THE EFFECTS OF THE TRANSCRIPTION FACTOR SRY1 ON LEFT VENTRICULAR FUNCTION

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THE EFFECTS OF THE TRANSCRIPTION FACTOR SRY1 ON LEFT VENTRICULAR FUNCTION

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Thesis

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ABSTRACT

It has been found that there are gender differences in the cardiovascular system that result with the male gender being at an advantage after a myocardial infarction (MI) (Norton et al, 2008). In an effort to understand the cause of these differences, researchers have investigated the effects of testosterone on the heart, but have not been able to come to a consensus. Sry, which is also known as the testis determining factor, has been shown to be expressed in the adult Rattus norvegicus heart (Ely et al, 2007), and could be responsible along with testosterone to account for the differences. The aim of the current study was to investigate the effects of the transcription factor Sry1 on left ventricular function. Left ventricular function was evaluated by performing heart isolation procedures 21 days post Sry1 administration. Coronary flow, heart weight, diastolic, and systolic pressures were evaluated. Our lab had previously shown that Sry transcripts are co-expressed with tyrosine hydroxylase in the heart (Milsted et al, 2004), and so we hypothesized that Sry1 could possibly affect heart function by a catecholamine and a testosterone mechanism. Plasma testosterone and heart norepinephrine levels were measured in order to determine a possible mechanism. The administration of exogenous Sry1 into the heart resulted in the castrate+Sry1 group having increased left ventricular systolic (p<0.001), and diastolic (p<0.001) pressures compared to the castrate group. The sham+Sry1 had significantly higher diastolic pressures than the castrate group (p=0.009). Diastolic and systolic contractility and relaxation, heart weight, and coronary flow, were
similar for all treatment groups. There was a trend of a positive correlation between increased levels of norepinephrine in Sry1 treated animals when compared to controls. The cast+Sry1 group had 14% more heart norepinephrine content than the cast group, and the sham+Sry1 group had 17% more NE than the sham group. Our results suggest that the transcription factor Sry plays a role in cardiovascular function through a catecholamine mechanism.
TABLE OF CONTENTS

LIST OF TABLES...........................................................................................................vii

LIST OF FIGURES..........................................................................................................viii

CHAPTER

I. INTRODUCTION..............................................................................................................1

Hypothesis.......................................................................................................................2

II. REVIEW OF THE LITERATURE....................................................................................4

The Heart.........................................................................................................................5

Sry Effects.......................................................................................................................7

Testosterone Effects......................................................................................................9

III. METHODS..................................................................................................................12

Castration.......................................................................................................................12

Exogenous Sry1 Delivery.................................................................................................13

Heart Isolation & Function Analysis..............................................................................13

Plasma Testosterone, Heart TH and NE Content.........................................................14

IV. RESULTS.....................................................................................................................16

V. DISCUSSION.................................................................................................................27

Effects of Sry1 on LV function......................................................................................28

Other Sry1 Effects.........................................................................................................31
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ventricular weight and coronary flow</td>
<td>26</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure                                                                                                                             Page
1. Comparison of Left Ventricular Diastolic Pressure………………………………………………….18
2. Comparison of Left Ventricular Systolic Pressure………………………………………………..19
3. Left Ventricular Diastolic Contractility……………………………………………………………20
4. Norepinephrine Heart Content……………………………………………………………………..21
5. Comparison of Coronary Flow…………………………………………………………………………..22
6. Comparison of Left Ventricular Diastolic % Gain (0.05mL-0.10mL)……………………………23
7. Comparison of Left Ventricular Systolic % Gain (0.05mL-0.10mL)………………………………24
8. Plasma testosterone levels……………………………………………………………………….25
CHAPTER I
INTRODUCTION

The presence of Sry transcripts in the sexually indifferent genital ridges of the developing mammalian embryo determines the formation of the testis, which produce the hormone testosterone (O’Neill et al, 1996). Researchers have discovered Sry expression in several adult rat organs including the brain, heart, adrenal medulla, and kidney, leading to the belief that its functions are not limited to testis determination (Ely et al, 2007). Previously, our lab has shown that Rattus norvegicus possesses several Sry loci on the Y chromosome (Turner et al, 2007). Multiple Sry loci were identified by Southern blot autoradiographs, and by amplification and sequencing using primers that included complete Sry coding region and flanking sequences. The multiple Sry copies are thought to be conserved for because they are functional. If they were not functional, they would decay due to accumulating mutations, as selection is expected to maintain only one copy of Sry for testis determination.

Blood pressure control is one of the additional functions why selection might be maintaining multiple Sry copies. Spontaneously hypertensive rats which have an earlier pubertal rise of testosterone and an increase of coronary collagen, have a Y chromosome that was demonstrated to have a locus that raises blood pressure (Seachrist et al, 2000, Ely et al, 2007). The Y chromosome was also demonstrated to have an effect on the catecholamine pathway in the kidney (Ely et al, 2007). For example, backcrosses of
WKY rats with SHR male rats were not only found to have elevated blood pressure, but they also had an increased renal NE turnover rate that was similar to SHR males.

Sry transcripts were shown to be co-expressed with tyrosine hydroxylase in the adult heart, brain, kidney, and adrenal glands (Ely et al., 2007). These are the tissues where a gene responsible for blood pressure control could potentially be expressed and have cardiovascular effects. The administration of exogenous Sry1 to the kidney and adrenal glands was shown to cause an increase in blood pressure.

Increased norepinephrine turnover rate in the heart was shown to result in increased tyrosine hydroxylase (TH) synthesis (Ely et al., 2000). Tyrosine hydroxylase is the rate limiting enzyme in catecholamine synthesis, and plays an important role in blood pressure control.

Further research is necessary to elucidate the mechanism by which Sry affects cardiovascular function. One of the possible mechanisms involves testosterone. Research has shown that there are gender related differences in cardiac function (Kuhar et al., 2007), and thus it is possible that testosterone plays a role in creating those differences.

Hypothesis

The hypothesis of the following proposal is:

Endogenous Sry acts through both testosterone and catecholamine mechanisms to influence heart function. In addition, it is predicted that administration of Sry1 to gonadally intact rats will increase plasma testosterone levels, leading to an increase in heart mass and coronary flow. Also, Sry will cause an increase in heart tyrosine hydroxylase, norepinephrine content, and left ventricular function in castrated rats.
Thus, the following research will examine the effect of exogenous Sry
administered to the left ventricle on myocardial function in the Wistar Kyoto (WKY) rat
model.
CHAPTER II

REVIEW OF THE LITERATURE

In order to better understand the potential effects of Sry on the heart, it is important to review literature that discusses Sry evolution, as well as an elucidation of its function in other organs. The mechanisms by which Sry performs its functions on the heart are not clear, but it is likely that one of them involves testosterone. As such, a review of testosterone effects is necessary. The following literature review will focus on three main topics: the heart, Sry, and testosterone effects.

The mammalian heart has been shown to differ in anatomy and function between the genders. In order to better understand these differences, it is important to review literature that addresses cardiovascular function investigated both invivo, as well as invitro.

The role of Sry is thought to go beyond testis determination. Sry has been shown to increase tyrosine hydroxylase transcriptional activity, and affect catecholamine synthesis. It is also thought to play a role in human nervous system differentiation.

Sry could be affecting the heart through increased plasma testosterone levels. Testosterone has been shown to not only determine male characteristics, but also have an effect on the cardiovascular system. Further investigation is necessary as researchers have found conflicting results of the effects of testosterone on the cardiovascular system.
While some have found it to have beneficial effects, others have found it to have no effect. The present study will hopefully shed some light on the matter.

The Heart

The mammalian heart consists of four chambers, with the left ventricle being made up of a thick muscular wall that generates high pressures. In vivo, the left ventricle ejects blood across the aortic valve and into the aorta during contraction. The pressure and volume relationship in the ventricles is nonlinear, with compliance decreasing with increasing volume (Klabunde et al, 2004). Invitro, an isolated heart can be perfused via the Langendorff apparatus (Kuhar et al, 2007). This involves introducing a canula in to the aorta above the semilunar valve which closes with perfusion due to retrograde flow, resulting in the coronary arteries being perfused. The pressure above the heart is kept constant, and a pressure catheter inserted into the left atrium and mitral valve to the left ventricle is used to measure left ventricular pressure.

Testosterone decreases in concentration with age, and has been shown to affect cardiovascular function and mass (Hayward et al, 2001). A necropsy study that excluded primary cardiac pathology revealed that with men, the measured ventricular mass decreased as age increased. Testosterone is thought to be causing the increased ventricular mass by impacting cardiac myocytes which possess androgen receptors, thereby causing them to hypertrophy (Modena et al, 1999). The ventricular hypertrophy occurs as a result of testosterone facilitating the proliferation of vascular smooth muscle cells. Testosterone treatments were also observed to result in 2 fold increases in myocyte nuclear hypertrophy, and it was further demonstrated that testosterone plays a role in cardiac contractility (Golden et al, 2004). Removal of testosterone by a gonadectomy in
rats resulted in a decrease in cardiac contractility performance. The reestablishment of testosterone in the same rat by hormone replacement therapy prevented the negative effects.

Some of the anatomical differences that have been shown to be present between the genders include left ventricular morphology, chamber function, and hemodynamics (Norton et al, 2008). Data from patients who had either normal left ventricle function (NLV), or systolic heart failure (HF), and had been catherized with a micromanometer tipped catheter was retrospectively analyzed. The results showed that men had a significantly greater prevalence of coronary artery disease than women in both NLV and HF groups. Several hemodynamic differences were observed in gender in the NLV group that were not observed in HF group, including left ventricular end diastolic pressure, and pulmonary capillary wedge pressure. The differences in mean pulmonary arterial pressure were attributed to lower right ventricular filling pressure in women. Women also had lower right atrial pressure and pulmonary capillary wedge pressure. The lack of gender differences in hemodynamics in the HF group suggests that there is an altered response to sex hormones after an MI. A cardiovascular altered response to testosterone after an MI could possibly be one of the reasons why researches have not been able to agree on testosterone’s cardiovascular effects.

Cardiac hypertrophy after a myocardial infarction has been shown to decrease pump function possibly due to cardiac remodeling of noninfarcted tissue in a normotensive rat (Norton et al, 2008). What has not been investigated is whether viable tissue in a hypertensive rat is susceptible to alterations in systolic function post MI. Post pubescent SHR and WKY rats had the left anterior descending coronary artery ligated
(Norton et al, 2008). This procedure resulted in a moderate size MI with low postoperative mortality. Factors measured to determine hypertrophy were degree of LV dilation, systolic chamber, and myocardial dysfunction. SHR were found to have reduced regional myocardial systolic function in viable tissue of the left ventricle 6 to 7 months after a moderate sized MI. The decreased function was shown to not be attributed to increased scar tissue, LV dilation, excessive apoptosis or necrosis, as previously thought. Further investigation is necessary, but upregulated Gi proteins were thought to be causing an adrenergic stimulus, therefore causing a reduction in the contractile response. Also, post MI, SHR has enhanced iNOS which through reactive oxygen species could be causing the reduced myocardial systolic function. The results from this study, along with the fact that spontaneously hypertensive rats have an earlier pubertal rise of testosterone than WKY rats (Seachrist et al, 2000, Ely et al, 2000), suggest that the mechanism by which Sry acts on the heart does not involve testosterone, but further research is necessary.

Sry Effects

Some of the earliest work on the testis and development was done in 1947 by Jost who castrated embryonic rabbits and found that they all developed as females (O’Neill et al, 1996). Consequent work showed that male development proceeds from the testis, which is the male gonad. An examination of karyotypes of individuals with congenital defects such as Turner’s and Klinefelter’s syndrome led to the consensus that the mammalian Y chromosome possesses a testis determining factor, TDF. Further research resulted in unequivocal identification of Sry as the mammalian sex-determining gene. Sry was shown to possess a protein known as the high mobility group HMG, which provides
Sry the ability to bind DNA in a sequence specific method. (Foster et al, 1992, Jacobs et al, 1959, Giese et al, 1994) Sry binds to DNA and maybe alters local chromatin architecture, suggesting that it acts as a transcription factor. (O’Niell et al, 1996)

Although not well characterized, Sry is known to have at least one open reading frame that encodes a protein which has a DNA binding motif (Behlke et al, 1993). Further research in 1993 by David Page and colleagues revealed that Sry is encoded by a single exon on the Y chromosome. Their methods included sequencing of the Y chromosome, Sry isolation, and rapid amplification and cloning of 5’ and 3’ ends. The template used was of human testis poly (A), and Sry specific oligonucleotides were used as primers. Their results suggested that Sry transcripts contain a complete open reading frame that possesses an exon that encodes the entire protein.

More recently, our lab has shown that Sry can act as a transcription factor (Milsted et al, 2004). The study investigated whether Sry could affect Th which is the rate limiting enzyme in catecholamine synthesis, and plays an important role in blood pressure control (Milsted et al, 2004). PC12 cells were co-transfected with a Sry expression vector. The results showed that Th promoter transcription in the cell’s activity was shown to increase after co-transfection of the Sry expression vector. A mutation at the Sry-Th interaction site resulted in a decrease in the Th transcriptional activity. Thus, Sry was shown to regulate TH promoter activity, and therefore affect blood pressure. The Sry-Th interactions were shown to be also true when exogenous Sry was delivered to the adrenal medulla. Sry increased adrenal medulla Th activity, which resulted in an increase in blood pressure and plasma NE.
Sry has been shown by RT-PCR to be expressed in the brain. Its expression in the brain has led to the belief that it plays a role in the differentiation of the human nervous system. RT-PCR analysis of human subjects showed that Sry was transcribed in the adult male hypothalamus and cortex, but not in the adult female. These results suggest that Sry is possibly involved in regulatory mechanisms that can affect neuron sex specific properties (Mayer *et al*, 1996).

Testosterone Effects

The effects of testosterone are controversial with some researchers finding no beneficial or deleterious effects, while others have shown it to impact cardiovascular vasodilation and contraction (Thompson *et al*, 2002). Acute testosterone administration to men with coronary artery disease was shown to produce no difference in systolic blood pressure, heart rate, or perfusion, but there was a difference in estrogen levels. It is possible that in the study, high levels of estrogen interfered with the effects of testosterone. The enzyme aromatase which converts testosterone to estradiol, could have led to an increase in estrogens levels which could have affected the effects of testosterone.

Testosterone’s vasodilatory effects were demonstrated in an in vivo experiment. Testosterone can cause vasodilation via a mechanism that involves an increase in nitric oxide formation (Golden *et al*, 2004). A study was done in which testosterone was infused into the arteries of anesthetized pre-pubertal pigs that had an electromagnetic flow meter probe placed around the anterior descending artery (Molinai *et al*, 2002). An increase in coronary flow was observed, while there was no significant change in arterial pressure, suggesting that the vasodilation was an effect of testosterone. The infusion of
saline at the same rate as that of testosterone did not result with an increase in coronary flow, while an increase in the concentration of testosterone resulted in an increased magnitude of response of blood flow. A catheter connected to a pressure transducer was used to measure the left ventricular pressure, which was shown to not be significantly affected by testosterone intra-arterial infusion. However, the effect of testosterone is still controversial.

Although the exact mechanism by which testosterone affects vascular cells is unknown, it was demonstrated that vascular cells possess steroid hormone receptors (Shabsigh et al, 2005). In one study, subcutaneous testosterone pretreatment resulted in an increase of coronary flow in isolated rat hearts that had myocardial injuries when compared to controls (Kuhar et al, 2007). The hearts were isolated, and a canula was inserted into the aorta. They were then perfused with Krebs-Henseleit solution, while connected to Langendorff’s apparatus with a pressure catheter in the left ventricle. While animals that had been pretreated with testosterone subcutaneously had increased coronary flow during reperfusion when compared to controls, there were no significant differences in diastolic and systolic pressure in the left ventricle.

One of the other organs that testosterone has been shown to have an effect on is the brain. The possession of androgen receptors in the brain provides a mechanism for testosterone to affect brain function. The brain not only has androgen receptors, but it is also capable of synthesizing them. Androgens direct neurotrophic factors and are involved in learning, memory, and cognitive behavior. The effects are thought to be related to circulating testosterone levels, versus being a result of early imprinting on the brain (Gouchie et al, 1991). Androgen levels and spatial ability were shown to have a
curvilinear relationship with females who had high testosterone levels and males with normal testosterone levels having the best performance.

As research advances, the role of testosterone is constantly being shown to go beyond sexual differentiation. Low testosterone levels can be linked to high triglycerides, fibrinogen, plasminogen activator, body mass index, waist circumference, and serum leptin levels. This data suggest that low testosterone levels are a part of a multidimensional metabolic syndrome characterized by disorders such as hypertension and obesity. Studies also found men with diabetes to have significantly lower levels of testosterone (Ridwan et al, 2005).
CHAPTER III

METHODS

WKY rats were bred in-house at the University of Akron. All animals received a standard 12:12 light: dark cycle, were fed standard rat chow and tap water *ad libitum*. They were maintained in compliance with the regulations set forth by NIH and approved by the University of Akron Institutional Animal Care and Use Committee. WKY males 4 weeks old were divided into 4 groups, n=6, and had the following treatments: group 1- castrated, group 2- sham surgery, group 3 - castrated plus exogenous Sry1 delivered, group 4- sham surgery plus exogeneous Sry1 delivered.

Castration

WKY males (weeks old) were anesthetized with Sodium Pentothal, (50 mg/kg, i.p.) and either had a castration or sham surgery performed. Surgeries were done using a midline incision on ventral side with removal of the testis for the castration. The sham animals had testis withdrawn and then relocated intact. After surgery, penicillin (2500 IU i.m.) was injected for post-operative care in all rats.
Exogenous Sry1 Delivery

A pilot study was done to ensure a mastery of the Sry1 delivery technique. The animals were anesthetized with Sodium Pentothal (50 mg/kg, i.p.) and had blue dye injected through the chest wall into the left ventricle. The hearts were then immediately dissected and examined for the presence of the dye.

After the successful completion of the pilot study, the study to evaluate the impact of Sry1 on the heart proceeded. An amount of 50 µL of Sry1 gene (50µg/100µL) was delivered by injection through the chest wall with a 25 gage needle to the left ventricle of all group 3 and 4 animals six weeks post castration or sham surgery. All rats were anesthetized with Sodium Pentothal (50 mg/kg, i.p.). After surgery, Penicillin (2500 IU i.m.) was injected for post-operative care in all rats.

The Sry1 expression construct (Sry1/pcDNA3.1) was prepared by cloning bp #1-1048 of Sry1 (Genbank accession # AF274872) into XbaI and EcoRV sites of pcDNA3.1 (Invitrogen). Immediately after injecting the Sry1, the injection area was gently rubbed in a circular motion.

Heart Isolation & Function Analysis

Sry has its peak blood pressure effect 21 days post delivery. As such, three weeks post Sry delivery, the animals were anesthetized with Sodium Pentothal (50 mg/kg, i.p.) and administered Heparin (1cc). Hearts were removed making sure to retain as much of the aorta as possible. The atria were removed, and hearts placed into ice cold Krebs Henseleit solution to reduce contractility. The contents of the Krebs Henseleit solution in millimolars are 119 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 14 glucose (Ely et al,1992). The atria were trimmed, hearts weighed and then
mounted by inserting a canula into the aorta. Krebs Henseleit solution continuously bubbled with (95/5) O₂/C O₂ to prevent precipitation of reagents, and maintained at 37°C was perfused with a constant flow by in a retrograde fashion. This resulted in the coronary arteries receiving the entire perfusate via the ostia at the aortic root (Skrzypie-Spring et al, 2007, Sellke et al, 1998).

To eliminate any variables that could result from different heart rates, the hearts were paced using bipolar pacing at 240 beats/min (Grass Instruments, Quincy, MA). Krebs Henseleit solution was perfused into the hearts at a starting flow of about 12mL/minute. Coronary flow was obtained by measuring timed volumes of perfusate. Ventricular systolic and diastolic pressure measurements were obtained by inserting a balloon tipped catheter connected to a pressure transducer (Statham P23 db, Gould, Cleveland, OH) into 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, creating a balloon (Ely et al, 2007). The catheter was connected to a pressure transducer and a physiograph. Three volumes were used to obtain data for Starling curves: 0.05ml, 0.10ml, and 0.12ml. The volumes were filled into the balloon using a 1cc syringe. The hearts were frozen for later NE analysis.

Plasma Testosterone, Heart TH and NE Content

Plasma testosterone, norepinephrine heart and plasma levels were measured 3 weeks after gene delivery. Testosterone levels were analyzed by an assay by RIA (Bio-Rad Laboratories, Hercules, CA) (Ely et al, 2004). Plasma samples were collected by retro-orbital puncture, and norepinephrine levels determined by HPLC with electrochemical detection (Ely et al, 2007, Foti et al, 1987). NE was extracted from
plasma using a Tris buffer that consisted of 1 M Tris, 10nM sodium metabisulfite, 20mM EDTA, 25 mg LC-alumina A, and was further prepared according to procedures used by Ely et al 1998. The left ventricles of the hearts were homogenized in mobile phase solution for norepinephrine analysis, and in 0.25M sucrose for TH analysis (Ely et al, 2007, Norton et al, 2008, Behlke et al, 1993, Nagatsu et al, 1979). The composition of the mobile phase was citric acid-35mM, sodium acetate- 90mM, octyl sodium sulfate-690µM, EDTA- 130 µM, and 10% methanol. The pH was adjusted to 4.7.

The following statistics were calculated: two way analysis of variance for coronary flow, left ventricular systolic and diastolic pressure, left ventricular systolic and diastolic slope, and heart weight. Unpaired t-tests were performed for percent heart weight gain, and plasma testosterone, and NE content. All pairwise multiple comparison procedures used the Holm-Sidak method, with an overall significance of 0.05.
CHAPTER IV
RESULTS

The administration of exogenous Sry1 into the heart was shown to have an impact on heart function. A comparison of left ventricular pressure between the castrate, castrate+Sry1, sham, and sham+Sry1 groups revealed a trend that showed that there were increased left ventricular pressures in the cast+Sry1 group when compared to the cast group, and also in the sham+Sry1 group when compared to the sham group (p = 0.002)(Fig. 1,2). Further analysis showed that the cast and the cast+Sry1 groups had the most significant differences in left ventricular systolic and diastolic pressures (p<0.001) (Fig. 1). The castrate+Sry1 group had higher left ventricular systolic and diastolic pressures than the castrate group. Although the sham+Sry1 and castrate groups had no left ventricular systolic functional differences between them, the sham+Sry1 had significantly higher diastolic pressures (p=0.009) (Fig. 1,2). The slopes for the diastolic and systolic left ventricular pressures were similar for all treatment groups.

LV functional differences which exist between the cast and sham groups were not observed in the cast+Sry1 and sham groups. Paired comparisons between the cast+Sry1 and sham+Sry1 groups, as well as between the sham and sham+Sry1 groups indicated no significant differences in LV function (Fig. 3). As Expected, increased balloon volume created significant increases in the LV function in all research groups regardless of
treatment (Starling effect). There were no significant differences observed for coronary flow or heart weight.

Plasma testosterone levels were not affected by the administration of Sry1 into the heart (Fig. 8). The cast and cast+Sry1 groups had similar plasma testosterone concentrations, and the sham group had the highest concentration. In the castrate and castrate+Sry1 group, a trend of a direct relationship between Sry1 levels and NE levels was observed (Fig. 4). The cast+Sry1 group had 14% more heart NE content than the cast group, and had higher left ventricular systolic and diastolic pressures. The sham+Sry1 group had 17% more NE than the sham group. Although there were no significant differences observed in percent coronary flow in any of the groups, the sham group had the highest absolute coronary flow (Fig. 5) Although there were no significant differences, the sham+Sry1 group had the highest heart weight water gain of 49% after perfusion, while the other three groups had similar gains with the cast group gaining 42%, the cast+Sry1 group 41%, and the sham group gaining 38%.

The castrate+Sry1 group had a higher percent gain in systolic and diastolic pressure from the 0.05mL to 0.10mL balloon volumes (Fig. 6,7).
Figure 1. Comparison of left ventricular diastolic pressure in castrate, castrate + Sry1, sham, and sham + Sry1 groups. Measurements taken 21 days post gene delivery. Means, ± S.E.M. ANOVA for all treatment groups (p<0.05). ANOVA for cast vs. cast+Sry1 += (p = 0.001). Balloon volume ANOVA for all groups (p = 0.001).
Figure 2. Comparison of left ventricular systolic pressure in castrate, castrate + Sry1, sham, and sham + Sry1 groups. Measurements taken 21 days post gene delivery. Means, ± S.E.M. ANOVA for all treatment groups (p<0.05). ANOVA for cast vs. cast+Sry1 + = (p = 0.008). Balloon volume ANOVA for all groups p = 0.001.
Figure 3. Comparison of left ventricular contractility for castrate, castrate + Sry1, sham, and sham + Sry1 groups. Means, ± S.E.M., ANOVA showed no significant differences.
Figure 4. Comparison of heart NE content for castrate, castrate + Sry1, sham, and sham + Sry1 groups. Means, ± S.E.M., ANOVA showed no significant differences.
Figure 5. Comparison of coronary flow in castrate, castrate + Sry, sham, and sham + Sry1 groups. Measurements taken 21 days post gene delivery. Means, ±S.E.M., ANOVA showed no significant differences.
Figure 6. Comparison of left ventricular diastolic pressure percent gain in castrate, castrate + Sry1, sham, and sham + Sry1 groups. Measurements taken 21 days post gene delivery. Means, ± S.E.M., ANOVA showed no significant differences.
Figure 7. Comparison of left ventricular systolic pressure percent gain in castrate, castrate + Sry1, sham, and sham + Sry1 groups. Measurements taken 21 days post gene delivery. Means, ± S.E.M., ANOVA showed no significant differences.
Figure 8. Comparison of plasma testosterone levels in castrate, castrate+Sry1, sham, and sham+Sry1 treatment groups. Measurements taken 21 days post gene delivery. Means, ± S.E.M., ANOVA showed no significant differences.
Table 1. Ventricular weight and coronary flow.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Ventricular Weight (g)</th>
<th>Initial Coronary Flow (mL/min)</th>
<th>Final Coronary Flow (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrate</td>
<td>0.90</td>
<td>10.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Castrate+Sry1</td>
<td>0.90</td>
<td>10.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Sham</td>
<td>0.94</td>
<td>11.28</td>
<td>6.4</td>
</tr>
<tr>
<td>Sham+Sry1</td>
<td>0.94</td>
<td>10.8</td>
<td>6.4</td>
</tr>
</tbody>
</table>
CHAPTER V
DISCUSSION

It has been found that there are gender differences in the cardiovascular system that include function, LV morphology, and hemodynamics (Norton et al, 2008). These differences result with males being at an advantage post MI, and understanding the mechanism could help provide better treatment for the females. The differences are not static, and have been shown to change from women having cardiovascular function protected before an MI, to men having more of their cardiovascular function preserved post MI. However, once the heart has suffered a MI, the structure and reactivity of the heart changes (Nortion et al, 2008). Men experience less adverse effects, and have better survival rates post MI (Mitoff et al, 2007). Some of the differences are thought to be a result of the actions of sex hormones (Mosca et al, 1997). Although estrogen has been shown to have cardio protective actions before an MI, cardiovascular disease is the number 1 cause of death in females (Kato et al, 2003). Pre-menopausal women are at a lower risk of heart disease than their male counter parts, and post menopausal women are at a comparable risk to their male counterparts. Estrogen’s cardio-protective actions could also be masking symptoms of disease. The Framingham Heart Study reported that two thirds of sudden deaths due to cardiovascular disease in women occur in those with no previous symptoms of disease, compared with half the sudden deaths in men (Kato et al, 2003).
In attempting to explain the male advantage post MI, researchers have looked at the cardiovascular effects of testosterone, but have not been able to reach a consensus. Therefore, it is possible that something is working along with testosterone to account for the differences. This other factor would have to be capable of influencing heart function, and be solely expressed in males. Our results suggest that the transcription factor, Sry plays a role in cardiovascular function.

Effects of Sry1 on LV function

Cardiac output is equal to the heart rate multiplied by the stroke volume (McGavock et al, 2003). Contractility, which has a big impact on the stroke volume, is the ability of the cardiac muscle to generate force, and is a good indicator of left ventricular function. All hearts were paced at identical rates to eliminate heart rate variability, and thus contractility and pressure were the focus of our evaluation of left ventricular function. Increases in left ventricular contractility were interpreted as beneficial, and increases in diastolic pressure indicated stiffness and were not interpreted as beneficial. This was a logical extension of previous research that demonstrated that prolonged strenuous exercise which puts higher demands on the heart, is associated with enhanced left ventricular systolic function secondary to increased contractility (McGavock et al, 2003).

The current study found that the exogenous administration of Sry1 into the left ventricle increased left ventricular pressure in the castrate + Sry1 group when compared to the castrate group. The impact of LV function by exogenous Sry1 shows that the mechanism by which Sry impacts cardiovascular function was shown to not rely solely on testosterone since there was no significant difference in function between the castrate
and gonadally intact animals. Interestingly, Sry1 affects LV function by increasing diastolic and systolic pressures, but did not affect their contractility. Some abnormalities occur in either diastolic or systolic ventricular pressures (Carabello et al, 2002). Diastolic dysfunction accounts for 30 to 50% of all cases of heart failure with preserved LV systolic function, and is thought to be part of the pathophysiology of hypertrophic cardiomyopathy (Vasan et al, 2003). Hypertrophic cardiomyopathy is characterized by increased diastolic pressures, prolonged relaxation, abnormal stiffness, and impaired left ventricular filling. Normally during diastole, the relaxation of the left ventricular wall tension results in decreased pressures that generate a pressure gradient. The pressure gradient causes rapid filling that is influenced by the rate of decrease in pressure.

Although the castrate +Sry1 group had a significant 32% increase in left ventricular diastolic pressure, and a 28% increase in systolic pressure compared to the castrate group, the lack of other significant differences in function suggest that heart function was not seriously compromised. No significant differences were observed in heart weight, coronary flow, and relaxation for all treatment groups. The increased diastolic and systolic pressures observed in the castrate+Sry1 group were not associated with differences in coronary flow, plasma testosterone levels, heart weight, or amount of water gained by the heart. Not only where the diastolic and systolic pressures higher in the castrate+Sry1 group than they were in the castrate, but the percent gains in pressure with increasing balloon volume were also higher for each balloon volume.

Even though our results suggest that the differences in heart function produced by Sry1 do not involve a testosterone mechanism, further research is needed. It was our expectation that some of the Sry1 that was injected into the heart would reach the testis
through the circulatory system, and thereby stimulate them to produce more testosterone. Most likely, the Sry1 was cleared from the body before it reached the testes. The increased levels of norepinephrine heart content between the castrate and castrate+Sry1 group suggests that the mechanism by which Sry1 affects the heart involves a catecholamine mechanism. Sry is coexpressed with tyrosine hydroxylase in the heart, adrenal glands, and kidneys, and it is possible that it is influencing the organs using the same catecholamine mechanism. Sry regulates tyrosine hydroxylase gene transcription, and causes increased levels of adrenal Th content, plasma NE content, and kidney NE turnover rate (Ely et al., 2007, Milsted et al., 2004). Our results are in agreement with this as we observed increased levels of NE in the castrate+Sry1 group compared to the castrate group, and in the sham+Sry1 group, compared to the sham group.

The similarity of LV function in the castrate + Sry1 group compared to sham group suggests that Sry1 plays a role in LV function that does not rely solely on a testosterone mechanism. LV differences in function were not seen solely in the castrate and castrate+Sry1 group. There was an increase in LV diastolic pressure in the sham+Sry1 group compared to the sham group that indicated that Sry was affecting the resting phase of the cardiac cycle. An increase in diastolic pressure indicates chamber stiffness, and has been found to indicate diastolic dysfunction when accompanied by normal ejection fraction (Varma et al., 2000).

In the absence of testosterone, exogenous Sry was demonstrated to restore heart function to normal levels through a catecholamine mechanism. The mechanism involves Sry increasing tyrosine hydroxylase levels, thereby resulting in increased norepinephrine
levels. The higher norepinephrine levels cause an increase in glucose and muscle readiness, resulting with increased ventricular pressures (Turner et al, 2002).

Other Sry1 Effects

There were no major differences observed in percent coronary flow values, but the trend indicated that the sham group had a higher coronary flow value at each balloon volume when compared to all of the other treatment groups. The lack of differences in percent coronary flow could be attributed to the fact that the sham + Sry1 group had higher initial coronary flow values. Although, we did not detect cardiovascular functional differences in the sham + Sry1 group, the increased coronary flow indicated that there was an increase in cardiac activity, and oxygen consumption in this group (Klabunde, 2005).
CHAPTER VI

CONCLUSION

As previously stated, the WKY rat possesses multiple copies of the Sry gene that are thought to have functions in addition to testis determination. Until now, none of these other functions had been investigated, and thus our knowledge about them is limited. This study evaluated the impact of the administration of exogenous Sry1 into the heart. The main finding was that the castrate+Sry1 group had higher diastolic and systolic pressures than the castrate group suggesting that Sry1 is impacting function.

Further evidence of the effects of Sry in-vivo could be provided by two future research experiments. One would involve analyzing the cardiovascular function of mice that had Sry1 expression selectively knocked out in the heart, and another would involve administering Sry1 to female rats post MI and analyzing cardiovascular function. This would provide further evidence that Sry1 impacts heart function by a catecholamine mechanism.

One of the limitations of the study include having a reduced sample size as some of the plasma samples and hearts collected at the beginning of the experiment could not be used to get viable results.

The administration of exogenous Sry1 to the left ventricle resulted with an increase in heart and plasma norepinephrine levels in the castrate+Sry1 group compared
to the castrate group, and in the sham+Sry1 group compared to the sham group. The castrate+Sry1 group had higher diastolic and systolic pressures that are thought to be a result of the increased norepinephrine levels. Testosterone levels were not affected by exogenous Sry administration, and thus coronary flow and heart weight remained unaffected. Although our results suggested that the mechanism by which Sry1 acts on the heart does not involve a testosterone mechanism, it is still a possibility that should be researched further.
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Varma N., Eberli F., Carl S., Apstein C. Increased Diastolic Chamber Stiffness During Demand Ischemia Response to Quick Length Change Differentiates Rigor-Activated From Calcium-Activated Tension Circulation 2000, 101: 2185-2192.
APENDICES
## APPENDIX A

### Summary of Heart Weight and Perfusion Volumes

<table>
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<tr>
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<th>Final Perf.</th>
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### APPENDIX B

Summary of Diastolic, Systolic and Coronary Flow Measurements

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