Chronic Swim Stress Attenuates Endocrine Response and Improves Cardiac Function in Mice Lacking the Angiotensin II Type 1a Receptor

Najat Khalifa Almahroug
Wright State University

Follow this and additional works at: https://corescholar.libraries.wright.edu/etd_all

Part of the Pharmacology, Toxicology and Environmental Health Commons

Repository Citation
Almahroug, Najat Khalifa, "Chronic Swim Stress Attenuates Endocrine Response and Improves Cardiac Function in Mice Lacking the Angiotensin II Type 1a Receptor" (2013). Browse all Theses and Dissertations. 1158.
https://corescholar.libraries.wright.edu/etd_all/1158

This Thesis is brought to you for free and open access by the Theses and Dissertations at CORE Scholar. It has been accepted for inclusion in Browse all Theses and Dissertations by an authorized administrator of CORE Scholar. For more information, please contact corescholar@wwwlibraries.wright.edu, library-corescholar@wright.edu.
CHRONIC SWIM STRESS ATTENUATES ENDOCRINE RESPONSE AND IMPROVES CARDIAC FUNCTION IN MICE LACKING THE ANGIOTENSIN II TYPE 1a RECEPTOR

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

NAJAT KHALIFA ALMAHROUG
MD, Tripoli University, Medical Collage, Tripoli, Libya 2001

2013
Wright State University
WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

Date: September 3, 2013

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY
SUPERVISION BY Najat K. Almahroug ENTITLED Chronic Swim Stress Attenuates
Endocrine Response and Improves Cardiac Function in Mice Lacking the Angiotensin II
Type 1a Receptor BE ACCEPTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF Master of Science.

_________________________________
Roberta L. Pohlman, Ph.D., Thesis Chair

Norma C. Adragna, Ph.D., Interim Chair
Pharmacology & Toxicology

Committee on Final Examination

_________________________________
Roberta L. Pohlman, Ph.D.

_________________________________
Michal Kraszpulski, Ph.D.

_________________________________
Nadja Grobe, Ph.D.

_________________________________
R. William Ayres, IV, Ph.D.
Interim Dean, Graduate School
ABSTRACT

Almahroug, Najat Khalifa. M.S., Department of Pharmacology & Toxicology, Wright State University, 2013. Chronic Swim Stress Attenuates Endocrine Response and Improves Cardiac Function in Mice Lacking the Angiotensin AT1a Receptor.

Stress is a stimulus that causes an often abrupt but always large change in autonomic activity and hormone secretion. Angiotensin II (AngII) is one of the main neurohormonal mediators that are stimulated by stress. AngII mediates most of its relevant biological effects via AngII type1 receptor (AT1R) activation. Stress increases tissue and circulating levels of AngII which contributes to stimulation of adrenal AT1R, mediating stress-induced hormone release. AngII through the activation of AT1R not only elevates blood pressure but also has a patho-physiological relevance in cardiac remodeling. The goal of this study was to assess the influence of AT1 subtype a receptor (AT1aR) on adrenal and cardiac function during chronic swim stress. Adult male AT1a knockout (KO) and wild type (WT) mice (n= 6 per group) were used; groups were exercise KO (KOEX) and exercise WT (WTEX) and control KO and WT. Exercise paradigm was swimming, 1 hour 3d/wk for 7 weeks. Urine and plasma corticosterone (CORT) were measured by radioimmunoassay. Urinary catecholamines were measured using high pressure liquid chromatography with electrochemical detection. Cardiac function was assessed by echocardiography (Echo). Results showed that plasma CORT was lower in KOEX and WTEX as compared to control groups (3 fold decreases). Urinary CORT was used to
show the dynamic response to exercise stress. Urinary CORT decreased in KOEX after 2 hour from the swimming session compared to the WTEX. These data also showed that KOEX had lower urinary CORT at baseline as compared to the baseline in the WTEX. Urinary catecholamines results indicate that there were no significant differences in Norepinephrine and Epinephrine responses between the KOEX and WTEX. Distance and Velocity significantly increased in KOEX as compared to WTEX. Echo showed a higher ejection fraction (EF %) in KOEX (75%) vs. WTEX (55 %). Assessment of mitral valve function showed a higher A-wave velocity in KOEX as compared to WTEX. AT1a deficient mice showed improved cardiac response and reduced adrenal stress response to chronic exercise. In conclusion, AT1aR is an important mediator of stress induced cardiac dysfunction during exercise training.
TABLE OF CONTENTS

I. INTRODUCTION AND REVIEW OF LITERATURE ................................................. 1

   Renin-angiotensin system (RAS) ................................................................. 4
   Angiotensin II (Ang II) ................................................................................... 5
   Angiotensin II receptors (ATRs) ................................................................. 7
   Angiotensin II type 1a receptor (AT1aR) ..................................................... 10
   Transgenic mouse model ............................................................................. 12
   Swimming as stress model ......................................................................... 13

II. HYPOTHESIS AND SPECIFIC AIMS ................................................................. 14

III. MATERIALS AND METHODS ......................................................................... 15

   Animals ........................................................................................................ 15
   Swimming protocols ..................................................................................... 15
   Body composition measurement ................................................................ 16
   Echocardiography (Echo) ........................................................................... 16
   Urine collection ............................................................................................. 18
   Cage change induced stress experiment ............................................... 18
   Food and water consumptions .................................................................. 18
   Distance and velocity measurements ....................................................... 19
   Oxygen consumption (VO₂(ml/min)) ....................................................... 19
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The neuronal and hormonal responses to stress</td>
</tr>
<tr>
<td>2.</td>
<td>Renin-angiotensin system (RAS)</td>
</tr>
<tr>
<td>3.</td>
<td>Signal transduction by Angiotensin AT1 Receptor</td>
</tr>
<tr>
<td>4.</td>
<td>Representative swimming of mice</td>
</tr>
<tr>
<td>5.</td>
<td>Representative distance and velocity measurements</td>
</tr>
<tr>
<td>6.</td>
<td>Representative oxygen uptake (VO$_2$ (ml/min))</td>
</tr>
<tr>
<td>7.</td>
<td>Effects of chronic swimming exercise on body weight</td>
</tr>
<tr>
<td>8.</td>
<td>Effects of chronic swimming exercise on percent of body fat</td>
</tr>
<tr>
<td>9.</td>
<td>Effects of chronic swimming exercise on food intake</td>
</tr>
<tr>
<td>10.</td>
<td>Effects of chronic swimming exercise on water consumption</td>
</tr>
<tr>
<td>11.</td>
<td>Effects of acute cage change/handling stress on urinary corticosterone</td>
</tr>
<tr>
<td>12.</td>
<td>Effects of acute cage change/handling stress on urinary catecholamines</td>
</tr>
<tr>
<td>13.</td>
<td>Effects of acute swim stress on urinary corticosterone</td>
</tr>
<tr>
<td>14.</td>
<td>Effects of chronic swimming exercise on urinary corticosterone</td>
</tr>
<tr>
<td>15.</td>
<td>Effects of chronic swimming exercise on plasma corticosterone in WT mice</td>
</tr>
<tr>
<td>16.</td>
<td>Effects of chronic swimming exercise on plasma corticosterone in KO mice</td>
</tr>
<tr>
<td>17.</td>
<td>Effects of cage change/handling induced stress during first week swim exercise</td>
</tr>
<tr>
<td>18.</td>
<td>Effects of cage change/handling induced stress after 7 week swim exercise</td>
</tr>
<tr>
<td>19.</td>
<td>Effects of chronic swimming exercise on urinary catecholamines</td>
</tr>
<tr>
<td>20.</td>
<td>Effects of chronic swimming exercise on adrenal catecholamines</td>
</tr>
</tbody>
</table>
21. Distance and velocity measurements ................................................................. 44
22. Quartile distance measurements .................................................................... 45
23. Oxygen uptake (Vo₂(ml/min)) .......................................................................... 46
24. Percent fractional shortening (FS %) ................................................................. 47
25. Left ventricular end systolic area (LVESA) ....................................................... 48
26. Ejection fraction (EF %) .................................................................................. 49
27. Left ventricular end systolic diameter (LVESD) ............................................... 50
28. Left ventricular end diastolic area (LVEDA) .................................................... 51
29. Posterior Wall Thickness (PWT) ....................................................................... 52
30. Left Ventricular Mass (LVM) ........................................................................... 53
31. Wave A (late diastolic filling) ........................................................................... 54
31. Wave E (early diastolic filling) .......................................................................... 54
32. E/A Ratio (EF %) ............................................................................................ 55
33. Cardiomyocyte quantification via H&E analysis .............................................. 56
34. Representative Picosirius Red Staining (Aorta) .............................................. 57
35. Collagen quantification via Picro-sirius Red staining ........................................ 58
36. Representative H&E Staining (WAT) ............................................................... 59
37. Adipocyte size via H&E analysis ...................................................................... 60
ACKNOWLEDGEMENT

First of all we thank our GOD for establishment of this work. I would like to express my sincere appreciation to my supervisor, Dr. Roberta L. Pohlman, for providing me an opportunity to work with her and for her guidance, encouragement, and continual support. Special thanks go to my committee members, Dr. Michal Kraszpulski and Dr. Nadja Grobe for their support, guidance, and helpful suggestions. Their assistance has served me well and I owe them my heartfelt appreciation.

I would like to give my wholehearted appreciation to my husband, Khalifa, who supported me with his love and encouragement to pursue this advanced degree. He has been a constant source of strength, with unlimited patience and understanding. Without his support, I would never have been able to accomplish this work. I am truly thankful for having him in my life. I also thank my wonderful and lovely children: Togaa, Alhosain, Jenna, and our new addition Aasm, who have always loved me unconditionally and for understanding those weekend mornings when I was writing my thesis instead of playing games. I hope that one day they can read my thesis and understand why I spent so much time in front of my computer.

My deepest gratitude goes to my family in Libya for their unflagging love and support throughout my life. I would like to express my appreciation to my country Libya for providing a financial support for my research. Members of Dr. Morris’ Lab also deserve my sincerest thanks for their friendship and assistance that has meant more to me than I could ever express. Finally, I would like to express my gratitude to all those in the
Department Pharmacology and Toxicology who gave me the opportunity to complete my Master’s Degree at Wright State University.
DEDICATION

This thesis is dedicated

to

my husband, Khalifa Khebreesh
I. INTRODUCTION AND REVIEW OF LITERATURE

Chronic exposure to stress produces an increase in allostatic load and is considered the main contributor in the development of cardiomyopathy and neuropsychiatric diseases. In addition, stress may worsen a chronic illness, such as diabetes and cancer (McEwen, 1998, 2007; McEwen et al., 1999). For that reason, understanding the mechanism of stress and how the organism reacts is important in the prevention of very serious consequences resulting from over-activation and misregulation of neural or neuroendocrine responses.

As early as 1926, the concept of stress was used in biological and psychological fields to refer to a mental strain or a state of threatened homeostasis (Gunther, 1981). It was Hans Selye who first coined the term “stress” in 1946 as a “non-specific response of the body to any demand”. In 2005, Day, defined stress as “the multisystem response of the body to challenges that overwhelm or threaten to overwhelm the selective homeostatic mechanisms of the organism”. Stressful situations induce a physiological and behavioral change as a response mechanism. In order to react to the stress, many regulatory systems help to maintain balance within the body and to support optimal health. The sympatho-neural and the sympatho-adrenomedullary systems respond by releasing the “fight or flight” hormones, norepinephrine and epinephrine, respectively, first described by Cannon, 1929(as reviewed by Gunther, 1981), and followed by secretion of adrenal corticoid from the hypothalamic-pituitary-adrenocortical (HPA) axis (Selye, 1976). The HPA-axis controls stress by negative feedback mechanisms with the
release of cortisol in humans and corticosterone in rodents. These hormones block the glucocorticoid and mineralocorticoid receptors in the anterior pituitary, the hypothalamus, and the hippocampus, suppressing hormone production and secretion. These systems produce the key neuroendocrine responses to stress (Kvetnansky et al., 2009) (Figure 1). Concurrently, the Renin-Angiotensin System (RAS) plays a fundamental role in the stress response (Saavedra et al., 2011, 2012).
Figure 1: The Neuronal and Hormonal Responses to Stress
Renin-angiotensin system (RAS)

Since its discovery in 1897 by Tigerstedt and Bergmann (as reviewed by Phillips et al., 1999), the RAS has emerged as playing a vital role in the regulation of blood pressure and control fluids and electrolyte balance (Millar-Craig et al., 1978) (Figure 2). Moreover, the RAS plays an important role in the pathogenesis of cardiovascular and renal disorders. The classical RAS is a hormonal cascade initiated by sympathetic activation of β-adrenergic receptors leading to an increased synthesis of renin (rate-limiting enzyme) in the juxtaglomerular cells of the kidney, the most important site for renin release. Renin acts on the circulating substrate, angiotensinogen, and undergoes a proteolytic cleavage to form the decapeptide angiotensin I (AngI). Angiotensinogen is a glycoprotein with a molecular weight of 58 kDa, synthesized and secreted constitutively mainly by the liver (Dévényi et al., 1968; Kageyama et al., 1984). The inactive metabolite of angiotensinogen, Ang I, is hydrolyzed by angiotensin converting enzyme (ACE) to yield a biologically active octapeptide angiotensin II (Ang II). ACE is a membrane-bound exopeptidase, which is primarily present on endothelial cells. In recent years, the understanding of RAS has become more complex after the discovery of new angiotensin peptides such as angiotensin (1-9), (1-7) and (1-5) and enzymes such as ACE2. During stress, activation of the local RAS is associated with circulating Ang II, including that in the brain (Saavedra, 1992), anterior pituitary, and adrenal glands (Jezova et al., 2003; Saavedra, 1992).
**Angiotensin II (Ang II)**

Ang II is an active metabolite, liberated from the inactive decapeptide Ang I via proteolytic activity of ACE. Ang II was first described as a circulating hormone (Braun-Menendez et al., 1940; Page et al., 1940). Besides being a peripheral hormone, Ang II is a neuropeptide, essential in controlling blood pressure, fluid homeostasis, and the stress response (Peach et al., 1966; Yang et al., 1996). The most well known functions of the RAS are largely attributed to this vasoconstrictor peptide (Page, 1987). Ang II is highly expressed in the brain and modulates stress responses by binding to the angiotensin II type 1 receptor (AT1R). A study conducted on rats, revealed that brain Ang II plays a significant role in the regulation of sympathetic adrenomedullary system activity during stress, through the stimulation of corticotrophin releasing hormone (CRH) mRNA in the paraventricular nucleus (PVN). They also postulated that the brain Ang II is not involved in the acute response of the HPA-axis during stress, but may affect long-term adaptive responses of the HPA-axis (Jezova et al., 1998).

Studies revealed that Ang II increases catecholamine synthesis and release in bovine adrenomedullary cells (Foucart et al., 1991; Stachowiak et al., 1990). Later, studies demonstrated important interactions of Ang II with the sympathetic nervous system and baroreceptor reflexes in controlling blood pressure (Reid, 1992). Ang II activates the neurosympathetic and adrenomedullary branches to secrete norepinephrine and epinephrine, respectively, and facilitates sympathetic neurotransmission by stimulating sympathetic nerve endings (Reid, 1992).
Figure 2: Renin-Angiotensin System (RAS)
**Angiotensin II receptors (ATRs)**

Ang II acts through two main receptors, namely, type 1 receptor (AT1R) and type 2 receptor (AT2R). Both G protein-coupled receptors are seven-transmembrane domain receptors with a molecular mass at about 40-42 KDa, (Kambayashi et al., 1993; Sasaki et al., 1991), however, there is only a 34% amino acid similarity (Clauser et al., 1996). The most recognized physiological actions of Ang II depend mainly on AT1R stimulation (Timmermans et al., 1993; Saavedra, 1999; de Gasparo et al., 2000). AT1R is present in almost all tissues including brain, heart, liver, adrenals, lung, kidney, skeletal muscle, and adipose tissues. AT1R isoforms were first cloned from the genomic library from an inbred strain of mouse (Balb/c) (Sasamura et al., 1992). AT1R in the brain is highly expressed in the circumventricular organs including the subfornical organ (SFO) and HPA, the key areas responsible for the stress response. During stress, sympathetic stimulation increases peripheral and central Ang II (Yang et al 1993, 1996) leading to “up regulation” of AT1R in the paraventricular nucleus (PVN) and the median eminence from where CRH is released to the portal system to stimulate anterior pituitary adrenocorticotropic hormone (ACTH) secretion (Tsutsumi et al 1991; Aguilera et al 1995; Israel et al., 1995; Jöhren et al., 1997). Up regulation of AT1R is the main factor in modulating secretion of corticotrophin-releasing hormone (CRH). Finally, ACTH then causes adrenal corticoid secretion, the trademark hormone of the stress reaction (Sumitomo et al., 1991; Aguilera et al 1995).

AT2R is predominant in fetal tissues and adult adrenal glands where it is highly expressed in the medulla and zona glomerulosa (Israel et al., 1995). Studies have reported the presence of AT2R in the rat’s locus coeruleus (Tsutsumi et al., 1991) and in large
quantities in the adrenal medulla and zona glomerulosa (Israel et al., 1995), but the exact roles of AT2R in the regulation of the stress response is still a matter of debate.

In 2002, studies demonstrated that AT1R and AT2R have counter regulatory effects in the biosynthesis of catecholamines in porcine adrenomedullary chromaffin cells; AT2R via Ang II activation inhibits production of cGMP, which in turn, may reduce protein kinase G activity. This reduction in enzyme action leads to a decrease in tyrosine hydroxylase enzyme (TH) activity, a rate-limiting enzyme in catecholamine synthesis, while the stimulation of AT1R elevates expression and activity of TH and increases intracellular catecholamine levels (Takekoshi et al., 2002).
Figure 3: Signal Transduction by Angiotensin AT1 Receptors.
Angiotensin II type 1a receptor (AT1aR)

In contrast to humans, two different subtypes have been found for AT1R in rodents; AT1a and AT1b (Kakar et al., 1992; Sandberg et al., 1992; Sasamura et al., 1992; Inagami et al., 1994). Both receptor subtypes show a 93.9% amino acid identity that makes it difficult to distinguish between them pharmacologically (Iwai et al., 1992). They have the same binding affinities and signal transduction (Van Esch et al., 2010). However, in the adult mouse, AT1a and AT1b receptors (AT1aR, AT1bR) are differentially expressed. AT1bRs are abundantly expressed in tissues of the adrenal glands, brain, and testes, while the AT1aRs are mainly expressed in kidney, heart, brain, liver, adrenal glands, ovaries, lung, and adipose tissue (Burson et al., 1994). Studies have demonstrated that mice lacking the genes that encode for the AT1aR have a significant reduction in resting blood pressure of about 20 mm Hg and an attenuated response to Ang II (Ito et al., 1995; Chen et al., 1997; Oliverio et al., 2000). This finding suggests that the AT1aR is a main receptor subtype involved in blood pressure regulation in mice. It has been reported that administration of Ang II to AT1aR KO mice produces a pressor response signifying that the AT1bR may participate in control of blood pressure in mice lacking the AT1aR (Oliverio et al., 1997).

Studies on mice lacking the AT1aR demonstrated a large reduction of Ang II in kidney and adrenal tissues and three-fold higher plasma Ang II levels suggesting the important role for AT1aR in mediating the uptake of circulating Ang II (Li et al., 2008). In 2010, Van Esch reported that AT1aR KO mice had a 5-fold increase of plasma Ang II and renal Ang II reduced by half as compared to the control with no changes in cardiac morphology.
Activation of the AT1aR by Ang II can also increase production of reactive oxygen species (ROS), superoxide and peroxynitrite, in the vascular wall through stimulating membrane-associated NADPH oxidase (Cheng et al., 2005). Studies conducted on mice deficient for AT1aR demonstrated a marked increase in life span with less oxidative damage and cardiovascular injury as compared to the wild-type (WT) mice (Benigni et al., 2009). They also postulated that the increases in the life span in the AT1aR KO mice may be due to reduced mitochondrial damage as a result of attenuation of oxidative stress and up regulation of the survival genes, nicotinamide phosphoribosyltransferase and sirtuin 3 in the kidney. These findings, therefore, suggest a role for AT1aR signaling in the aging process (damage to cells by ROS), and may offer the possibility that AT1R blockers (ARBs) may prolong life in humans through actions slowing the aging process.

ARBs are commonly prescribed for the treatment of hypertension (Werner et al., 2008), diabetes, and modulation of the cerebrovascular blood flow during stroke (Nishimura et al., 2000). In addition, studies done in rats revealed that pretreatment with candesartan (ARB), reduced the sympathoadrenal and hormonal response to acute isolation stress (Armando et al., 2001; Pavel et al., 2008), protected gastric mucosa from ulceration and inflammation during cold-restraint stress (Bregonzio et al., 2008), prolonged lifespan, and reduced sympathetic and HPA-axis stimulation in spontaneously hypertensive rats (Baiardi et al., 2004). These results demonstrate that AT1R plays an important role during stress. Ultimately, these observations could be of therapeutic benefit in treatment of anxiety and stress-related disorders (Saavedra et al., 2005).
Another study in adult rats demonstrated the presence of AT1aR and AT2R in micro-vessels as well as in skeletal muscle fibers (Lindeman et al., 2001). Recently, it was reported that AT1aR KO mice exhibited improved whole body and skeletal muscle function despite impaired recovery in muscle mass following myotoxic injury (Murphy et al., 2012). Based on this it has been postulated that ARBs could have a beneficial effect for enhancing skeletal muscle performance after injury. A study conducted on genetic models demonstrated that inhibition of ACE and reduced bradykinin degradation enhanced muscular performance (Jones et al., 2002, 2003). Collectively, these findings support the idea that the use of a combination treatment of AT1R and ACE inhibitors, in addition to their antihypertensive effects, can improve skeletal muscle performance, which could have implications for treatment of muscular disorders such as sarcopenia and Duchene muscular dystrophies.

Transgenic mouse models

Animal models are useful tools to understand human physiology and disease. As mice have a high similarity at the cellular and molecular levels to humans, they are a good model for clinical investigation (Waterston et al., 2002). Pharmacologic agents are often used to study the disease process. The main drawbacks to using pharmacologic agents are the difficulty in targeting specific tissues and the effects are often incomplete due to lack of specificity of the agents for their targets. For example, losartan (ARB) blocks the AT1R perfectly, but is unable to differentiate between the subtypes of that receptor (Lake-Bruse et al., 2000). Hence, development of an ideal animal model, which mimics the action of ARBs, is essential for a better understanding of the physiology and pathophysiology of angiotensin receptors. In 1989, Capecchi, Evans, and Smithies,
discovered a technique to generate mice in which a specific gene is deleted, known as a knockout mice, for which they were awarded the 2007 Nobel Prize in Physiology or Medicine (Abbott, 2007). In the present study, a gene targeting technique was used with specific deletion of the gene encoding the AT1aR, which has been used in previous experimental studies.

Swimming as a stress model

Physical training programs have been recognized as an important and safe strategy for prevention and treatment of cardiomyopathies. Exercise increases cardiac vascularity by developing more collateral vessels of the coronary artery, which assists blood circulation during cardiac infarction. Physical exercise is considered a eustress; a type stress to which the organism responds in a positive way via a series of reaction mediated by the activation of the HPA and the sympathetic nervous system (Viru, 1984). In response to the physical stress, tissue adaptation takes place and leads to an increase in the stress threshold, making the body more tolerant to prolonged physical activity (Mueller et al., 2002).

Swimming exercise is considered one type of physical stress that challenges homeostasis; a stress condition that affects many systems in the body, mainly cardiovascular and pulmonary systems, to adapt to a higher level of function for a lower energy cost (Mastorakos et al., 2005). Chronic stress is considered a risk factor for the development of cardiovascular and psychiatric disorders such as depression and anxiety (McEwen, 2008). In the present study, chronic swimming exercise was used as a stress model to study the consequences of prolonged and repeated stress on adrenal and cardiac function in transgenic mice.
II. **Hypothesis and Specific Aims**

*Hypothesis:*

Mice Lacking Angiotensin II Type 1a Receptor Show Attenuated Endocrine Response and Improved Cardiac Function during Chronic Exercise Stress.

*Specific Aims:*

1. To test the hypothesis that urinary corticosterone could be used as a biomarker for the detection of stress induced adrenal secretion in conscious mice.

2. To test the hypothesis that lack of the Angiotensin II Type 1a receptor improves physiological and metabolic responses to chronic exercise stress.

3. To test the hypothesis that deficiency of the Angiotensin II Type 1a receptor protects the animals from stress-induced cardiac dysfunction during exercise training.
III. MATERIALS AND METHODS

**Animals**

Adult male AT1aR KO and wild type (WT) mice (n= 6-8 per group) were used in this study which lasted for 7 weeks. Groups were divided into exercise (KOEX and WTEX) and control (KOCon and WTCon) mice. KO mice (C57BL/6 background; Harlan, Indianapolis, IN) were bred in the Wright State University Laboratory Animal Resource Center. Breeding stocks were provided by Drs. Coffman and Gurley (Duke University, Durham, NC, USA). Mice were housed individually at 22°C under a 12 h light/12 h dark cycle with *ad libitum* access to water and standard laboratory chow [29% protein, 17% fat, 54% carbohydrate [Teklad 8640 (Teklad Animal Diets, USA)]. All experimental procedures were approved by the Wright State University Animal Care and Use Committee.

**Swimming protocol**

Mice were habituated to water over a four-day period during week 1. Mice initially swam for 20 minutes gradually increasing the time spent in the water by 10 minutes/session until 60 min was attained. Chronic swimming stress continued for three days a week for seven weeks. Mice freely swam in a plastic tank with a surface area of 53 cm and a depth of 33 cm filled with fresh tap water each day the mice swam (Figure 4). The water was gently circulated by using a small pump (5 L/min; EHEIM GmbH & Co. KG, Deizisau, Germany) to encourage continuous swimming. Water temperature was maintained at 22-25°C. A maximum of three to four mice were allowed to swim at the same time. The swimming session was performed during the light cycle of
the mice between 10.00AM to 11.00AM to avoid variation in urine and plasma corticosterone levels. Urine samples were collected at baseline before swimming (pre-stress) and two hours after swimming (post-stress). After each exercise session, mice were dried by a soft washcloth adjacent to a heat lamp. They were then returned to their home cages, which sat near the heat lamp. When the mice completed grooming, cages were returned to the breeding room with access to food and water for the rest of the day. The sedentary mice were housed under the same conditions and were handled each day during the same time that the exercise mice swam. Two of eight KOEX mice were unable to complete this course of swimming, but all other animals were trained successfully.

**Body composition measurements**

Body composition was measured using nuclear magnetic resonance designated Echo-MRI (Echo Medical Systems, Houston, TX, USA). A conscious mouse was placed in a clear plastic cylinder (4.7 cm diameter) and held in position with a plastic plunger to avoid any movements. The cylinder was placed in the MRI machine and the mouse was scanned for a two-minute period. Printouts of the procedure yielded data for whole body fat, percent body fat, lean tissue mass, free water, and total body water. Once all the measurements were completed, the mouse was returned to its home cage.

**Echocardiography (Echo)**

The method for the echocardiography measurements has been described in detail previously (Alghamri et al., 2013). These measurements were made on the mice at week 1 and week 7. Briefly, Echo analyses were performed using a Siemens ACUSON Sequoia 512 ultrasound system (Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania) with an ACUSON 15 megahertz transducer (model 15L8, Siemens.
Medical Solutions USA). Mice were anesthetized with 3% isoflurane (Isf) in oxygen (1 L/min; Surgivet Anesco Isotec 4) in an anesthesia induction chamber. Anesthesia was maintained 1.5% Isf in 100% O2 at a flow rate of 1 L/min via a small nose cone throughout the procedure. Anesthetized mice were transferred to a heating pad to maintain body temperature and placed in a supine position at a 60° angle. Chest hair was removed from the neckline to mid chest level using a commercial gel (Nair, Church & Dwight CO, Inc, Township, New Jersey). The transducer was coated with pre-warmed ultrasound transmission gel and positioned at a 90° angle to the chest (Aquasonic100, Parker Laboratories Inc., Fairfield, New Jersey). The heart was imaged in a two-dimensional (2D) imaging mode (B-mode) in the parasternal long and short-axis views. M-mode Echo (2D M-mode) measurements were taken to obtain fine measurements of cardiac dimensions and contractility. Using 2D M-mode tracing, left ventricular (LV) end-diastolic diameter (EDD) and end-systolic diameter (ESD) were recorded in all mice. Fractional shortening (FS) was calculated with the standard equation $FS (%) = \frac{LVEDD - LVESD}{LVEDD} \times 100$. Ejection fraction (EF %) was calculated from end-systolic and end-diastolic volumes using the modified Simpson’s formula of areas and major axes (performed by Echo device). Measuring flow patterns across the mitral valve using pulse wave Doppler (PWD) mode allowed two characteristic waves to be observed. Wave E, which represents early diastolic filling and wave A, which is consistent with late diastolic filling or atrial contraction. Once all measurements were completed, electro-gel was wiped from the chest and restraints were removed from the mouse. The mouse recovered on the heated pad in an upright position and was after complete recovery returned to its home cage.
Urine collection

Urine testing is a simple and non-invasive technique used for the identification of biomarkers in the diagnosis and prognosis of many diseases. Spot urine samples were collected in a large, clean weigh boat by holding the mouse firmly by the base of the tail and gently massaging the abdomen until the bladder was voided. Urine was stored on dry ice during the collection and then aliquoted and stored at -80°C until analysis. Urine was collected at baseline (before swim stress), and two hours after swim stress on Friday of each week for 7 weeks.

Cage change induced stress experiment

Moving a mouse from a dirty to clean cage has been characterized as an appropriate way to determine an acute psychological stress response (Rasmussen et al., 2011). Each cage change experiment was performed at the same time of day. Mice were transferred gently by picking up the tail. Urine was collected at baseline (before cage change), 15 and 30 min after cage change. Corticosterone and catecholamine (epinephrine and norepinephrine) release were later evaluated from the urine to determine stress level.

Food and water consumption

Food and water consumption was measured over a three-day period during week 1 and week 7. On day 1, 100 g of standard laboratory chow and 200 ml of water were given to each mouse. On day 3, food and water were again measured and total consumption recorded as a difference between day 1 and day 3 after the amount of any food spilled was taken into account.
**Distance and velocity measurements**

Individual mice were filmed using Shuttle Soft system software (Loligo Systems, Tjele, Denmark) and a U-Eye camera (National Instruments, Austin, TX, USA). The camera was placed approximately 3 feet above the circular swim tank. Mice were individually filmed swimming continuously for 5 min (Figure 5). Following the swimming session, animals were dried by a soft cloth and were returned to their home cages. From the recorded data, average swimming velocity (cm/sec) and total swim distance (cm) were computed using Shuttle Soft system software.

**Oxygen consumption (VO₂ (ml/min))**

Rate of oxygen uptake (VO₂ ml/min) in mice was determined during a single swimming session using a calibrated metabolic gas analysis system (Moxus modular oxygen uptake system; AEI Technologies, Pittsburgh, PA, USA). The gas analyzers were calibrated before every test session. The oxygen analyzer has a one-point calibration (20.95%); the carbon dioxide analyzer has a two-point calibration (0.04% and 5%). Rate of airflow was measured with an F-4140 Mass Flow Meter (AEI Technologies, Pittsburgh, PA, USA).

Exhaled air volume was collected during a 10 min swim. Room air was drawn through the metabolic chamber with pressure generated by a suction pump from the Moxus system. Water flow through the metabolic chamber was generated using a 5 L/min pump (EHEIM GmbH & Co. KG, Deizisau, Germany) and water temperature was maintained between 23-25°C (Figure 6).

The amount of oxygen consumed (VO₂) per minute was determined using the difference between the amount of oxygen inspired (VIO₂) and the amount of oxygen
expired \((\text{VEO}_2)\) where \(\text{VO}_{2\text{STPD}} = \text{VIO}_{2\text{STPD}} - \text{VEO}_{2\text{STPD}}\). The following variables were recorded at 30-second intervals: gas temperature, water pressure at a given gas temperature, barometric pressure, volume exhaled (VE), the fractional concentration of oxygen in inspired air \((\text{FI}_O_2 = 0.2093)\), fractional concentration of carbon dioxide in inspired air \((\text{FI}_C_0_2 = 0.0004)\) fractional concentration of oxygen in exhaled air \((\text{FE}_O_2)\), and the fractional concentration of carbon dioxide in expired air \((\text{FE}_C_0_2)\). The metabolic rate was calculated with the standard equations \(\text{VO}_2= (\text{VE}_O_2)(\text{FI}_O_2 - \text{FE}_O_2), \text{VCO}_2= (\text{VE}_C_0_2)(\text{FI}_C_0_2 - \text{FE}_C_0_2)\). Metabolic variables were measured at ambient temperature and pressure saturated (ATPS), but reported as standard temperature and pressure dry (STPD).

**Corticosterone Radioimmunoassay**

Radioimmunoassay (RIA) was first introduced to examine the concentration of insulin in plasma (Yalow & Berson, 1996). Since that time, it has become a widely used technique to measure the levels of hormones in biological fluids. RIA is a highly sensitive technique for analyzing corticosterone (CORT) concentrations in plasma and urine. It is a competitive binding assay dependent upon a radioactive isotope (iodine), to create a radioactive signal to measure CORT levels.

**Urinary Corticosterone RIA**

Spot urine samples were collected during the first and seventh weeks of the experiment, urine was collected at baseline (before swim stress), and two hours after swim stress. Urinary CORT concentration was measured using the ImmuChem™ double antibody corticosterone \(^{125}\text{I}\) RIA kit (MP Biomedical, Orangeburg, NY, USA). The standard curve controls, and samples were assayed in duplicate according to the manufacturer’s instructions with slight modification. The assay required less than 5 µl of
urine diluted (1:100) with steroid diluent (phosphosaline gelatin buffer (pH 7.0 ± 0.1). The assay was performed using 10×75 mm tubes. An adequate amount (50µl) of corticosterone$^{125}$I was added to all tubes followed by 50 µl of the corticosterone antibody. All samples were vortexed and incubated at room temperature for 2 hours. At the end of incubation, 200 µl of precipitating antibody (mixture of PEG and Gout anti-rabbit gamma globulin contain TRIS buffer) was added to all tubes. The assay tubes were vortexed and centrifuged at 2300-2500 RPM for 15 min at 4°C to separate bound $^{125}$I corticosterone from unbound. The supernatant was discarded before the radioactivity was counted in an automatic gamma counter machine (Micromedic 4/200 plus systems, Inc. Huntsville, Alabama, USA). The average concentration of CORT were obtained from the standard curve of the known concentrations of CORT and expressed in ng/ml.

**Plasma corticosterone RIA**

RIA for plasma CORT samples were performed using the same procedure as described above. After decapitation, trunk blood was collected in heparinized test tubes and centrifuged. Plasma samples were aliquoted and stored at −80°C until RIA measurement occurred.

**Catecholamine determinations in urine and adrenal glands using HPLC-ECD**

Catecholamines in urine and adrenal supernatants were measured using high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) (Bioanalytical Systems 4 C amperometric detector). Extraction of urine samples via activated alumina (Sigma Chemical Co. product A-9003) was conducted in 1.5 ml polypropylene micro tubes using 50 mg of alumina, 200 µL of 0.05N HCl containing 5 mM sodium metabisulfite, 20 µL urine, 40 µL of 50 ng/mL dihydroxybenzylamine
(DHBA) as an internal standard and 200 μL of 0.2 M Tris buffer. Tris buffer contained Trizma Base {tris (hydromethyl) aminomethane} reagent grade (Sigma Chemical Co. product T-1503) and distilled deionized water with pH adjusted to 9.0. Tris buffer was added carefully at 200 μL aliquots to raise the pH to 8.50 ± 0.05. Each tube was then tapped lightly and inverted after the addition of Tris buffer. The catecholamines were allowed to absorb the activated alumina during 1 min of vortexing. The tubes were centrifuged at 3000 RPM for 2 minutes at 4°C and the supernatant discarded. The remaining alumina was washed three times with 1 mL of distilled deionized water where each wash step consisted of vortexing, centrifuging, and discarding the supernatant. The catecholamines and internal standard was then eluted from alumina with 200 μL 0.1 N HClO₄ containing 0.1 mM sodium metabisulfite. All the samples were vortexed for 15 s, centrifuged for 1 min followed by the supernatant being separated into two aliquots and stored at -80°C until detection by HPLC-ECD. The standard extraction was made in the same way as the samples extractions except a 125 ng/mL extraction standard was used instead of using DHBA and urine samples. A standard containing 50 ng/ml of catecholamines, norepinephrine (NE), epinephrine (EPI), 2, 3-dihydroxbenzoic acid (DHBA), and dopamine (DA), was injected in the system twice a day, before and at the end of analyzing the samples. By comparing the peak amplitude from the samples to the standard, the concentrations of catecholamines in the samples were determined. The concentrations of NE, EPI, and DA were expressed as ng/mg of tissue and ng/ml for urine.

The adrenal glands were placed into pre-weighed 1.5 mL micro tubes, weighed, and homogenized in 800 μL of 0.2N perchloric acid (HClO₄) then sonicated for about 5 s,
two times. The samples were then transferred to 2ml 0.45 µm filter tubes and centrifuged for 30 min at 13,000 RPM. The supernatant was aliquoted and stored at -80°C until analysis with HPLC-ECD.

**Picrosirius Red staining for collagen fibers:**

This procedure uses Weigert’s iron hematoxylin staining kit (ENG Scientific, Inc, Clifton, New Jersey) for collagen types I and III. Heart and aorta tissues were fixed with paraformaldehyde and then send to the AML Laboratories (Inc., Belair Road Baltimore, MD, USA) where the paraffin-embedded done. The staining procedure was performed in-house (Dr. Morris Lab). The slides were stained for 8 min, and rinsed with tap water for 10 min. Picrosirius red stain containing 5 grams of Direct Red # 80 and 500 mL of saturated picric acid solution (Sigma-Aldrich, St. Louis, MO) was applied for 1.5 hr, followed by a wash in acidified water (2 changes), dehydrated with xylene, and cover-slipped with Permount mounting medium. Light microscopy was used to detect collagen types I and III. Quantification was performed by Metamorph ® (ver.7.6.3) image analysis software.

**Cardiac cell size quantification using haematoxylin and eosin (H&E) stain method:**

Haematoxylin and eosin (H&E) is a common staining method used in preparation of histology slides. H&E stains collagen in muscle fibers pink and the nuclei blue.

**Cardiomyocyte size quantification:**

After decapitations, heart tissues were collected and fixed in 4% paraformaldehyde (PFA). Tissues were embedded in paraffin, sectioned at a thickness of 5 µm, and stained with H&E done by AML Laboratories Inc., Belair Road Baltimore, MD, USA. Slides were imaged by a Leica Microsystems® DMR microscope at 20X.
magnification. Three different locations of a left ventricular heart section image were filmed in gray scale. The quantitation of the images were done by Metamorph® image analysis software (Ver. 7.6.3) using the combination of the Threshold selection tool and the Integrated Morphometric Analysis (IMA) tool kit. Data were averaged and cell size as computed as a total tissue area (pixels) per nucleus.

Measuring fat cells size

Epididymal white adipose tissues were fixed in 4% PFA and embedded in Paraffin to be sectioned at 5 µm thick and stained with H&E (AML Laboratories Inc., Belair Road Baltimore, MD, USA). Slides were examined and adipocytes measured using Metamorph® version 7.6 (Molecular Devices Inc., Sunnydale, CA). From each mouse, four tissue sections were used for analysis with three digital images made of each section.

Statistical Analysis

All data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using SAS® 9.3 (SAS Institute Inc., Cary, NC) or PRISM® 5.0 (Graph Pad Software, Inc., La Jolla CA). The Student’s t-test, 1-way, 2-way, or 3-way ANOVAs were used when appropriate. Alpha level was set at ≤0.05. Modified Tukey post hoc or Bonferroni correction tests were used to compare means. The Bonferroni correction test is a method used to offset the problem of multiple comparisons and control the family wise error rate. The Statistical Consulting Center at Wright State University provided complex statistical analyses.
Figure 4: Representation of swimming the mice
Figure 5: Representation of Distance and velocity measurements

Figure 6: Representation of oxygen uptake (VO$_2$) (ml/min)
IV. RESULTS

General physiologic and body composition parameters

To examine the stress-induced changes in AT1aR KO mice (KOEX) and wild type mice (WTEX), the following parameters were monitored at week 1 and after 7 weeks of swimming exercise.

a) Body weight: KOEX have a large body weight compared to the WTEX at week 1 and after 7 weeks of swimming exercise. Results showed that there was no effect of exercise on the body weights of KOEX and WTEX mice (Figure 7).

b) Percent fat: KOEX mice have a % body fat larger than the WTEX at week 1 and after 7 weeks of swimming exercise. There was no effect of exercise on the % body fat of KOEX and WTEX mice (Figure 8).

c) Food intake: Results showed that 7 weeks of swimming exercise had no effect on food intake in KOEX mice. Similarly, duration of swimming exercise showed no effect on food intake in WTEX mice (figure 9).

d) Water consumption: Results showed that 7 weeks of swimming exercise had no effect on water intake in both groups (Figure 10).

Effects of Acute Stress on Mouse HPA- Axis

a) Cage change/Handling stress: There was a significant increase in urinary CORT at 15 and 30 min (2.7 and 2.8 fold inc. at 15 and 30 min), in WT mice as compared to the baseline (Figure 11). The data also showed that there was a significant increase in urinary NE (1.9 and 2.3 fold inc. at 15 and 30 min) and EP (3.6 and 4.9 fold inc. at 15 and 30 min) in WT mice as compared to the baseline (Figure 12). Results indicate that urinary CORT and catecholamines provide a good means to examine stress induced adrenal
secretion in conscious mice and using urine as a biomarker for detection of the stress response.

b) *Acute swim stress:* There was a significant increase in urinary CORT at 30 min post swim (5.9 fold increase) as compared to the baseline (Figure 13). Results from acute swim stress also showed that urinary CORT provide a good means to examine stress induced adrenal secretion in conscious mice.

**Evidence of HPA-axis habituation**

*a) Effects of chronic swimming exercise on urinary corticosterone concentration:* To investigate the role of HPA-axis in the stress response, urinary corticosterone levels were measured in urine samples using RIA. In the present study, urinary CORT was used to show the dynamic response to exercise stress. The present results show that after 7 weeks of swimming exercise, urinary CORT decreased in KOEX after 2hour from the swimming session compared to the WTEX. These data also showed that the KOEX mice had lower urinary CORT at baseline as compared to the baseline in the WTEX mice (Figure 14).

*b) Effects of chronic swimming exercise on plasma corticosterone concentration:* Plasma corticosterone levels were measured in plasma samples using RIA. There was a significant reduction in plasma corticosterone levels after 7 weeks of swimming exercise in wild type (WTEX) mice (Figure 15) and AT1aKO (KOEX) mice (Figure 16) compared to the control mice in the same group.

c) *Cage change/handling stress induced HPA stress response:* Again, comparing the stress responsiveness in KOEX and WTEX mice during the first week and after 7 weeks of swimming exercise found that the KOEX mice produced less urinary CORT in
response to the cage change/handling stress as compared to the WTEX mice during the first week (Figure 17). Cage change-handling-induced stress led to significantly decreased urinary CORT over 30 min after 7 weeks, suggesting that the chronic exercise produced adaptation in the acute stress-induced stimulation of the sympathetic system (Figure 18).

**Catecholamines determination in urine and adrenal glands**

*a) Urinary catecholamines measurements*: To detect the effect of chronic swimming exercise on changes in urinary catecholamines of KOEX and the WTEX mice, 100 µL aliquots of the spot urine collection were measured using HPLC-ECD. Results showed after 7 weeks of swimming exercise, no significant difference in excretion of urinary NE (Figure 19A) and urinary EP (Figure 19B) in KOEX mice compared to WTEX mice.

*b) Adrenal catecholamines levels measurements*: To detect the influence of chronic swimming exercise on catecholamines in the adrenal glands in KOEX and WTEX mice, aliquots of adrenal supernatants were measured using HPLC-ECD. Results showed after 7 weeks of swimming exercise, adrenal levels of catecholamines were not significant different between WTEX and KOEX mice (Figure 20).

**Distance and Velocity measurements**

There was a significant increase in the distance in KOEX mice as compared to the WTEX mice during swimming exercise (Figure 21). The data showed that there were significant increases in velocity in KOEX mice as compared to the WTEX mice during swimming exercise (Figure 22).
Oxygen uptake

Chronic swimming exercise had no effect on the oxygen uptake (VO₂ (ml/min)) in KOEX mice compared to the WTEX during swimming exercise (Figure 23).

Echocardiography Analysis

Effects of chronic swimming exercise on cardiac function in WTEX and KOEX mice

A 2D M-Mode Echo analysis was conducted before (baseline) and after 7 weeks of swimming exercise to assess cardiac functions in KOEX and WTEX mice. Chronic swimming exercise produced a significant decrease in percent fractional shortening (FS %) in WTEX mice from 35% to 27% (baseline vs. exercise) (Figure 24). Parasternal short axis 2D images showed a marked increase in left ventricle end systolic area (LVESA, cm²) in WTEX as compared to the baseline and KOEX mice. The mean change in LVESA in WTEX increased from 0.094 cm² at baseline to 0.116 cm² after 7 weeks of exercise (Figure 25). The end result was a reduction in EF% in WTEX as compared to baseline and KOEX mice. The mean changes in EF % in the WTEX mice were markedly decreased from 63.5% in the baseline to 54.2% after exercise (Figure 26). Results showed a trend in the WTEX mice toward a contractile dysfunction and decreased LV performance.

In contrast, LVESA was significantly decreased in KOEX mice from 0.111 cm² at baseline to 0.063 cm² after exercise (Figure 25). The left ventricular end systolic diameter (LVESD, cm) was also significantly decreased in KOEX mice as compared to the WTEX (Figure 27) after exercise, with no change in left ventricular end diastolic area (LVEDA, cm²) in both groups (Figure 28). The end result was a significant increase in EF % in KOEX mice as compared to the baseline and WTEX. The mean change was significantly increased from 57.18 % at baseline to 72.16 % after exercise (Figure 26).
Moreover, posterior wall thickness (PWT, cm) (Figure 29) and left ventricular mass (LVM) (Figure 30) were significantly increased in the KOEX mice as compared to the WTEX. The resulted LVM was normalized to the body weight. Transmitral Pulse Wave Doppler (PWD) mode showed a significant increase in A-wave velocity (cm/s) in KOEX mice as compared to the WTEX with no changes in E-wave velocity (cm/s) in both groups (Figure 31A, B). PWD imaging showed no significant changes in E/A ratio in both groups (Figure 32). Taken together, these results indicate an increase in pumping capacity and cardiac contractility in AT1aKO mice after chronic swimming exercise.

**Results of cardiomyocyte size quantification**

There were no significant differences in cardiomyocyte size between WTEX and KOEX mice after 7 weeks of swimming exercise, as measured using H&E staining method (Figure 33).

**Results of Collagen Quantification via Picrosirius Red Staining**

Collagen content within aortic tissue was visualized using polarized light imaging of Picrosirius Red stained sections (Figure 34). Quantification did not show significant differences in percent collagen in the aorta between WTEX and KOEX mice (Figure 35).

**Results of adipocyte size quantification**

There were no significant differences in cell size in white epididymal fat between WTEX and KOEX mice after chronic swimming exercise as measured using H&E staining method (Figure 36), (Figure 37).
Figure 7: Body weight measurement in KOEX and WTEX at week 1 and after 7 weeks of swimming exercise. Two-way ANOVA showed that swimming exercise resulted in no change in body weight of KOEX and WTEX mice and the KOEX mice have a large body weight compared to the WTEX at week 1 and week 7 of swimming exercise; *p<0.001 vs. WTEX. Data are represented as mean±SEM of group size (n=6-8).
Figure 8: Percent fat in KOEX and WTEX at week 1 and after 7 weeks of swimming exercise. Two-way ANOVA showed that swimming exercise had no effect on % body fat of KOEX and WTEX mice and the KOEX mice have a large % body fat compared to the WTEX at week 1 and week 7 of swimming exercise; *p<0.001 vs.WTEX. Data are represented as mean±SEM of group size (n=6-8).
Figure 9: Food intake measurement in KOEX and WTEX at week 1 and after 7 weeks of swimming exercise. Two-way ANOVA showed that swimming exercise had no effect on food intake in KOEX mice. Similarly, duration of swimming exercise showed no effect on food intake in WTEX mice. Data are represented as mean±SEM of group size (n=6-8).
Figure 10: Water intake measurement in KOEX and WTEX at week 1 and after 7 weeks of swimming exercise. Two-way ANOVA showed that swimming exercise had no effect on water intake in both groups. Data are represented as mean±SEM of group size (n=6-8).
Figure 11: Cage change/handling stress test was performed in WT mice. One-way repeated measures ANOVA showed that urinary CORT was significantly increased at 15 (*p<0.05 vs. baseline) and 30 min (*p<0.001 vs. baseline) after cage change. Data are represented as mean±SEM of group size (n=6).
Figure 12: Cage change/handling stress test was performed in WT mice. Repeated measure two-way ANOVA resulted in a significant increase in urinary NE after 15 min and EPI at 15 and 30 minutes without affecting kidney DA levels. *p<0.05 vs. baseline. Data are represented as mean±SEM of group size (n=6).
*Figure 13*: Acute swim stress was performed in WT mice. One-way ANOVA showed that the acute swim stress resulted in a significant increase in the urinary CORT after 30 min in WT mice as compared to the baseline. *p*<0.05 vs. baseline. Data are represented as mean±SEM of group size (n=6).
Figure 14: Urinary CORT levels in KOEX and WTEX mice after 7 weeks of swimming exercise. Two-way ANOVA showed decrease in urinary CORT in KOEX mice at baseline and after 2hour as compared to the WTEX. *p<0.05 vs.WTEX. Data are represented as mean±SEM of group size (n=6-8).
Figure 15: Unpaired t-test showed that there is a significant decrease in plasma CORT in WT exercise (WTEX) as compared to the WT control (WTCon) after 7 week of swimming exercise. *p<0.05 vs.WTCon.

Figure 16: Unpaired t-test showed that there is a significant decrease in the plasma CORT in KOEX mice as compared to the KOCon after 7 week of swimming exercise. *p<0.05 vs. KOCon. Data are represented as mean±SEM of group size (n=6-8).
*Figure 17:* Two-way ANOVA showed that there was a significant decrease in urinary CORT level of the KOEX mice at baseline and after 30 min cage change test as compared to the WTEX during the week 1 of swimming exercise; *p*<0.05 vs. WTEX.

*Figure 18:* Two-way ANOVA showed that there was a significant decrease in the urinary CORT levels of the KOEX mice at baseline and after 30 min cage change test as compared to the WTEX after 7 weeks of swimming exercise; *p*<0.05 vs. WTEX. Data are represented as mean±SEM of group size (n=6-8).
**Figure 19:** Urinary catecholamines concentration in KOEX and WTEX mice after 7 weeks of swimming exercise. Two-way ANOVA showed that there was no significant difference in either norepinephrine (A) or epinephrine (B) in KOEX mice as compared to the WTEX. Data are represented as mean±SEM of group size (n=6-8).
**Figure 20:** Influence of 7 weeks swimming exercise on the content of adrenal catecholamines of KOEX and WTEX mice. Two-way ANOVA showed that there was no significant difference in the adrenal levels of catecholamines in KOEX as compared to the WTEX. Data are represented as mean±SEM of group size (n=6-8).
Figure 21: Unpaired t-test showed that there was a significant increase in distance (cm) for KOEX mice as compared to WTEX during swimming exercise. *p<0.01 vs. WTEX. Data are represented as mean±SEM of group size (n=6-8).
**Figure 2**: Quartile distance swam for KOEX and WTEX mice. Repeated measures two-way ANOVA showed that there was a significant increase in velocity (cm/s) in KOEX as compared to WTEX during swimming exercise *p<0.05 vs. WTEX. Each bar represents mean±SEM of group size (n=6-8).
Figure 23: Unpaired t-test showed that there was no significant difference in oxygen uptake (VO₂ (ml/min)) in KOEX compared to WTEX mice during chronic swimming exercise. Data are represented as mean±SEM of group size (n=6-8).
**Figure 24:** Percent fractional shortening (FS %) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA showed that swimming exercise resulted in a significant decrease in FS% in WTEX mice as compared to the KOEX and baseline; *p<0.05 vs. KOEX; †p<0.05 vs. baseline. Data are represented as mean ± SEM of group size (n=6-8).
**Figure 25:** Left ventricle end systolic area (LVESA) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA using a Bonferroni’s posthoc test showed a significant increase in LVESA in WTEX mice as compared to KOEX and baseline; *p<0.05 vs. KOEX; †p<0.05 vs. baseline. Data are represented as mean ± SEM of group size (n=6-8).
Figure 26: Ejection Fraction (EF %) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA showed that swimming exercise resulted in a significant decrease in EF% in WTEX mice as compared to the KOEX and baseline; *p<0.05 vs. KOEX; † p<0.05 vs. baseline. Data are represented as mean ± SEM of group size (n=6-8).
Figure 27: Left ventricle end systolic diameter (LVESD) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA using a Bonferroni’s posthoc test showed a significant decrease in LVESD in KOEX mice as compared to WTEX mice; *p<0.05 vs. WTEX. Data are represented as mean ± SEM of group size (n=6-8).
Figure 28: Left ventricle end diastolic area (LVEDA) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA showed that there was no change in EDA in both groups. Data are represented as mean ± SEM of group size (n=6-8).
Figure 29: Posterior wall thickness (PWT) at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA using a Bonferroni’s posthoc test showed a significant increase in PWT in KOEX mice as compared to WTEX mice and baseline; *p<0.05 vs. WTEX; †p<0.05 vs. baseline. Data are represented as mean ± SEM of group size (n=6-8).
Figure 30: Unpaired t-test showed that there was a significant increase in left ventricular mass (LVM) in KOEX compared to WTEX after 7 weeks of swimming exercise *p<0.05 vs. WTEX. The resulted LVM was normalized to the body weight (BW). Data are represented as mean ± SEM of group size (n=6-8).
**Figure 31:** Assessment of mitral valve function at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA showed a higher A-wave velocity in KOEX as compared to WTEX (A); *p<0.01 vs. WTEX; †p<0.05 vs. baseline, with no change in E-wave velocity in both groups. Data are represented as mean± SEM of group size (n=6-8).
**Figure 32:** E/A ratio at baseline and after 7 weeks of swimming exercise in KOEX and WTEX mice. Two-way ANOVA showed that there was no change in E/A ratio in both groups. Data are represented as mean± SEM of group size (n=6-8).
Figure 33: Unpaired t-test showed that there was no difference in cardiomyocytes area in KOEX and WTEX after 7 weeks of swimming exercise. Each bar represents mean±SEM of group size (n=6-8).
Figure 34: Representative images of aortic sections stained for collagen using Picrosirius Red in KOEX (A) and WTEX (B). Images were taken at (10X) magnification using polarized light microscopy. Histological examinations did not show significant differences in percent collagen in the aorta between KOEX and WTEX mice.
**Figure 35:** Quantification of aortic collagen contents in KOEX and WTEX after 7 weeks of swimming exercise. Unpaired student t-test showed that there was no difference in collagen percentage (collagen %) between groups. Each bar represents mean±SEM of group size (n=6-8).
Figure 36: H&E staining of the white epididymal fat of the KOEX (A) and WTEX (B) after 7 weeks of swimming exercise. Histological examinations did not show significant differences in cell size in white adipose tissues between groups. Images were taken at (20X) magnification.
Figure 3: Unpaired t-test showed that there was no difference in cell size in white epididymal fat in KOEX and WTEX after 7 weeks of swimming exercise. Each bar represents mean±SEM of group size (n=6-8).
IV. DISCUSSION

The renin angiotensin system (RAS) plays a pivotal role in the regulation of blood pressure and in maintaining homeostasis. Previous studies revealed that AT1aR deletion produced a significant reduction in resting blood pressure (Chen et al., 1997; Oliverio et al., 2000). This is consistent with results from (Phillips et al., 2008) that infusion of Ang II into key brain nuclei raised blood pressure, an effect blocked by the ARBs or AT1R deletion. Moreover, many studies showed that Ang II also participates in the stress response (Yang et al., 1993, 1996). Several lines of evidence show that pharmacological inhibition or deletion of AT1aRs produced attenuation in the stress response. In studies conducted to determine whether or not Ang II and AT1R played important roles in the regulation of the stress response, it was found that the AT1Rs were increased in the rat’s brain by isolation stress experiments and ARBs prevented this response (Armando et al., 2001; Saavedra et al., 2005). Additionally, pretreatment with ARB (Losartan) provided protection from stress produced by immobility or forced swimming (Saavedra et al., 2007).

The aim of the present study was to establish the functional and structural outcomes in mice lacking the AT1aR and to determine the effects of chronic stress on adrenal and cardiac function in AT1aR knockout mice (AT1aR KO) and whether physical stress, in the form of swimming exercise, has an effect on HPA-axis response habituation in these mice. To confirm whether urinary CORT could be used as a biomarker for the detection of stress induced adrenal secretion in conscious mice, the
influence of acute stress on the mouse HPA-axis was made by measuring urinary CORT and catecholamine response to cage change stress in adult male C57 BL/6 mice. Additionally, urinary CORT response was measured after acute swim stress. Results showed an improved cardiac and reduced adrenal stress response to chronic stress in AT1a deficient mice. In addition, it was found that urinary CORT and catecholamine measurements provide a good means to examine stress induced adrenal secretion in conscious mice.

In studies where AT1aKO mice were used, an understanding of the essential role of AT1aR in regulation of blood pressure has been elucidated (Ito et al., 1995). Therefore, to explore AT1a’s role in the stress response, the AT1aKO strain was used. Consistent with many findings, 7 weeks of swimming exercise resulted in quantifiable increases in urinary CORT compared to baseline levels in KOEX mice as compared to the WTEX. In addition, results showed that the urinary CORT decreased in KOEX mice after 2 hour from the swimming session compared to the WTEX. These data also showed that the KOEX mice had lower urinary CORT at baseline as compared to the baseline in the WTEX. These results showed that the rate and ultimate extent of habituation were enhanced in KOEX mice more than the WTEX mice and attenuation in the adrenal response in KOEX mice might support the hypothesis that AT1aR plays an important role in the regulation of the enhanced HPA-axis stress response. In the present study, the KO mice were a good experimental model to investigate role of AT1aR in stress reaction response and the chronic swimming exercise can produce a classical stress response.

However, there are few studies which examined the stress response in AT1aR KO mice. Several lines of experimental studies reported the beneficial effects of
candesartan (ARBs) in blocking the peripheral and central AT1 receptors (Nishimura et al., 2000). This notion is supported by a study conducted on rats where peripheral administration of ARBs reduces HPA axis activation and central and peripheral sympathetic response to isolation stress (Armando et al., 2001). Another study reported that ARBs prevent the hypothalamic corticotropin-releasing factor response to isolation stress in rats demonstrating that the urinary and adrenal corticosterone was significantly lower in animals pretreated with the candesartan than in rats treated with vehicle. The authors postulated that the activation of AT$_1$ receptors is required for the HPA axis response to isolation (Armando et al., 2007). Due to the limited number of studies available on the AT1aR KO mice, further research needs to be done to provide a definitive answer on the role of AT1aR in control of stress response.

The present study aimed to develop a murine model of physical stress using AT1aKO mice to investigate role of AT1aR mediated Ang II actions in adrenal and cardiac stress response and characterize them by measuring distance and velocity. Individual mice were filmed using Shuttle Soft system software and a U-Eye camera as mentioned in the method section. The swimming mouse was filmed continuously for 5 min. From the recorded data, average swimming velocity (cm/sec) and total distance (cm) swum were computed. Data results found a significant increase in the distance and velocity in KOEX mice as compared to the WTEX. Moreover, there was a significant difference in the activity between KOEX and WTEX mice; the KOEX mice were more active and swum faster and were able to go a longer distance during the swimming sessions as compared to the WTEX. Interestingly, the greater swimming speed of the KOEX mice was visually evident when observing the swimming session. The WTEX
mice swam more slowly and could not keep a steady pace throughout the swim session. These data was supported by a study done on AT1aR KO mice where circadian rhythms of wheel running and drinking activity were significantly higher in KO mice as compared to the WT. Although KO and WT mice showed comparable levels of running and drinking during the first week of experiment, the KO mice ran a greater distance each week thereafter. The authors speculated that the ‘amplitude’ of running and drinking rhythms was higher in KO mice (Mistlberger et al., 2001). In a different study performed in our laboratories, blood lactate concentration was found to be significantly lower at five and 60 min after wheel exercise in AT1aKO mice as compared to the WT (Alhajoj, 2013).

At the beginning of exercise, the glycolytic energy pathway produces lactic acid, which then dissociates to form lactate and hydrogen ions (H+), which leads to an increase in acidity. Studies show that accumulation of blood lactate on the muscle performance may cause muscle fibers to fail due to an increase in hydrogen ions (Fitts, 1988; Poortmans, 2003). Decreases in blood lactate levels and maintenance of normal pH in the KOEX mice could be a plausible explanation for why the KO mice were able to swim greater distances. Quick adaptation of the metabolic pathways where less lactate is produced (aerobic transition) and/or the conversion of lactate to pyruvate may lessen some of the damaging effects of stress by enhancing habituation response of HPA-axis to the repeated stress in KO mice; ultimately reducing the amount of CORT produced to which the body is exposed. This could be another reason why the KOEX mice are more active during each swimming session as compared to the WTEX.

The results of the present study demonstrate that chronic swimming exercise facilitates an adaptive process of HPA-axis response habituation in AT1aKO mice. This
finding agrees with previous studies and indicates the participation of Ang II and the AT1R in the stress reaction mediated by the HPA-axis and sympatho-adrenal axis (Shelton, 2007). This same response is also supported by another experimental study which showed that peripheral administration of the ARBs prevented central and peripheral sympathetic stimulations and abolishes the HPA-axis activation during isolation stress. These findings suggest further the possible involvement of circulating Ang II in the stress responses via the activation of the AT1R (Pavel et al., 2008).

Moreover, it has been shown that central Ang II is associated with HPA-axis activation and attenuation responses to stress and anxiety (Aguilera et al., 1995). The present study, therefore, is supported by previous studies, which demonstrate that Ang II, through AT1aR stimulation, is an important stress hormone, and ARBs, in addition to their antihypertensive effects, may be considered as a novel treatment of stress-related disorders.

In agreement with the results of the above studies, the present results demonstrate the attenuation in the HPA-axis response in KOEX mice after 7 weeks of chronic swimming exercise as compared to the WTEX mice at the adrenal level due to a significant decrease observed in the responsiveness to the acute cage stress experiment. Collectively, findings point toward AT1aR as an important modulator to the maladaptive stress response resulting from chronic swimming exercise and supporting the idea that Ang II is an important stress hormone (Yang et al., 1996). A major finding in the present study is that CORT levels are decreased in response to repeated stress in the AT1aKO mice. Once again, this finding supports the idea that the activation of the AT1aR is important for development of full stress response induced HPA-axis stimulation. The
improved HPA responses to chronic stress in AT1a KO mice indicate that blockade of the AT1aRs might induce therapeutic advantages in the stress-related disorders such as depression and anxiety.

The present study aimed also to investigate the effects of chronic swimming exercise on cardiac function in AT1aR KO mice. The AT1aR isoform is highly expressed in the heart (Burson et al., 1994). Local Ang II acts within cardiac tissues through the influence of protein synthesis and cellular growth (Aceto et al., 1990; Lindpaintner et al., 1991; de Gasparo et al., 2000). A growing body of evidence has suggested that Ang II through the AT1R may be involved in development of cardiac hypertrophy (Weber et al., 1991; Sadoshima et al., 1993; Yamazaki et al., 1995; de Gasparo et al., 2000).

A study by Patten et al. (2003) confirmed the beneficial effects of inhibition of RAS with ARBs on myocardial hypertrophy after myocardial infarction. The results of the present study Echo analyses show a decrease in LV performance in WTEX mice after 7 weeks of swimming exercise. In contrast, KOEX mice presented improvement in cardiac contractility. Percent of FS, one of the echocardiographic indexes of LV systolic function, was measured and showed a marked reduction in WTEX mice as compared to the baseline and KOEX. Cardiac fractional shortening percent represents the change in the functional diameter of the LV following a cardiac cycle. Decreases in the % FS serves as an indicator of reduced cardiac performance (Syed et al., 2005). Moreover, EF % was markedly decreased in WTEX mice as compared to the baseline and KOEX. Ejection fraction represents the volumetric portion of blood that is pumped from the heart each cardiac cycle. A decrease in its value also indicates that the function of the heart decreases. ESA was greater in WTEX mice as a sign of an increased stress response.
Increases ESA in WTEX mice may be considered as an early indication of cardiac pathology. The Echo data exhibited systolic dysfunction in the WTEX mice demonstrated by a decrease in FS % and EF %. Thus, the Echo data indicate that the AT1a receptor plays an important role in stress induced cardiac dysfunction during exercise training.

In agreement with present results, two studies reported that AT1 receptor expression increases in response to exercise training induced cardiac hypertrophy without any alterations in the other components of the RAS. Moreover, treatment with Losartan prevented this adaptive hypertrophy (Oliveira et al., 2009; Fernandes et al., 2011). Based on their results, the authors postulated that the AT1 receptor stimulation is necessary for the development of cardiac hypertrophy in response to exercise training. This notion is supported by a study that demonstrated that the local RAS is activated by hemodynamic overload and that the AT1 receptor might play an essential role in the development of cardiac hypertrophy induced by stress (Dostal, 2000). This finding agrees with Fernandes et al. (2011) where swimming exercise training caused an increase in cardiac AT1 receptor levels with decrease in other components of the RAS including AGT and Ang I levels, ACE activity and protein expression in rats, as well as Ang II levels. Moreover, Angiotensin converting enzyme 2 (ACE2) and Ang (1-7) levels were increased in the hearts of trained rats. The authors suggested that there was exercise-induced cardiac hypertrophy via the cardiac AT1 receptor. They also postulated that the increased ACE2 and Ang (1-7) in the heart by exercise could counteract the classic cardiac RAS. The counterbalancing effect increases vasodilatation in response to the demands of swimming exercise for increasing the transport of blood and oxygen supply to the exercising cardiac muscle (Fernandes et al., 2011). This could also be a plausible explanation for
mechanism through which the heart in KOEX mice protects itself from the cardiac
dysfunction induced by stress during exercise training. Zou et al., 2004 reported that,
AT1 receptor converts mechanical stress into a biochemical signal inducing LVH. The
mechanism of increase AT1 receptor expression may be related to an independent action
of the AT1 receptor. AT1 receptors have no direct cell signaling pathway to tyrosine
kinase and the MAPK pathways for cell growth but have an indirect pathway to stimulate
epidermal growth factor receptor (Kagiyama et al., 2002, 2003).

A study done on transgenic animals demonstrate that increased expression of AT2
receptors after myocardial infarction maintained cardiac function (Yang et al., 2002). In
another study, it was reported that the exercise training in rats leads to a physiological left
ventricular hypertrophy associated with increased in cardiac AT1 and AT2 receptors
expression (Fernandes et al., 2011). It has been known that, although the AT1 and AT2
receptors have the same ligand binding affinity, they may serve opposing functions in the
heart (Dostal, 2000; Reudelhuber et al., 2007).

Horiuchi et al. (1999) reported that the AT2 receptor associated with
dephosphorylation and inactivation of growth factor-activated MAPK may provide a
protecting role in the heart. Moreover, AT2 receptor activates nitric oxide and bradykinin,
leading to a vasodilation (Tsutsumi et al., 1999). Based on these results and the results of
the present study, it can be speculated that the increased AT2 receptor expression in heart
during exercise training may be increase vasodilatation and reduced vascular resistance
which might increase blood and oxygen transport to the exercising cardiac muscle to
facilitate high cardiac performance. We postulated that this could be a possible
mechanism through which the heart in KOEX mice protects itself from the cardiac dysfunction induced by stress during exercise training.

Shikata et al. (2003) demonstrated the beneficial effects of inhibition of RAS with ACE inhibitors or ARBs on progression of cardiac hypertrophy and improving cardiac performance. AT1 receptor blockade, in addition to the major effect of hindering the action of Ang II, stimulates the cardiac AT2 receptor and accelerates the processing of Ang II by the action of ACE2, inducing vasodilator and anti-fibrotic effects and conferring therapeutic benefits on patients with cardiac disease (Keidar et al., 2007).

A study by Pereira et al. (2009) reported that exercise training attenuates deleterious cardiac remodeling and also preserves cardiac function in cardiovascular disease. These beneficial effects may be due in part to the exercise training-induced attenuation of the RAS. These authors also demonstrated that aerobic exercise training reduced cardiac Ang II levels and ACE activity in a genetic model of sympathetic hyperactivity-induced heart failure, while it increased ACE2 expression and prevented exercise intolerance and ventricular dysfunction with little impact on cardiac remodeling (Pereira et al., 2009). Taken together, these data provide evidence that reduced cardiac RAS explains at least in part the beneficial effects of exercise training on cardiac function.

Histology did not show any evidence of left ventricular pathology in either group. Collagen levels in the aortic tissues were not increased in either of the exercise groups. Typically, increases in collagen levels in the heart indicate diminished cardiac performance (Lutgens et al., 1999). Although collagen could not be observed in the heart of the KOEX and WTEX mice does not mean that cardiac dysfunction did not occur. Previous studies have reported that LV hypertrophy can occur in mice without increased
collagen formation (Holtwick et al., 2003). There were no significant differences in the cardiomyocytes between WTEX and KOEX mice as measured in the H&E staining method. Therefore, the increase in LV mass in KO mice as compared to WT may be an adaptive response to chronic exercise training.

As mentioned previously, there was a trend toward a contractile dysfunction in the WTEX mice and an increase in pumping capacity in the KOEX mice during exercise training. This finding agrees with another clinical study where treatment with ARBs significantly decreased mortality rates in subjects with cardiac failure more than those who were treated with ACE inhibitors (ACEIs) (Pitt et al., 1997). These authors also postulated that ARBs offers superior cardiac protection over ACEIs in patients with cardiac failure by decreasing sudden death. The present study results coupled with these findings allow speculation that the cardioprotective effects of ARBs might be partly mediated by its influence on AT1αR. It may then be concluded that AT1α signals are an important mediator of stress induced cardiac dysfunction during exercise training.

The present findings of this study suggest the potential of ARBs with regular exercise as a strategy for treatment of many cardiac problems. Results showed that chronic exercise improves cardiac function in the KO mice while exerting a negative impact on the WT mice. The reason for this difference could be attributable to attenuation in the stress response by absence of AT1αR signals. Data from previous research demonstrating a decrease in blood pressure and LV loading in the KO mice (Ito et al., 1995; Chen et al., 1997; Oliverio et al., 2000). This could be one reason to explain the advantageous effects of KO mice in the prevention of LV changes after prolonged or
repeated stress. This could also explain why KOEX mice exhibit improvement in cardiac performance as compared to the WTEX mice during exercise training.

The findings from the present study suggest that AT1aR KO mice demonstrated improvement in cardiac performance without myocardial hypertrophy and greater exercise endurance in response to chronic exercise. These results suggest that the AT1aR is an important mediator of exercise induced cardiac dysfunction during exercise training. AT1aR may play important role in maladaptation in the heart after prolonged or repeated stress. Hence, it follows that decreased LV performance in WT mice during swimming exercise might cause detrimental effects on cardiac function under prolonged or repeated stress, resulting in heart problems. However, due to the limited number of studies available on AT1aKO mice, further investigation is required to clarify whether AT1aR signals is really necessary for induced cardiac dysfunction in response to the stress during exercise training.
VI. CONCLUSION

The results in the present study demonstrate that the Angiotensin type 1a receptor knockout mice show an improvement in cardiac performance, increased pumping capacity, increased cardiac contractility without myocardium hypertrophy and greater exercise endurance in response to chronic exercise. Exercised AT1aKO mice exhibit less negative impact of prolonged or repeated stress on health as compared to the wild type mice by enhancing habituation of the CORT response ultimately reducing the amount of CORT to which the body was exposed. The improved HPA responses to chronic stress in AT1aR KO mice indicate that blockade of the AT1aRs might induce therapeutic advantages in stress-related disorders. These observations may offer a rationale for exploring the role of AT1aRs in the stress response and for the use of ARBs as a therapeutic tool to maintain homeostasis under long-lasting stress conditions. Moreover, ARBs could also be considered as adjunct therapy in the treatment of anxiety and other stress related disorders. Finally, we can conclude that AT1a receptor signals are an important mediator of stress induced cardiac dysfunction during exercise training.
APPENDIX A

Effects of acute stress on mouse hypothalamic pituitary adrenal axis

Najat Almahroug, Raquel Oliveria, James B. Lucot, Mariana Morris, Roberta Pohlman.
Boonshoft School of Medicine, Wright State University, Dayton, OH

Stressful situations induce physiological and behavioral changes in order to maintain homeostasis. The goal was to assess the influence of acute stress on the mouse hypothalamic – pituitary adrenal axis (HPA). We measured the urinary corticosterone (CORT) and catecholamine response to cage change stress in adult male C57 BL/6 mice (15 weeks age, n=6/group). We also measured the CORT response to swim stress. Mice were housed under standard conditions. For the cage stress, animals were transferred to a clean cage with new bedding for 15 min. The swim test was conducted for 15 minutes in 22-25°C water. Water was circulated using a pump in order to encourage continuous swimming. Urine samples were collected at baseline (pre stress), 15 and 30 min post stress. Urinary CORT was measured using radioimmunoassay. Catecholamines were measured using HPLC plus electrochemical detection. Creatinine was measured to normalize values for renal function. For cage change stress, there were increases in CORT (2.7 and 2.8 fold inc. at 15 and 30 min), norepinephrine (1.9 and 2.3 fold inc. at 15 and 30 min) and epinephrine (3.6 and 4.9 fold inc. at 15 and 30 min). For the swim stress, there were increases in CORT at 30 min post stress (5.9 fold). Results showed that urinary CORT and catecholamine provide a good means to examine adrenal function in conscious mice.

Poster presented at the 2012 Ohio Valley Chapter Society of Toxicology; Columbus, Ohio
APPENDIX B

Effects of chronic swim stress on cardiac function in Angiotensin AT1a Receptor knockout mice. Najat Almahroug, Ahmad Alhajoj, Mahmoud Alghamri, Roberta L. Pohlman, Lynn Hartzler, Mariana Morris
Boonshoft School of Medicine, Wright State University, Dayton, OH

There is growing consensus that exercise training reduces cardiovascular risk. Moreover, exercise training has been recognized as an important and safe strategy for prevention and treatment of heart failure. Angiotensin II (Ang II) through the activation of Ang type I receptor (AT1R) has patho-physiological relevance in cardiac remodeling. The goal was to assess the influence of chronic swimming stress on the cardiac function in AT1aR knockout mice. Adult male AT1R KO and wild type (WT), (15 weeks age, n=6/group) were used. The swim test was conducted for 60 minutes, 3 days a week for 7 weeks in 22-25°C water. Cardiac function was assessed by echocardiography (Echo), at week1 and at the end of the study, week7. Distance, velocity and maximum oxygen consumption (VO2 Max (ml/min)) were recorded. Results showed that there was a significant higher ejection fraction (EF %) in AT1R KO exercise (AT1RKOE) versus WTEX (75 % versus 55 %). Distance (cm) and Velocity (cm/s) significantly increased in AT1R KOEX as compared to WTEX, \( F (1, 32) =9.35, p < 0.01 \). The (VO2 Max) analysis showed no differences between the groups (AT1R KO EX versus WTEX). The exercise produces a greater change in pumping capacity in AT1aRKO.

Poster presented at the 27th Meeting of the Ohio Physiological Society, Wright State University, Dayton, Ohio.
APPENDIX C

Effects of Chronic Swim Stress on Endocrine and Cardiac Function in Angiotensin AT1a Receptor Knockout Mice
Najat Almahroug, Ahmad Alhajoj, Mahmoud Alghamri, Mariana Morris, Roberta L. Pohlman
Boonshoft School of Medicine, Wright State University, Dayton, OH

Stress is an event or stimulus that causes an often abrupt but always large change in autonomic activity and hormone secretion. Angiotensin II (ANG II) is one of the main neurohormonal mediators that are stimulated by stress. The goal of this study was to assess the influence of chronic swim stress on adrenal and cardiac function in AT1 receptor knockout mice. Adult male AT1 receptor knockout (KO) and wild type (WT) mice were challenged to moderate exercise and were compared to sedentary groups (n=6/group). Exercise paradigm was swimming, 1 hour 3d/wk. for 7 weeks. Urine and plasma corticosterone (CORT) were measured by radioimmunoassay (RIA). Urinary catecholamines were measured using high-pressure liquid chromatography (HPLC) with electrochemical detection. Cardiac function was assessed by echocardiography (Echo). Results showed that plasma CORT was lower in both KOEX and WTEX as compared to sedentary groups (3 fold decrease). Urinary CORT was used to show the dynamic response to exercise stress. The stimulatory change was 10 fold higher in WTEX as compared to 5 fold in KOEX. Urinary catecholamines results indicate that there are no significant differences in Norepinephrine (NE) and Epinephrine (EP) responses between the KOEX and WTEX. The swimming distance (cm) as well as the swimming velocity (cm/s) were significantly higher in KOEX as compared to WTEX, \( F(1, 32) = 9.35, p < 0.01 \). In addition, Echo results showed a higher ejection fraction (EF %) in KOEX versus WTEX (75 % versus 55 %). Assessment of mitral valve function showed a higher A-wave velocity in KOEX as compared to WTEX. We concluded that ANG II AT1 receptor deficient mice showed improved cardiac response and reduced adrenal stress response to chronic exercise.

Poster presented at the Experimental Biology (EB) meeting 2013; Boston.


Alhajoj, A. M. (2013). Role of AT1a receptor in cardiac function and acid-base homeostasis during exercise endurance [Abstract].


Physiology. Regulatory, Integrative and Comparative Physiology, 303(3), R321-R331. 
doi:10.1152/ajpregu.00007.2012


