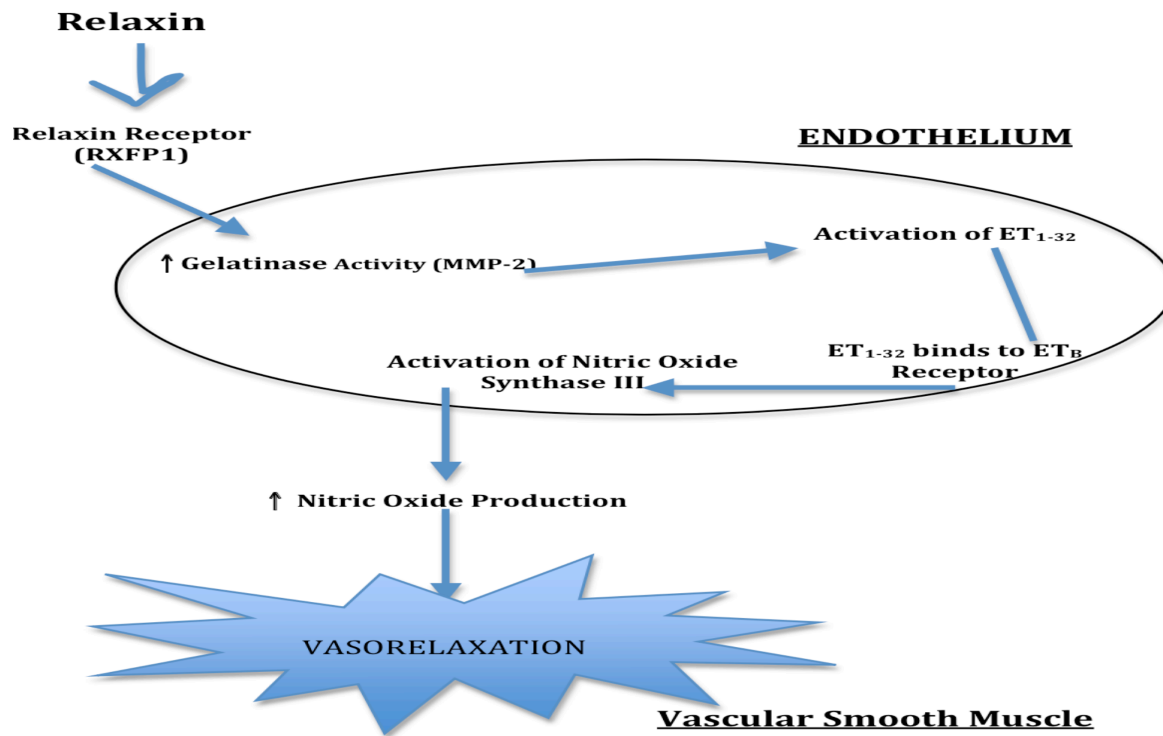
Figure 1: Nitric Oxide Pathway. A pathway similar to Figure 1 was first published by Conrad *et al.* to explain the underlying cellular mechanism when relaxin is introduced during pregnancy (39). The main ideas in this pathway are when relaxin is introduced to the body and vessels, eNOS is released by the endothelium, which increases NO production, and results in vasorelaxation of the vessel.

L-arginine is released from the endothelium when combined with oxygen, it activates the endothelial nitric oxide synthase (eNOS) receptor, causing the release of eNOS (33). When eNOS is released, it causes nitric oxide formation. When NO is combined with L-citrulline, it causes activation of soluble guanylate cyclase in

vascular smooth muscle. Guanylate cyclase then causes the activation of the second

messenger cyclic GMP (cGMP). cGMP is what causes smooth muscle to relax,

resulting in the vasodilation of the blood vessel. cGMP and L-arginine are

responsible for the inhibition of platelet aggregation and adhesion to the endothelial

cells of a vessel (33).

Dr. Murad, and two other doctors, won the Nobel Prize for Physiology and

Medicine in 1998 for this discovery about NO. Dr. Ferid Murad discovered the role of

nitric oxide (NO) in 1978 when he was examining how nitroglycerin works on blood

vessels. Dr. Murad found out that nitrates cause the release of NO, relaxes smooth

muscle cells, resulting in vasodilation (29). The vascular endothelium mediates a

blood vessels ability to change in responses to pressure. NO is a major endothelial

mediator that causes smooth muscle to change architecture in responses to changes

in pressures (30). NO is produced by the endothelium in response to mechanical

forces such the flow of blood and pressure. NO is also responsible for inhibiting

platelet adhesion and platelet aggregation, elicited by endothelium-derived relaxing

factor (31, 32). Endothelium-derived relaxing factor (EDRF) is released by both

arteries and veins, and possesses identical biological and chemical properties as NO

(32).

All of this suggests that relaxin is a suitable candidate for use as a hormone

replacement therapy from a vascular standpoint. The aim of this study is to evaluate

the therapeutic potential of relaxin on the coronary arteries in postmenopausal

females as well as the contribution of relaxin as a potential hormone replacement

therapy on a postmenopausal animal model (aged female spontaneously hypertensive rat) which will mimic the human condition.

I plan on studying the effects of relaxin as a potential therapeutic for coronary artery and coronary disease via the following two hypotheses:

Hypothesis 1: Relaxin administration to aged spontaneously hypertensive female rats (SHR) improves coronary vascular function.

Hypothesis 2: Relaxin administration to aged SHR females improves cardiac performance.

CHAPTER III

MATERIALS AND METHODS

Animal Model

Postmenopausal spontaneously hypertensive female rats were used as the model for a number of reasons. This model mimics the physiology of postmenopausal women because of the increasing age, decreased ovarian hormone production and hypertension (9). At 16-18 months, these rats have high blood pressures, low plasma estrogen levels, increased plasma renin activity, and increased markers of oxidative stress (9). The SHR (spontaneously hypertensive rats) females were purchased from Harlan Sprague Dawley (Indianapolis, Indiana). These aged SHR females were housed in the University of Akron Research Vivarium at the University of Akron, Akron, Ohio. This study was done in accordance with IACUC protocol 11-7B. Non-estrous cycling aged (postmenopausal) females were studied at 16-18 months. Cessation of cycling was confirmed via vaginal smears. An Alzet model 2ML1 osmotic pump (Durect Corporation, Curpertino, CA USA) containing rhRLX (recombinant human relaxin), was inserted subcutaneously above the right shoulder under isoflurane anesthesia. The pump delivered 4 μg/hr rhRLX for 5-7 days prior to the experiment. Half of the aged SHR's were treated with RLX and the other half of the aged SHR's were vehicle treated (controls).

Prior to sacrifice, the SHR females were brought down from the vivarium to the surgery room in the lab. Females were weighed. Heparin saline mixture was prepared in a 15 ml falcon tube at 1000 U/ml. Heparin saline was mixed by adding 0.0843g of heparin sodium in 15 ml of 85% sterile saline (0.85 NaCl), or sterile water. The mixture was spun until all heparin sodium dissolved and then was stored at 4°C until needed. Fifteen minutes prior to sacrifice, a 1 ml intraperitoneal injection of heparin saline was administered to prevent intravascular and intracoronary blood clotting.

At sacrifice, aged SHR females were anesthetized with isoflurane (5% Iso in 100% O_2). An incision was made in the abdomen and cut up the linea alba, through the rib cage and sternum. The heart was removed by cutting the ascending aorta and then placed in cold Krebs-Hensleit solution (per liter, 119 mmol of NaCl, 4.7 mmol of KCl, 2.5 mmol of $CaCl_2$, 1.2 mmol of $MgSO_4$, 1.2 mmol of KH_2PO_4, 25 mmol of $NaHCO_3$, 14 mmol of glucose) for isolated heart experiments or cold 1X 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffered physiological saline solution (HEPES-PSS, pH 7.4) for isolated artery experiments.

Langendorff Procedure

The isolated heart experiment is also referred to as the Langendorff heart perfusion procedure. The Langendorff procedure used warm Krebs-Heinsleit solution ($\sim$ 37°C, pH 7.4) as the buffer. The heart had the atria removed, as well as any other connective tissue on the heart and aorta, and was hung by the ascending aorta on a cannula and tied off using suture. Krebs Henseleit solution was

continuously bubbled with (95/5) O_2/CO_2 to prevent precipitation of reagents and maintained at 37°C. On the cannula, the isolated heart was perfused with a constant flow in a retrograde fashion. This resulted in the coronary arteries receiving the entire perfusate via the ostia at the aortic root (11). Perfusion of the buffer solution had an approximate starting rate of 12 ml/minute. Coronary flow was obtained by measuring timed volumes of perfusate. The sinoatrial (SA) node was crushed for the purpose of manually regulating the heart rate. A bipolar electrical stimulus (Grass Instruments, Quincy, MA) was set at 240 Bpm causing the heart to beat at a controlled rate. The heart had a balloon attached to a 1 ml syringe filled with water, which was placed in the left ventricle. The balloon was made by folding a 1 inch square of non-elastic plastic (Saran® Wrap) around Tygon tubing (R3603). The tubing was pulled back slightly and secured using suture, creating a balloon (12). Three different volumes were used to obtain data for the Frank-Starling curve: 0.05 ml, 0.10 ml, and 0.15 ml. Attached to the balloon was a pressure transducer, which was connected to a polygraph data collection system on a computer, which recorded the pressure changes of the heart. This allowed for ventricular systolic and diastolic pressure measurements. The WinDaq (DI-720-Di-7x0) was used to record heart data (DATAQ Instruments, Akron, OH). This data was collected and analyzed for statistical significance. After running the Langendorff experiment, the septal coronary artery was dissected out of the heart. Our lab has removed the septal coronary artery after the Langendorff before and seen no adverse effects to the vessel.

Isolated Septal Artery Experiments

   In the isolated artery experiment, the right ventricle was resected and the septal coronary artery was harvested from the cardiac tissue surrounding the artery under a dissecting microscope. The septal artery segment removed from the right ventricle was then placed in an isobaric pressurized arteriograph (Living Systems, Burlington, VT) in cold HEPES-buffered physiological saline solution (HEPES –PSS) consisting of (mmol/liter): sodium chloride 142, potassium chloride 4.7, magnesium sulfate 1.17, calcium chloride 2.5, potassium phosphate 1.18, HEPES 10, dextrose 5.5, and the pH was adjusted to 7.4. It was then mounted on 2 microcannulae suspended in the chamber. The septal artery was tied with 2 separated suture threads on each microcannula, and placed on an inverted microscope. The myogenic response was examined using an isobaric pressure arteriograph under a no-flow state under constant pressure. Pressure was regulated and measured using a pump and pressure transducer. Following equilibration, the artery's initial diameter was recorded via an electronic filar microscope (Lasico, Los Angeles, CA, USA). The buffer was changed with a warm HEPES-PSS buffer and was equilibrated for 30 minutes, then a conditioning stretch (100 mmHg) was performed. After a 15 minute equilibration period, the buffer was changed with warm HEPES. The artery was exposed to pressure increases starting at 10 mmHg for 10 minutes, then 20 mmHg, 40 mmHg, 60 mmHg, 80 mmHg, and 100 mmHg for 4 minutes each respectively. Vessel diameters were measured with an electronic filar. These data resulted in active diameters at each given pressure step.

After active diameters were measured, the buffer was changed from HEPES to a HEPES passive buffer. Passive buffer was made with 10^{-4} M papaverine and EGTA in calcium-free HEPES-PSS. Passive buffer was put into the chamber, and after a 10 minute equilibration period, the pressure was changed 10 times every 2 minutes. The pressure changes start at 0 mmHg and diameters of the artery were measured at 3 mmHg, 5 mmHg, 10 mmHg, 20 mmHg, 40 mmHg, 60 mmHg, 80 mmHg, 100 mmHg, and 120 mmHg. The diameter was measured from wall to wall of the artery, as well as wall thickness using a Filar microscope. The passive experiment was used to measure the distensibility of the coronary artery. The distensibility was used to characterize the stiffness of the material components that comprise the vascular wall.

By definition, the myogenic response is the intrinsic response of an artery to change in intraluminal pressure (35). In the coronary artery experiments, this phenomenon was examined by looking at myogenic tone generation, which is how much tone is generated from the isolated artery under different pressures. By comparing active and passive diameters I can determine the amount of tone in a given artery via the following equation: Percent tone of vessel diameter = $((D_{Active} - D_{Passive}) / D_{Passive})$ X 100 where D_{Active} represents the active diameter at a certain intraluminal pressure and $D_{Passive}$ represents the passive diameter of a vessel at a certain pressure [10, 20, 40, 60, 80, or 100 mmHg].)

Statistics

For myogenic tone, the data were expressed as percent tone generation in active diameter to the passive diameter at each pressure step. The statistical analyses used, were of mean effective concentrations, as well as threshold and maximal responses, were conducted using repeated measures 2-way analysis of variance (ANOVA) with Tukey's post-hoc test. Significance was determined with a P-value <0.05. Langendorff contractility was evaluated using a repeated measures, 2-way ANOVA. Significance was determined with a P-value <0.05. Langendorff relaxation and coronary flows were analyzed using a one-way ANOVA. Significance was determined with a P-value <0.05. Additionally, a MANCOVA was performed on the coronary flows. Significance was determined with a P-value <0.05.

CHAPTER IV

RESULTS

Contractility was measured in aged SHR with RLX at three different volumes (0.05 ml, 0.10 ml, and 0.15 ml). This was compared to contractility measured at the same volumes in the vehicle treated group. The data of rat weights and heart weights are summarized in Table 1. Figure 2 shows the differences in contractility between the relaxin and vehicle treated groups. Contractility was significantly lower in hearts from RLX treated animals compared to those from the vehicle treated controls at each balloon volumes (p= 0.046 by one way ANOVA). Table 1 displays the averaged weight and heart weight of isolated hearts, from aged, female SHR's treated with and without relaxin.

Left Ventricular Contractility
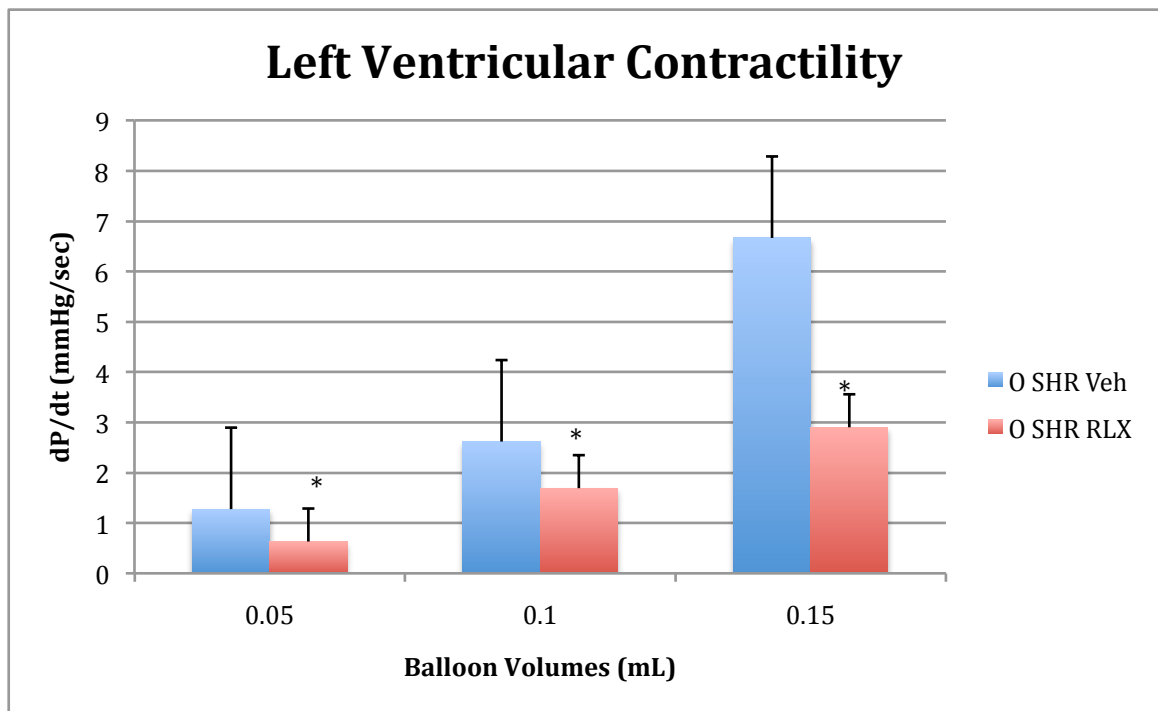
Figure 2: Left Ventricular Contractility. Differences in contractility for aged SHR

vehicle (n= 6) and aged SHR relaxin (n=5) groups. Data are expressed as means ±

S.E.M. between the two groups. A significant difference was found at all three

balloon volumes between relaxin and vehicle treated animals by two-way, repeated

measures ANOVA (p= 0.046).

* Indicates statistical significance.

Relaxation of the left ventricle was also measured in relaxin and vehicle treated aged SHR's. Relaxation was calculated by the change in pressure over the change in time ($-(\mathrm{d}P/\mathrm{d}t)$) at three different balloon volumes (0.05 ml, 0.10 ml, and 0.15 ml). This means relaxation should always be negative; however the magnitude of the change is the important observation. These are summarized in Figure 3. In the relaxin treated group the $\mathrm{d}P/\mathrm{d}t$ was less than the $\mathrm{d}P/\mathrm{d}t$ measured in the vehicle treated animals (Figure 3). Although this failed to reach statistical significance ($p= 0.087$ by one way ANOVA), the trend suggests a decreased ability to relax in the isolated hearts from relaxin treated animals. Table 1 displays the averaged weight and heart weight of the hearts from aged SHR's treated with and without relaxin.
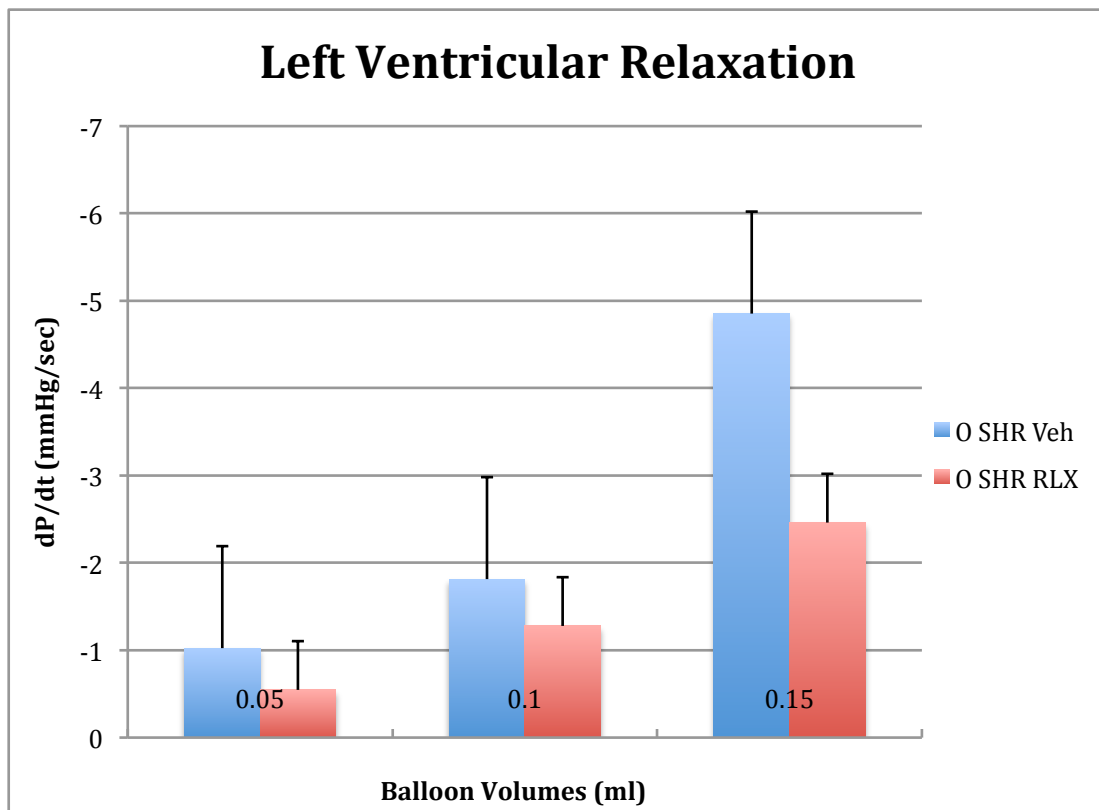
Figure 3: Left Ventricular Relaxation. Differences in cardiac relaxation for aged SHR

vehicle (n= 6) and aged SHR relaxin (n=5) groups. Data are expressed as means ±

S.E.M (p= 0.087).

*Relaxation occurs in the negative, graph flipped for easier viewing.

Coronary flows were measured in vehicle and relaxin treated aed SHR's.

Percent change was calculated by the difference of flow at the volume by initial flow,

over the initial flow multiplied by 100 (((F_{Volume}-$F_{Initial}$)/ $F_{Initial}$) x 100%) at the three

different balloon volumes (0.05 ml, 0.10 ml, and 0.15 ml). These data are

summarized in Figure 4. Figure 4 shows the averaged percent change of coronary

flows recorded from the Langendorff procedure. As the balloon volume increased,

the percent change of coronary flow decreased in both groups. Coronary flow curves

were not statistically different between relaxin and vehicle treated animals. In fact,

hearts treated with relaxin did not demonstrate a significant change between

coronary flow curves compared to the aged SHR vehicle group. However, a

MANCOVA was performed and a difference was found at the balloon volume of 0.10

ml between the slopes of vehicle treated group and relaxin treated group. At the

balloon volume 0.10 ml, another parameter may be affecting cardiac performance.

Table 1 shows the averaged initial coronary flow for both groups.
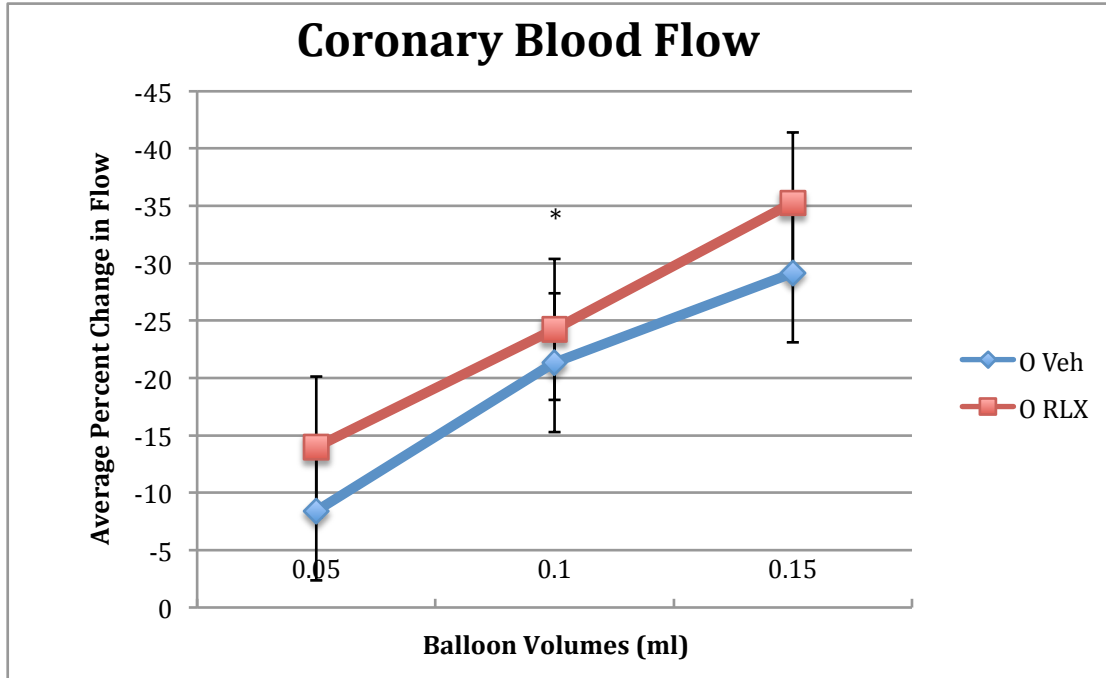
Coronary Blood Flow

Figure 4: Coronary Blood Flow. Average percent change in coronary flow measured in isolated hearts from aged SHR female rats treated with and without relaxin (vehicle). A one-way ANOVA showed no significance between aged hearts treated with relaxin and aged control hearts. A MANCOVA showed a significant difference between the slopes of the hearts treated with relaxin and aged control (vehicle) groups (p=0.036).

* Shows significance between groups.

Note: Percent change in flow occurs in the negative, graph flipped for easier viewing.

Table 1: Weight, Heart Weight, and Initial Coronary Flow Averages.

Rat ID	Weight (g)	Heart Weight (g)	Initial Coronary Flow (mL/min)
Aged SHR Veh (n=6)	236.4	1.481	14.22
Aged SHR RLX (n=5)	244.2	1.405	14.2

Myogenic tone was measured in isolated coronary arteries from relaxin and vehicle treated aged SHR's. In order to evaluate myogenic tone, the inner diameter was measured in isolated septal coronary arteries in active and passive buffer over a range of pressures from 10-100 mmHg. Percent tone was calculated by $(((D_{Active} - D_{Passive}) / D_{Passive}) \times 100)$. As shown in Figure 5, at each pressure step from 10- 100 mmHg, the percent tone was significantly greater in the aged SHR vehicles ($15.02 \pm 0.56\%$) compared to the aged SHR's treated with relaxin ($4.75 \pm 0.35\%$) ($p = 0.006$ by way of 2 way repeated measures ANOVA).

Coronary Artery Myogenic Tone

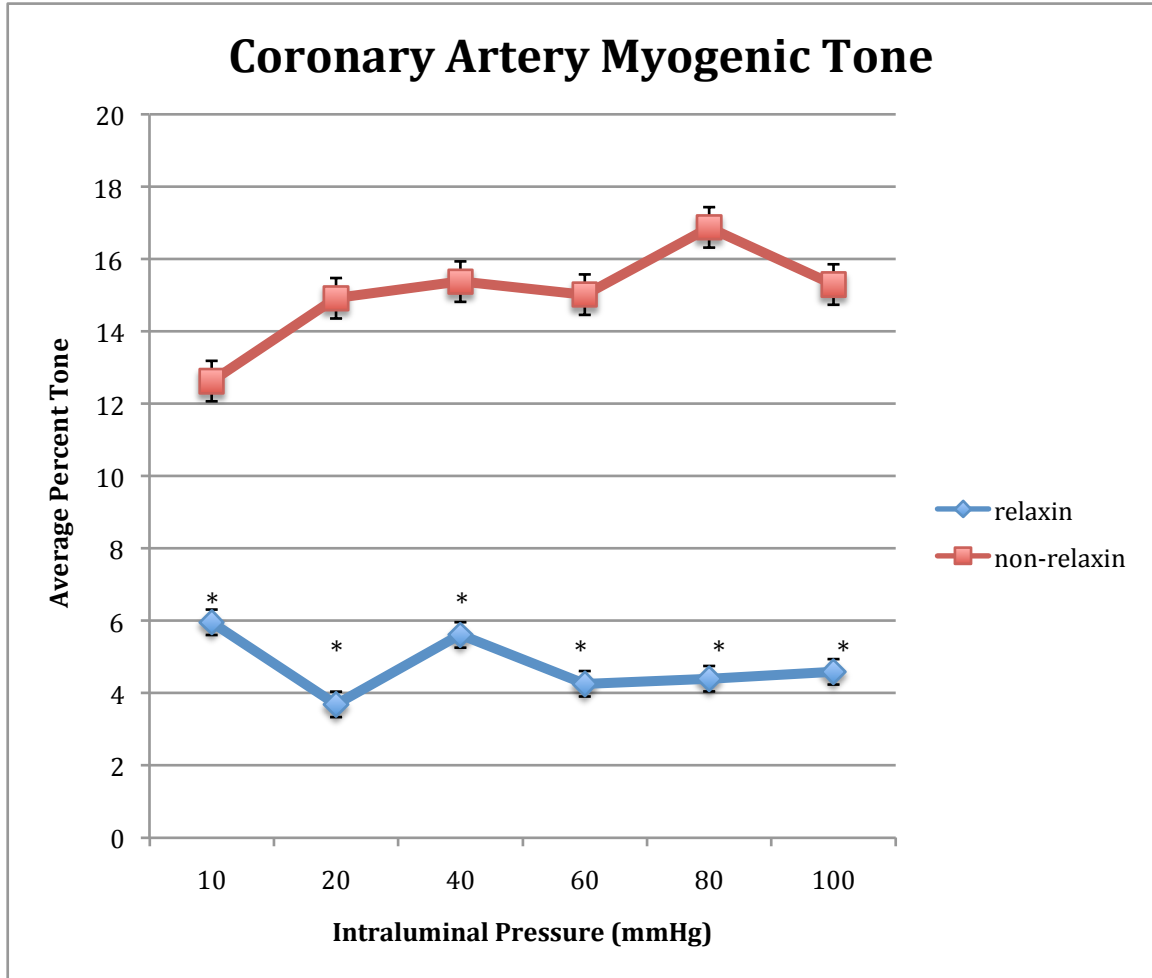Figure 5: Coronary Artery Myogenic Tone. Myogenic tone measured in aged SHR female rats treated with relaxin and without relaxin (vehicle). Each data point represents an averaged percent tone of coronary septal arteries at given pressures. Two-way repeated measures ANOVA indicated a significant decrease in percent tone in response to relaxin treated animals ($p< 0.006$).

* Indicates significance between groups.

CHAPTER V

DISCUSSION

Main Findings

The hypotheses tested in this study were: 1) relaxin administration to aged

spontaneously hypertensive female rats (SHR) improves coronary vascular function,

2) relaxin administration to aged SHR females, improves cardiac behavior. To test

the hypotheses, both isolated hearts and isolated coronary arteries were studied.

Relaxin significantly decreased myogenic tone in isolated coronary arteries as well

as decreased left ventricular contractility in isolated hearts from aged SHR rats, a

model of postmenopausal physiology.

Effects of Relaxin on Coronary Vascular Function

In isolated coronary arteries, myogenic reactivity was significantly decreased

in relaxin treated, aged SHRs compared to vehicle treated controls (Figure 5). This

finding is consistent with previous work demonstrating relaxin treatment reduces

vascular tone and reactivity (24). Spontaneously hypertensive male rats treated

with relaxin, increases cardiac output, reduces arterial load, and decreases systemic

vascular resistance (24). Furthermore, previous work by Xu *et al.*, showed in aged,

hypertensive rats, that relaxin reverses arterial remodeling and stiffening of the arteries (28). Relaxin treatment has been shown to reduce myogenic reactivity in renal and mesenteric arteries (34). The reductions in vascular tone observed during relaxin treatment may be beneficial in the hypertensive animals. It could result in an increase in blood flow, which would allow more oxygen to reach the tissue.

I expected coronary arteries from vehicle-treated aged SHRs to have a greater percent tone average at each pressure. This was expected because these animals are hypertensive, which could indicate greater peripheral resistance in this model. Previous work in the aged female SHR by Fortepiani et al. reported significantly increased mean arterial pressure compared to young female SHR's and aged male SHR's (9). In young SHRs, myogenic tone was significantly greater in isolated coronary arteries at pressures above 140 mmHg as compared age-matched WKY control rats (42). In a study by Albrecht *et al.*, precapillary resistance sized vessels in young, male SHR showed wall thickening and luminal narrowing (37).

Effects of Relaxin on Cardiac Performance

The current study found isolated hearts from aged SHR females treated with relaxin have a lower contractility compared to aged SHR females not treated with relaxin (vehicle). Contractility increased as the balloon volume increased (Figure 2). There have been studies done examining the effects of relaxin on the cardiovascular system in males and females, however, there haven't been studies examining the effects of relaxin in postmenopausal females with hypertension. In the current study, the relaxin group displayed a lower contractility, which may be beneficial.

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With relaxin present in the aged SHR's, this reduced the cardiac contractility by

approximately half compared to those from the vehicle group (Figure 2). The vehicle

group displayed a higher contractility, compared to the relaxin treated group, which

may not be beneficial. In fact, it may lead to heart failure over time since the hearts

isolated from the vehicle treated rats generated a greater force per contraction. This

could result in cardiac stress as the heart may need to use more force to pump the

blood. In aged hearts with hypertension, it may be better for the heart to have a

lower contractility because this allows the heart to pump blood efficiently, without

putting extra stress on the heart muscle. This is evidenced by data investigating

angiotensin-converting enzyme (ACE) inhibitors and β-blockers treatment for heart

failure. One study shows that there is a beneficial effect of ACE inhibitors on

myocardial mass and contractility in hypertension and heart failure, which is

believed to be related to the decrease in afterload (43). The mechanism of β-

blockers is not well known, however it is considered that β- blockers have an

antihypertensive action by reducing heart rate and cardiac contractility (44). This is

consistent with the hypothesis that relaxin is cardioprotective.

This study also showed a trend that aged SHR's treated with relaxin have

reduced left ventricular relaxation compared to aged SHR vehicle group (Figure 3).

The reduction of the left ventricular relaxation should be beneficial. Reduction of left

ventricular relaxation in hearts, in a hypertensive state, would reduce the risk of

heart failure and left ventricular hypertrophy as the heart performs more efficiently

without resulting in excess stress of the cardiac muscle. Prior studies have shown

the SHR's had a decrease stroke volume, cardiac output, and an increase in left

ventricular hypertrophy which means the heart could not stretch and fill adequately

(37). Excess cardiac stress could result in an increase in muscle of the left

ventricular wall (thicker), which would cause the left ventricle to become stiffer

(left ventricular hypertrophy). Although a significant difference was not found

between old SHR vehicles and old SHR relaxin treated animals in left ventricular

relaxation, the trend also suggests the relaxin is cardioprotective. If I increased my

sample size I would expect to see a statistical significance.

Coronary blood flow was not significantly different in relaxin-treated old SHR

compared to vehicle treated animals (Figure 4). This finding conflicts with a

previous report by Bani-Sacchi *et al*, in which it was found that relaxin increased

coronary flow in isolated guinea pig and rat hearts (17). However, that study was

conducted in young animals while my current study was performed in aged animals.

The authors reported that the relaxin induced coronary vasodilation was mediated

by nitric oxide (17). The lack of effect in the current study may be related to

impaired nitric oxide synthesis in the SHR (45). These aged hypertensive hearts may

have been overworked due to a lifetime of hypertension and therefore a longer

period of RLX may be necessary. A MANCOVA was performed comparing the slopes

of the two groups and a significant difference was found at 0.10 balloon volume.

This finding reveals that something else is occurring in the heart when a volume of

0.10 ml is placed in the left ventricle that does not involve relaxin, the balloon

volume, or percent change in flow. This finding needs to be further examined in

order to determine the reason behind this finding.

A decrease in coronary flow, left ventricular relaxation, and left ventricular contractility in the relaxin treated group compared to the vehicle treated group shows that relaxin treated hearts were pumping the same amount of blood out as the vehicle treated group. The relaxin treated hearts were working more efficiently compared to the vehicle treated hearts because the RLX treated group had a lower left ventricular contractility and relaxation compared to the vehicle treated group I interpret this to mean that the vehicle hearts were working harder than the RLX hearts, to pump the same amount of blood out. Indicating that relaxin has a potential cardioprotective effects on hearts from aged, hypertensive female rats.

Hypertensive heart disease is a group of disorders, which include heart failure, hypertensive heart disease, and left ventricular hypertrophy, that are the number one cause of death related to hypertension. Post-menopausal females have increased blood pressure, which makes the aged SHR an excellent model (9). Chronic hypertension can lead to heart failure which is when the heart does not generate enough force to move the required amount of oxygenated blood out of the chambers, which causes the pressure to increase in the heart. The heart wall hypertrophies in order to keep blood moving, as well as deal with the increased pressures in the heart. Due to the excess stretching, the contractions become stronger, which makes the wall of the left ventricle thicker, which eventually results in failure of the heart.

Cardiac output is equal to the stroke volume (SV) multiplied by the heart rate (HR) (36). Contractility, which has a large impact on stroke volume, is the ability of the cardiac muscle to generate force, and is a good way to measure the ability of left

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ventricular function. In this study, hearts were paced at the same rates in order to eliminate the variable of HR, which allows for contractility and pressure to be examined. Normally, increases in contractility are viewed as beneficial. However, in this case increases in contractility are not beneficial. Due to the SHR's being aged and hypertensive, an increase in contractility would negatively affect their hearts, which are working harder to deal with the hypertension.

Experimental Challenges

As in all experiments, there were some challenges to overcome. When attaching the heart to the Langendorff apparatus, it was difficult placing the cannula into the aorta without puncturing the atrioventricular valve. Another challenge faced during the Langendorff procedure was bringing the Krebs-Henseleit solution up to proper temperature and pH, and maintaining the temperature during the experiment. If the temperature were too high, the pH would decrease and eventually compromise the performance of the heart. The final challenge faced with the Langendorff apparatus occurred from the balloon. If there was an air bubble or leak in the balloon, it would cause pressure to drop and the data could not be recorded properly. In order to prevent the balloon from leaking, silicon rubber sealant was used around the balloon knot to maintain an airtight balloon.

An obvious experimenter challenge that arose was efficiency of removing the septal coronary artery from the heart and mounting the vessel. Removing the septal coronary artery from the cardiac muscle was a delicate process. The muscle had to be cut away from the coronary artery almost entirely in order to get a clear

measurement of the vessel. It has been shown that isolated arteries become more responsive to pharmacological agents as the skill of the experimenter and quality of the dissection improves (38). This is true because as soon as the vessel is removed from the organ and body, it slowly begins to die. It is important to isolate the vessel and mount it quickly in order to prevent damage to the endothelium. The tubing of the bath and cannula need to be checked to make sure the tubing is free of air bubbles because an air bubble can ruin the endothelium and kill the vessel.

Conclusions

The main objective of this study was to determine if relaxin administration to aged hypertensive female rats improves coronary vascular function and cardiac behavior. The main findings in the isolated hearts were that relaxin treatment decreased left ventricular contractility. This finding suggests that relaxin may be cardioprotective in a rodent model of postmenopausal physiology because a reduction in left ventricular contractility would reduce the workload for the aged, hypertensive heart. Relaxin also significantly decreased tone in the isolated coronary arteries. This could contribute to a decrease in coronary vascular resistance, which may result in improved perfusion of the myocardium. Although the results of this study suggest that relaxin may be cardioprotective in the post-menopausal model, further studies are needed. The mechanism of how relaxin works at the cellular level would be of interest to study males, hypertensive or non-hypertensive, and how the cardiovascular system responds to relaxin administration. Additionally it would be interesting to find ways to deliver relaxin

therapeutically in human patients with severely atherosclerotic, coronary blood

vessels of the heart. One of the ideas that came to mind is to look into a relaxin-

coated stent. The relaxin-coated stent would be placed in atherosclerotic blood

vessels.

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