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The Increase in Renal Sodium Excretion in Response to Angiotensin II Infusion in Exercised Female Rats is Dependent on a Rise in Renal Perfusion Pressure

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THE INCREASE IN RENAL SODIUM EXCRETION IN RESPONSE TO ANGIOTENSIN II INFUSION IN EXERCISED FEMALE RATS IS DEPENDENT ON A RISE IN RENAL PERFUSION PRESSURE.

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ABSTRACT

THE INCREASE IN RENAL SODIUM EXCRETION IN RESPONSE TO ANGIOTENSIN II INFUSION IN EXERCISED FEMALE RATS IS DEPENDENT ON A RISE IN RENAL PERFUSION PRESSURE. K. Janssen, P. Knoblich. (2009) Department of the Biological Sciences, Minnesota State University, Mankato, MN 56001.

Prior studies in this lab have shown that chronically exercised female spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats excrete a greater amount of sodium in response to angiotensin II (Ang II) infusion than do sedentary rats. The current study determined if the difference in renal sodium excretion persisted when the renal perfusion pressure (RPP) was held constant. Female SHR and female WKY rats were separated into sedentary and exercised groups at 4 weeks of age. The exercise group voluntarily exercised for at least eight weeks using an exercise wheel and time/distance monitor. At 13 weeks of age or older, rats were anesthetized and catheters were placed into the jugular vein, and carotid and femoral arteries. A noose, placed around the abdominal aorta, was continually adjusted to maintain a constant RPP during Ang II infusion. Ang II was infused at 0, 0.125, 0.5, and 2.0 μg/ml saline, each for 15 minutes. MAP and RPP were measured continually. Urine was collected during each 15 minute period and analyzed for sodium excretion. Sodium excretion did not significantly increase with the Ang II infusions when RPP was held constant, and no difference was found between the exercised and sedentary rats. The increase in renal sodium excretion observed in exercised female rats compared to sedentary appears to be due to a difference in the pressure natriuresis response.

TABLE OF CONTENTS

INTRODUCTION

High blood pressure, usually referred to as hypertension is a common disorder that is known to be a major risk factor for many diseases (8, 9, 38). Hypertension can lead to many complications, some of which include cardiovascular disorders such as left ventricular hypertrophy, stroke, ischemic heart disease, myocardial infarction and renal disease (17). If left untreated, the risk of these complications increases two to three fold (17).

The pathogenesis of hypertension is not fully understood, although researchers have proposed many different theories. Some of these theories include a defect in the control of sodium excretion by the kidney or a vascular defect (9). Overall, hypertension has three main contributing factors: 1) abnormal vascular tone, 2) abnormalities in blood volume and salt regulation, and 3) vessel wall remodeling (38). These three mechanisms rely on both nitric oxide (NO) and Angiotensin II (Ang II), and the balance between them is important in hypertensive end-organ injury (20, 38).

Regulation of Blood Pressure

Mean arterial pressure (MAP) or blood pressure is under neural, hormonal, and hemodynamic control (30). Two determinants of MAP, cardiac output and total peripheral resistance, are under constant regulation by both short and long term mechanisms (30). The short-term mechanisms control peripheral vascular resistance, cardiovascular capacitance, and cardiac performance (30). The long term mechanisms for

regulation of MAP, work by regulating blood volume, sodium balance, and extracellular fluid volume (ECFV) (30, 33).

Sodium and extracellular anions, including chloride and bicarbonate, make up the osmotically active solutes in extracellular fluid (30). The regulation of these extracellular solutes takes place in the kidneys, which under normal conditions are able to alter the sodium excretion rate (30). Blood volume and ECFV are closely associated (30). Cardiovascular receptors are able to detect changes in ECFV and blood volume and therefore can affect the kidneys by altering renal nerve sympathetic activity, or various hormone levels, such as atrial natriuretic peptide (30). The mean circulatory pressure (MCP) is the filling pressure in the cardiovascular system, and this pressure reflects blood volume. By directly acting on the venous return to the heart, and cardiac output, the MCP influences MAP (30).

MAP is controlled in the short term by the baroreflex response, a buffering mechanism that counteracts variations in MAP (24). The baroreceptors are located in the aortic arch and carotid sinus and are part of the afferent pathway for the reflex. Arterial baroreceptors respond to increased stretch and rate of change of stretch. Efferent pathways of the arterial baroreceptors are the cardiovascular sympathetic and parasympathetic nerves (27). Increased blood pressure stimulates the baroreceptors causing a signal to be sent to the cardiovascular center in the brain. The cardiovascular center then stimulates the parasympathetic nervous system to decrease heart rate. The

sympathetic activity is inhibited, causing vasodilation and a further decrease in heart rate (29).

Baroreflex resetting is a mechanism that allows increases in sympathetic activity and heart rate for any given blood pressure (29). When blood pressure remains high for more than a few days, the reflex response is reset to a higher operational pressure point; the baroreflex response is unable to buffer blood pressure and instead works to maintain the pressure at the new higher level (29).

The baroreflex however, is still unclear as to its long term effects on MAP (24). Many studies suggest that a damaged baroreflex contributes to higher sympathetic activity, leading to an abnormal regulation of sodium excretion and MAP (24). However, the lack of techniques available to study the long term effects of baroreflex sympathetic activity has clouded the issue (24). Theories have been proposed regarding alterations in arterial baroreflexes in hypertension: 1) these alterations are potentially important in long term regulation of blood pressure, 2) they represent a hereditary component of the pathophysiology of essential hypertension, 3) abnormalities in baroreflex control of the parasympathetic activity (heart rate) may not always predict alterations in baroreflex control of the sympathetic nerve activity and vascular resistance (29).

The Kidney

The kidney has an outer region known as the cortex and an inner region known as the medulla. Within these two regions, millions of kidney tubules or nephrons are found.

Each nephron has the same structure, including a glomerulus, Bowman's capsule, proximal tubule, loop of Henle, distal tubule, and collecting duct (Figure 1).

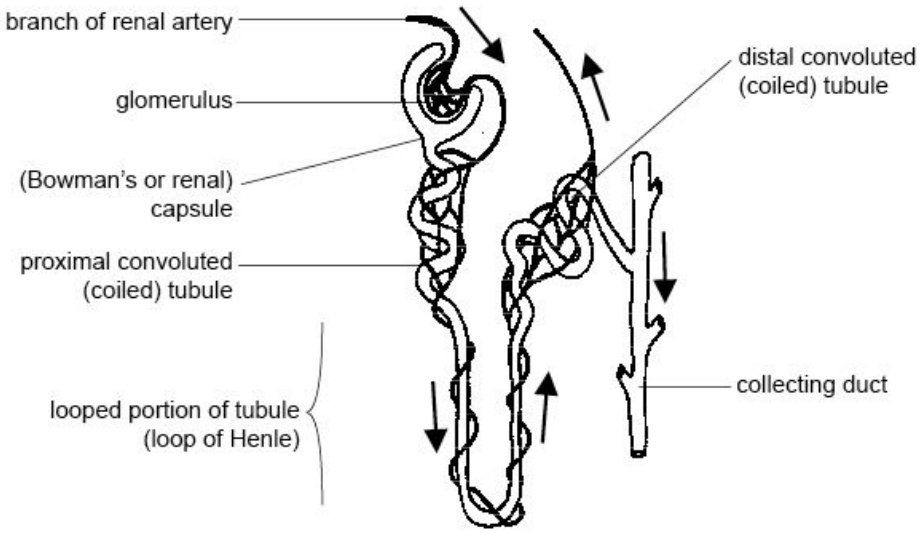

Figure 1The structure of a single nephron within the kidney. **(**http://en.wikibooks.org/wiki. Last updated 11/26/2007.**)**

Blood is brought to the kidneys via the renal artery, and an arteriole brings the blood to the nephron where filtration begins within the glomerulus. Blood pressure forces fluid out of the leaky capillary and fluid enters the tubular system. The filtrate starts moving through the proximal tubule where some water and ions are reabsorbed out of the tubule and back into the capillaries. The tubules may secrete other ions into the filtrate. The filtrate then reaches the loop of Henle, which creates an osmotic gradient in the renal interstitium. The loop of Henle then delivers the filtrate to the distal tubule and eventually the filtrate enters the collecting duct. Any substances not reabsorbed from the filtrate leaves the body as urine. The urine consists of urea, water and some salts. Urine

concentration depends on hormonal regulation of water reabsorption in the collecting duct.

If the body is dehydrated, causing a low blood volume, the kidneys will respond by increasing sodium reabsorption back into the blood. This will lead to an increase in plasma osmolarity and will stimulate the secretion of an anti-diuretic, such as antidiuretic hormone (ADH). Water will follow the reabsorption of sodium bringing the osmolarity back to normal, and causing a rise in the blood volume (27).

It is well accepted that renal function and high blood pressure (arterial pressure) are closely related (1, 6, 34). Renal vasoconstriction found in early stages of renal disease may lead to increased MAP and glomerular hyperfiltration (34). One study using rats showed that normotensive recipients of a renal graft from a genetically hypertensive donor developed post-transplant hypertension (30). Similar results were found in four different animal models.

Another study found that genetically hypertensive rats that had undergone a bilateral nephrectomy, had a reduction in MAP after receiving a kidney from a normotensive rat (30). Similar results have been described in humans. Recipients of grafts from genetically hypertensive donors required more antihypertensive medication than those who received kidneys from normotensive donors. Furthermore, patients that were dialysis-dependent due to hypertension-induced renal injury turned normotensive after receiving a kidney from a normotensive donor (1, 10). Although renal diseases are

known to be a cause of hypertension, many other factors, such as genetic predisposition and environment, may also contribute (1).

Regulation of Blood Pressure by the Kidney

Renal perfusion pressure (RPP) and sodium excretion have a direct relationship (30). Increases in RPP will produce natriuresis and diuresis (30). Natriuresis is the excretion of sodium ions in the urine by the kidneys, whereas diuresis is an increase in urine volume. The mechanism is not fully understood, however the proximal tubule, thick ascending limb of Henle, and collecting duct have all been studied as potential sites where perfusion pressure alters the tubular reabsorption response (33). One study concluded that tubular reabsorption can be influenced greatly by the level of MAP, independent of other neural or hormonal regulators of renal function (33). These results indicate that even small daily alteration in MAP, on the order of 5mmHg, may affect the regulation of sodium and water excretion on a short term basis. However, this study did not elucidate or identify the mechanism for the pressure-diuresis relationship (33, 38).

A damaged pressure-natriuresis relationship results in a higher blood pressure requirement to maintain the same level of sodium excretion (33, 38). The slope of the plot of blood pressure versus sodium excretion in urine is subject to change due to many factors (30). A steeper slope of the pressure-natriuresis relationship indicates a greater sodium excretion response to an increase in MAP (30). An increase in renal sympathetic tone, increased activity of the renin-angiotensin-aldosterone system (RAAS), and greater levels of circulating catecholamines have been shown to reduce natriuresis, leading to a

reduction in the slope (30). Catecholamines are hormones released by the adrenal gland as part of the sympathetic nervous system and include epinephrine and norepinephrine. Nitric oxide (NO) can also affect pressure natriuresis (30, 38). Increases in MAP stimulate intrarenal production of NO, which is known to both inhibit tubular sodium reabsorption and act as a vasodilator (30).

Renin-Angiotensin-Aldosterone System (RAAS)

Although inadequate volume regulation leads to hypertension, most forms of hypertension are more complex, involving increased production of vasoconstrictor substances that reduce renal blood flow and glomerular filtration rate, and enhance tubular sodium reabsorption (30). The most important system with regards to clinical significance in maintaining fluid volume and vascular resistance is the RAAS (7, 8, 9). The RAAS regulates blood pressure by affecting vascular resistance and controlling sodium excretion by the kidneys (7, 9, 11). If the RAAS is activated under the wrong conditions, such as salt abundance or various types of renal injury, it can play a primary role in hypertension (30).

The enzyme renin is formed by the juxtaglomerular cells in the kidneys in response to low blood pressure or low sodium. Renin cleaves angiotensinogen to form an inactive decapeptide, angiotensin I (Ang I), (11, 30, 37) Ang I is then converted to angiotensin II (Ang II), an active octapeptide, by angiotensin-converting enzyme (ACE) (38). The immediate effect of active Ang II is to act on the walls of arterioles causing them to constrict, therefore increasing vascular resistance and blood pressure (38). Ang II

also stimulates the release of aldosterone from the adrenal gland which, within a couple of hours, causes an increase in sodium and water reabsorption, raising blood volume and MAP (Figure 2) (2).

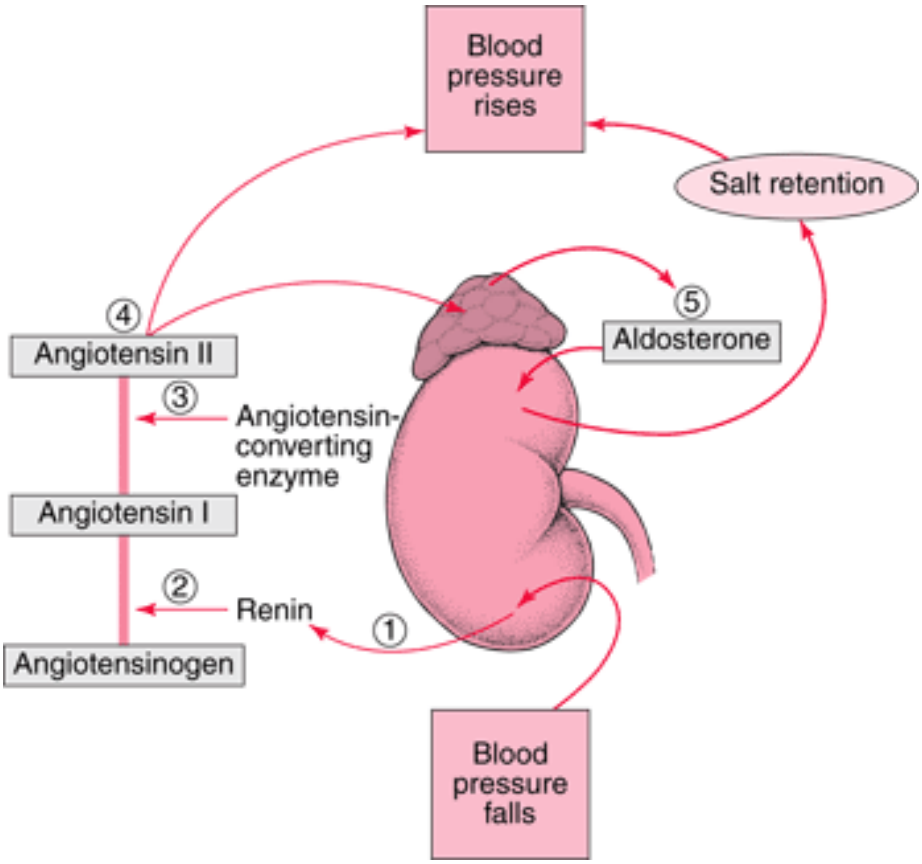

Figure 2 The renin-angiotensin-aldosterone system.
(https://eapbiofield.wikispaces.com; Chapter 42.**)**

Ang II plays an important physiological role in the regulation of blood pressure, plasma volume, sympathetic nervous activity, and thirst responses (Figure 3) (3). The circulating RAAS is important in short term regulation of the cardiovascular system and is activated under acute conditions, such as low blood pressure, low blood volume, and

hemorrhage (38). Ang II also has a pathophysiological role in cardiac hypertrophy, myocardial infarction, hypertension, and atherosclerosis (3).

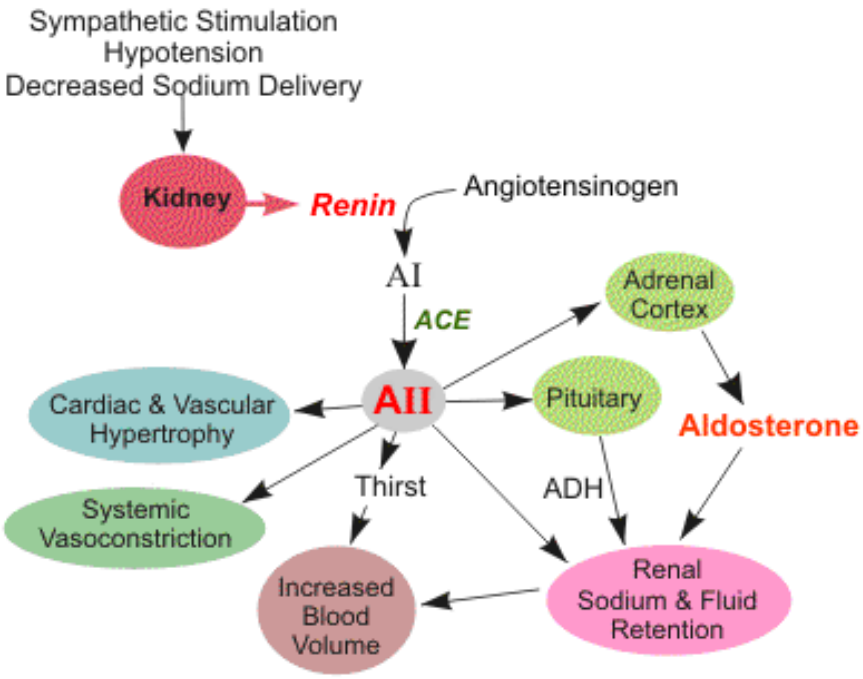

Figure 3 Angiotensin II and its effects within the body.

(www.cvphysiology.com Last updated: 1/1/2007.**)**

There are two different RAAS; classical (systemic), and local (tissue) (22). Classical RAAS synthesizes Ang II via circulating renal derived renin, which cleaves angiotensinogen to Ang I. Ang I is converted by ACE in the lungs to the active Ang II (3). Local RAAS is characterized by 1) the presence of RAAS components in tissues; angiotensinogen and the conversion enzymes, 2) synthesis of Ang II in specific tissues, and 3) binding Ang II to specific receptors found within localized tissues (22). The local system has been identified in the heart, kidneys, brain, pancreas, and reproductive, lymphatic, and adipose tissues (22).

Although the components of the RAAS are expressed in many tissues and organs, they are mainly found in the kidneys (11, 35). The components are found close to the renal vasculature and tubules, and include renin, receptors for renin to bind, and angiotensinogen (35). The renin receptor is a 350 amino acid protein with a single transmembrane domain (35). The receptor is found within the kidney in the glomerular mesangium and in the subendothelial layer of the renal arteries (35). Stimulation of the renin receptor causes cell hypertrophy and renal arteriolar vasoconstriction, suggesting that the receptor may have a role in the development of renal and cardiovascular diseases (11, 35).

The messenger RNA and proteins for the RAAS have been localized to proximal tubule cells (5). Ang II concentration within the lumen of the proximal tubule has been reported to be higher when compared to plasma concentration (5, 30). These findings support the conclusion that Ang II is produced in the proximal tubule cells and then is released into the lumen where it binds to receptors on the tubular cell membrane, or travels to more distal nephron segments (5, 35). Intrarenal levels of Ang II are relatively high when compared to plasma concentrations, even in nonhypertensive conditions, and independent of salt intake. The intrarenal levels of Ang II increase more in hypertensive conditions, and are regulated by many mechanisms. Low dietary salt intake, enhanced sympathetic tone, and different stress-related conditions all stimulate local renin production and release.

Ang II acts on two different plasma membrane receptors: angiotensin type 1 receptor (AT1) and angiotensin type 2 receptor (AT2). The AT2 mRNA has been identified in the adrenal gland, heart and brain of rats and the receptor has been identified in the kidney and heart of rats (28, 38). The receptor expression has been reported to decrease with age as its mRNA was found in fetal and neonatal rat kidneys, but was not detected in the adult kidney (28). However, immunohistochemical studies indicated the AT2 receptor is present in mature rats, just at reduced levels (28).

The functions and signaling pathways for AT2 are unclear (3). Research suggests AT2 may antagonize AT1 under physiological conditions by inhibiting cell growth and inducing apoptosis and vasodilation (3). Vascular vasodilatation induced by AT2 may be through the nitric oxide-bradykinin-cGMP cascade (35). The AT2 receptor may also play a separate role in counteracting the antinatriuretic effect of Ang II, by inhibiting renin synthesis (28, 35, 38).

Ang II primarily acts on AT1 receptors (10, 30). AT1 receptors can be found in the heart, kidney, blood vessels, adrenal glands, and cardiovascular control centers within the brain, all of which contribute to blood pressure homeostasis (9, 11). AT1 within the vascular system causes vasoconstriction, within the adrenal cortex leads to the release of aldosterone, and in the kidney leads to renal vasoconstriction and antinatriuresis (11). Blocking the AT1 receptor leads to a significant decrease in MAP (35).

As stated earlier, angiotensinogen mRNA is found in the proximal tubule and a positive amplification mechanism occurs within the renal proximal tubules, stimulating

synthesis and release of angiotensinogen. The release leads to increased formation of Ang II (30). Ang II then binds to AT1 and controls renal afferent and efferent arteriolar vasoconstriction, decreases glomerular filtration rate and renal plasma flow, and stimulates sodium and fluid reabsorption in the proximal tubules (28). This positive mechanism helps the kidneys retain sodium when levels of sodium intake are low, leading to an increase in blood pressure. However, this amplification may also contribute to unwanted elevations in intrarenal and intratubular Ang II (30).

Angiotensin II Effects on Heart Rate

Ang II has been shown to have two opposing effects on heart rate: 1) the angiotensin induced elevation in blood pressure stimulates the baroreceptor reflex, which slows the heart rate and 2) a central nervous system inhibition of vagal discharge which increases heart rate (15, 23). This has been demonstrated in sheep, dogs, and monkeys. However in humans, one study found an initial reflex bradycardia, (slowing of the heart rate), as systolic pressure rose during intravenous infusion of Ang II, but at the peak of the pressor response, tachycardia, (rapid heart rate), was present (15). Another study concluded that Ang II can increase heart rate when the baroreceptor control of heart rate is eliminated (23). Three possible mechanisms by which Ang II could cause a dose-dependent tachycardia include: 1) activation of cardiac sympathetic nerves or release of adrenal medullary catecholamines, 2) direct chronotropic, effect (direct action on the heart to increase heart rate), and 3) dose-dependent central inhibition of cardiac vagal tone. Vagus nerves, part of the parasympathetic nervous system, are in a constant state of

excitation and act to slow the heart rate. If the vagal tone decreases, heart rate increases, whereas if vagal tone increases, heart rate will decrease (36).

Angiotensin II and Nitric Oxide Interactions

The effects of NO appear to counteract Ang II vascular actions (38). The balance between Ang II and NO seems to have a greater influence on the medullary blood flow than the cortical blood flow (38). In normotensive and hypertensive rats, Ang II stimulates medullary NO production, thus preventing vasoconstriction of the medullary vessels (38). Recent research has shown that hypertension effects due to Ang II could be the result of a defective NO counterregulatory system in the medulla (38). NO may influence the regulation of MAP by altering the vascular tone of the cardiovascular system and also affecting the relationship between MAP and sodium excretion (30).

Spontaneously Hypertensive Rats

Spontaneously hypertensive rats (SHR) are a commonly used animal model for the study of hypertension. They are derived from a closed colony of Wistar-Kyoto (WKY) rats (31). SHR develop hypertension without any organic lesions in the kidneys or adrenal glands, with an incidence of hypertension at 100%. The hypertension that occurs is very severe, usually over 200mmHg, and a high incidence of hypertensive cardiovascular disease also occurs (31). The pressure natriuresis relationship is altered in

SHR, which require a higher renal perfusion pressure to excrete normal sodium and water volumes (21).

Studies that have compared short term renal responses to Ang II in anesthetized SHR to those of WKY rats, indicate that infusion of Ang II produces a greater decrease in renal blood flow and a greater increase in renal vascular resistance in SHR (21). These studies concluded that the SHR kidneys are hyperresponsive to Ang II (21). Some critics state that anesthesia could alter the affect of Ang II on blood pressure and hemodynamics differently in WKY versus SHR (21). However, previous studies that looked specifically at the renal response to Ang II in conscious SHR and WKY, found similar results when compared to the anesthetized rats (21).

Exercise and Hypertension

The exact mechanisms by which hypertension is reduced by exercise is still unknown (37). Exercise has been considered a possible treatment for mild forms of hypertension (17). In both hypertensive and normotensive men and women, moderate intensity exercise may decrease systolic and diastolic blood pressure by 5-7 mmHg and also decrease heart rate (17).

One study looked at daily exercise in SHR and concluded that exercise attenuated the development of hypertension and tachycardia (8). This result has also been shown in humans; endurance training with borderline or moderate hypertension seemed to reduce blood pressure (20). The reduction in blood pressure may be due to a stimulation of

endothelial NO formation (20). Another study found that chronic exercise in SHR lead to an increase in NO formation while preventing Ang II elevations (20).

Low intensity exercise training in SHR has been shown to decrease sympathetic tone to the heart, leading to a reduction in the heart rate (37). A specific study looked at the effects of low intensity exercise training compared to high intensity exercise in SHR. The results were 1) exercise training at 55% VO2 max (maximum oxygen uptake) produced a significant decrease in resting heart rate, cardiac output, and mean arterial pressure, but no change in total peripheral resistance, 2) exercise training at 85% VO2 max caused no change in cardiac output, heart rate, or mean arterial pressure (37). Although the experiment showed low intensity exercise decreased heart rate and cardiac output in SHR resulting in an attenuation of hypertension, they were unable to explain why high intensity exercise did not show the same results.

Exercise training has been demonstrated to increase aortic baroreceptor gain sensitivity in normotensive rats and SHR, resulting in an improvement of the baroreceptor sensitivity and response (4). This finding suggests that improving aortic baroreceptor sensitivity may lead to a more efficient MAP regulation. Exercise also been shown to increase spontaneous baroreflex sensitivity in both men and women (17).

Exercise and Angiotensin II Infusions

The research on the effects of exercise on Ang II is sparce. It is not clear if exercise affects the RAAS, thus producing antihypertensive effects through this system. A prior study in this lab showed that exercised hypertensive and normotensive female rats that received infused Ang II had a greater sodium excretion than sedentary controls. However, this study did not determine if the mechanism for the increased natriuresis was a direct effect by Ang II, or an indirect effect via a rise in MAP.

SUMMARY

Hypertension is diagnosed when systolic pressure is greater than or equal to 140 mmHg and/or diastolic is greater than or equal to 90 mmHg. Hypertension commonly predisposes the patient to many other cardiovascular diseases, such as coronary artery disease, congestive heart failure, and cerebrovascular disease. Cardiovascular disease is known to cause 31% of total mortality in the United States.

Mean arterial pressure (MAP) is a function of cardiac output and peripheral resistance, and is under constant neural, hormonal, and hemodynamic control. A short term mechanism to regulated mean arterial pressure includes the baroreceptor reflex response. Long term mechanisms include blood volume and sodium balance, which are regulated by the kidneys. A pressure natriuresis response that results in the elimination of additional sodium in the urine, when MAP increases, is believed to contribute significantly to the regulation of sodium and MAP. Individuals with hypertension have been suggested to have a poor pressure natriuresis relationship, requiring a higher arterial pressure to induce normal levels of sodium excretion.

The renin-angiotensin-alsosterone system (RAAS) is an important hormone system in the regulation of MAP and blood volume. Ang II is known to cause an immediate vasoconstriction of the arterioles leading to an increase in blood pressure, and also stimulates the adrenal gland to release aldosterone, causing sodium retention.

It is known that exercise has a blood pressure lowering effect in both humans and rats; however it is unknown whether exercise affects the renin-angiotensin-aldosterone

system. Prior studies in this lab have shown that exercise significantly improved sodium excretion in response to Ang II infusion in WKY and SHR female, but not male rats. The previous research lead to additional questions, such as, is the exercise induced increase in sodium excretion in response to Ang II the result of improved pressure natriuresis, or did exercise change the renal sodium response to Ang II? The present study was designed to determine if the exercise induced increase in sodium excretion persisted if blood pressure at the kidney was not allowed to rise with Ang II infusion. In other words, if pressure natriuresis was eliminated, did the difference in sodium excretion between the groups disappear?

PURPOSE

The purpose of this study is to determine if the increase in sodium excretion in response to Ang II that was demonstrated in prior studies in exercised females (WKY and SHR) is due to a difference in the direct kidney response to Ang II, or is simply due to an exercised-induced shift in the pressure-natriuresis relationship. In other words, did a change in the pressure-natriuresis relationship, rather than the Ang II itself, produce the greater sodium excretion in exercised versus sedentary female rats.

It is hypothesized when renal perfusion pressure (RPP) is held constant, eliminating pressure natriuresis, sodium excretion, in response to Ang II, will not differ between exercised and sedentary rats. Renal perfusion pressure will be held constant by placing an adjustable noose around the abdominal aorta just above both kidneys. The noose will be manually adjusted to maintain RPP at baseline values, as blood pressure rises in response to the infusion of Ang II.

Urine collected throughout the acute study will be analyzed to determine the sodium concentration. The amount of sodium excreted will determine whether the hypertensive and normotensive, exercised and sedentary rats, had an altered response to Ang II during the infusion of three different Ang II concentrations.

METHODS

Twenty female Wistar-Kyoto breed (WKY) rats and eighteen female spontaneously hypertensive rats (SHR) were selected from the Minnesota State University colony. The birth periods spanned over a six month period to allow for gradual testing during six months. At four weeks of age, each pup was weaned and randomly assigned to either an exercise or sedentary group, making sure littermates were split amongst the groups.

Exercise Group: At four weeks of age, rats were placed in individual cages with an exercise wheel (Pet Expo, Mankato, MN), and were allowed to exercise voluntarily for at least 10 weeks before the acute study. A magnetic counter and magnet were used to keep track of each rotation of the wheel, while a bicycle odometer was used to log running time and distance (*Nicollet Bike Shop,* Nicollet, MN). Counters were checked 2-3 times a week and a total weekly value was calculated for each rat, and an average was determined for each rat group.

Sedentary Group: At four weeks of age, sedentary rats were placed in individual cages without exercise wheels. The acute study was performed no earlier than 14 weeks of age.

Surgical Setup

After a minimum of 10 weeks of voluntary exercise, rats were subjected to the acute study. To minimize stress, rats were anesthetized in a chamber (isoflorane 3% in oxygen) and then injected intraperitoneally with *Inactin* (SIGMA, St Louis, MO) (100mg/kg). *Inactin*, a barbiturate anesthetic, is preferred due to its prolonged action and production of a stable blood pressure during anesthesia. Rats were prepped by shaving the ventral neck, lower abdomen, and medial left thigh. Body temperature was maintained at 37^{o}C by adjusting a slide warmer on which the rat was positioned, while monitoring with a rectal thermometer (ReliOn). A longitudinal incision was made on the ventral portion of the neck. A transverse incision was made in the ventral trachea and a breathing tube was inserted (PE 240), to aid in respiration throughout the procedure. The left carotid artery was isolated and a small saline filled tube (PE 50) attached to a three-way stopcock was inserted for the monitoring/recording of blood pressure. The left jugular vein was isolated, and another catheter (PE 50) was inserted for infusions of saline (12ml/kg/hr) or Angiotensin II (Ang II) in saline, using an infusion pump (ORION Sage, model M362). The left femoral artery was isolated and a saline filled tube (PE 50) attached to a three-way stopcock was inserted and advanced for the monitoring of renal perfusion pressure (RPP). A ventral midline incision was made to expose the bladder and abdominal aorta. The abdominal aorta was isolated cranial to the renal arteries and a suture was placed around the aorta. The suture was pulled through a section of tubing (PE 240) to create an adjustable noose. The suture ends and tubing end was externalized through a left lateral incision in the abdominal wall. Traction was placed on the suture to

tighten the noose as needed to prevent a rise in blood pressure at the level of the kidneys. An incision was made in the ventral bladder and a urine collection tube (PE 320), flamed to create a lip on one end, was inserted into the bladder and held in place by a purse-string suture.

Collection Periods

A 30 minute equilibration period was observed, following the surgical setup, during which infusion of saline was continued (12 ml/kg/hr). Once the equilibration period ended, the carotid and femoral blood pressures were recorded continuously. A baseline 15-minute period began once the equilibration period ended, and consisted of infusion using only the saline vehicle. RPP was averaged for the baseline period, and this pressure was maintained throughout the study by adjusting the aortic noose. After the baseline period, three 15-minute periods followed, each with an infusion of a different concentration of Ang II (0.125, 0.05, and 2.0 μg/ml saline, respectively), (2). The log of the Ang II concentrations when graphed on a dose response curve allowed for even spacing between each dose. The passage of Ang II through the tubing before reaching the rat's circulation resulted in a lag time. Therefore, the 15-minute time period was started at the point blood pressure began to rise, indicating Ang II had reached the tubing end and was in the rat's circulation. Urine was collected during each 15-minute period, volume was recorded, and urine was later analyzed for sodium concentration. Between the 15-minute periods of Ang II administration, blood pressures were allowed to return to baseline values. Urine collected between Ang II doses was discarded.

Computer Data

Mean arterial pressure, renal perfusion pressure, and heart rate were recorded on a computer using BIOPAC hardware and software. Data from the acute study was later analyzed using the BIOPAC software. The maximum blood pressure for each Ang II dose was recorded, along with the corresponding heart rate. An average of each was calculated for each Ang II dose for each rat group. Carotid blood pressure, RPP and heart rate were averaged every five minutes of each 15 minute Ang II infusion. The data was then grouped according to exercised or sedentary, WKY or SHR strain, and a group average for each five minute period at each Ang II dose was calculated.

Urine Collection and Sodium Determination by Flame Photometry

Total urine volume for each 15 minute period was recorded at the time of collection. For sodium concentrations, each sample whose initial volume was less than 0.30 ml was diluted to a final volume of 0.30 ml with distilled water, to provide a sufficient volume for the flame photometer (Corning 480 Flame Photometer with Sampler, Ciba Corning Diagnostics, Ltd.). Sodium concentration was determined by following the calibration and operation protocol of the flame photometer as outlined in the instruction manual. Calibration was performed each time the photometer was utilized, using distilled water (zero) and a urinary sodium and potassium calibration solution supplied by the company. Total sodium excretion for each collection period was calculated by taking the sodium concentration and multiplying by the final (undiluted) volume of the urine.

ANALYSIS

Sensitivity to Angiotensin II:

A dose response curve was created for each rat, plotting the log of the dose of Ang II against the maximum increase in systolic blood pressure. The slope determined the sensitivity of the cardiovascular system to Ang II. A regression curve for each treatment group was calculated and the slopes compared between the groups.

Sensitivity of the kidney to Angiotensin II:

A one way ANOVA (analysis of variance) was performed to analyze differences in sodium excretion between the two groups (exercise and sedentary) during the same time periods. A Tukey-Kramer was performed within each group to compare sodium excretion during each concentration of Ang II to the baseline (saline infusion). A two-way ANOVA compared the response over time between the two groups. An ANCOVA (analysis of covariance) compared the regression slopes used to determine if the dose response slopes were significantly different between exercised and sedentary rats.

RESULTS

Twenty female Wistar-Kyoto (WKY) rats (n= 10 for sedentary and exercised) and 18 female spontaneously hypertensive rats (SHR) (n= 10 sedentary, n=8 exercised) were studied (Table1). Each rat in the exercised groups ran for a minimum of 10 weeks (Figure 1). Mean running distances peaked at week five for SHR (65.19 km ± 6.12) and week six for WKY (64.51 km ± 7.33). After the peak, weekly running distances in the WKY remained relatively stable, while distances gradually decreased in the SHR, becoming significantly less than the WKY rats by week nine (Figure 1).

Mean Arterial Pressure and Sensitivity to Angiotensin II

Mean arterial pressure (MAP) increased with each successive dose of angiotensin II (Ang II) in both the sedentary and exercised groups of WKY and SHR. In the WKY, MAP significantly increased ($p< 0.05$) over baseline at the onset of 0.5 μg/ml Ang II infusions in both exercise and sedentary groups, and remained elevated throughout the rest of the study. No between group differences were found at any time period (Figure 2). In the SHR, MAP significantly increased ($p< 0.05$) over baseline at the onset of 0.125 μg/ml Ang II infusion in both exercise and sedentary groups and remained significantly elevated throughout the rest of the study as observed in WKY. MAP in the exercised SHR was significantly greater ($p <0.05$) than in the sedentary at several time points during the infusion of Ang II (Figure 3). However, the exercised SHR had a slightly higher baseline MAP, and the increase from baseline was not different between the exercised and sedentary rats.

In all rat groups, the peak MAP increased as Ang II concentration increased (Figure 4, 5). However, there was no significant difference when the slopes of the dose response curves were compared between sedentary and exercised groups in either the WKY or SHR.

Heart Rate

In the WKY, both sedentary and exercised groups showed no change in heart rate from baseline at any point. However, the exercised rats had significantly lower ($p< 0.05$) heart rates when compared to sedentary, beginning in the baseline period and extending to the 2.0 μg/ml dose of Ang II. Exercised WKY rats also showed a significantly lower heart rate when overall heart rates were compared to sedentary (Figure 6). In the SHR sedentary group, heart rate significantly increased ($p< 0.05$) over baseline at the onset of 2.0 μg/ml Ang II infusion and heart rate remained significantly elevated throughout the rest of the study. In the SHR exercised group, heart rate did not significantly increase over baseline, at any points. However, no significant between group differences were found in heart rate at any time period or in the pattern of heart rate over time (Figure 7).

Heart Rate at Peak MAP

The change from baseline in heart rate was measured at the peak MAP for each dose of Ang II as an indicator of the sensitivity of the baroreceptor. Both WKY sedentary and exercised rats had an increase in mean heart rate as Ang II infusion concentrations increased. Although a trend toward a greater increase in heart rate was apparent in the sedentary group, this was not significant (Figure 8). In the SHR, the magnitude of the increase in heart rate from baseline gradually increased with each Ang II dose in the sedentary rats, but decreased in the exercised rats during the 0.5 μg/ml Ang II infusion, and decreased below baseline during the infusion of Ang II at 2.0 μg/ml. The change in heart rate was significantly different ($p < 0.05$) between the exercised and sedentary SHR during the 0.5 and 2.0 μg/ml Ang II infusions (Figure 9).

Mean Renal Perfusion Pressure

WKY renal perfusion pressure (RPP) did not change from baseline at any time period in either group. RPP showed no between group differences at any time period (Figure 10). In the SHR, RPP did not change from baseline at any point in the study in either the sedentary or exercised group. The SHR exercised group had a higher RPP than sedentary, but reached significance ($p < 0.05$) only at a few time periods during 0.5 and 2.0 μg/ml Ang II infusions (Figure 11).

Urinary Sodium Excretion

Urinary sodium excretion did not change significantly from baseline values in either WKY or SHR. No between group differences were found in either WKY or SHR during any of the Ang II infusions (Figure 12, 13).

Urine Flow Rate

Urine flow rate did not change significantly from baseline values in either WKY or SHR. No between group differences were found in either WKY or SHR during any of the Ang II infusions (Figure 14, 15).

Table 1. WKY average age, weight, and running distance at the time of the acute study. Values are reported as mean ± standard error. The number of rats in each group is listed in parentheses.

Group	Age (wks)	Weight	Running Distance (wks)
WKY			
Sedentary	16.35	0.305	
(n=10)	±0.913	±0.012	
Exercise	17.3	0.327	13.9
(n=10)	±1.225	±0.01	±1.191
SHR			
Sedentary	18.7	0.214	
(n=10)	±0.609	±0.006	
Exercise	16.5	0.24	12.5
(n=10)	±0.586	±0.01	±0.606

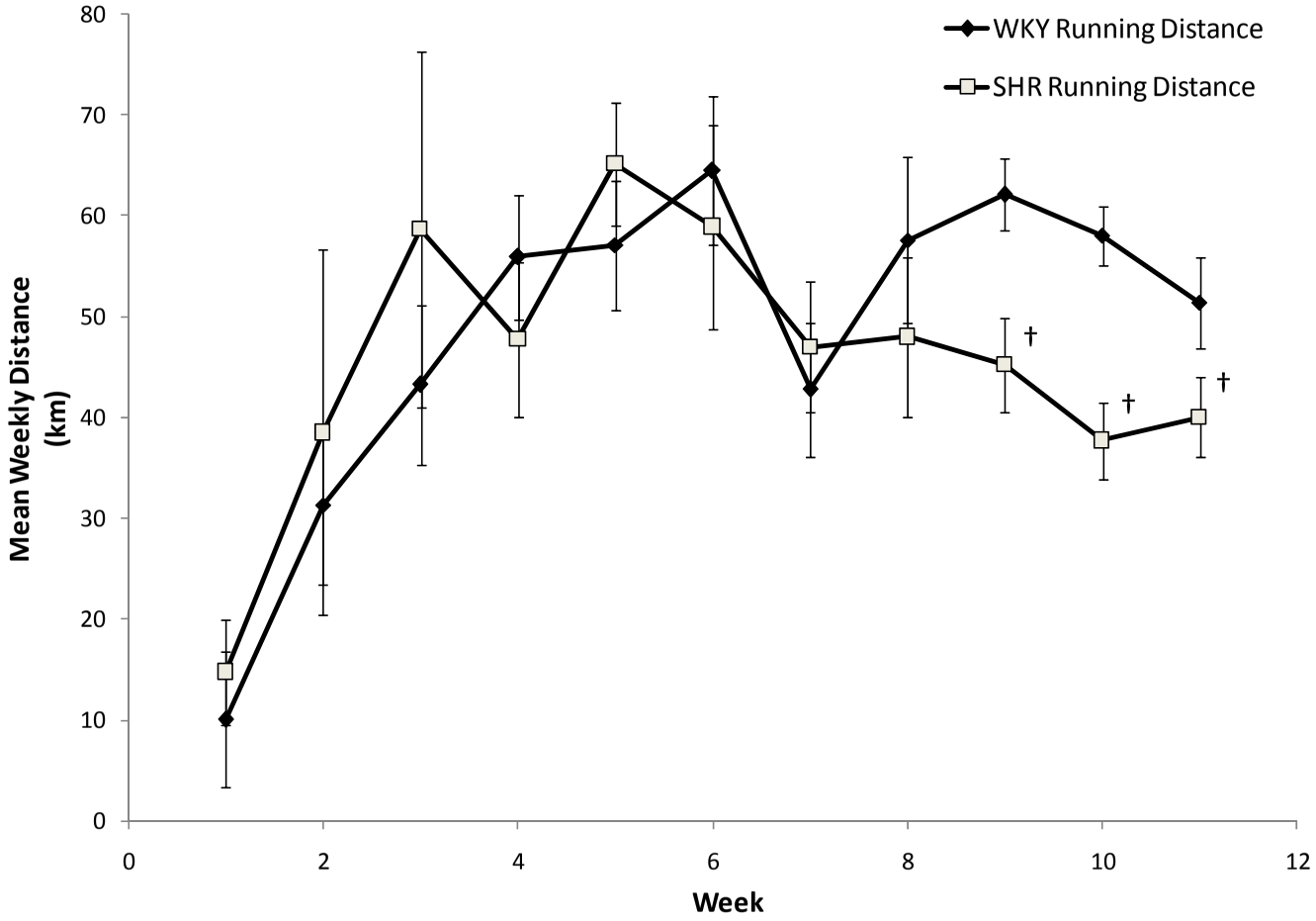

Figure 1: Weekly running distances in the WKY and SHR exercised rats. Values are reported as mean ± SE.

† $p < 0.05$ when compared to sedentary

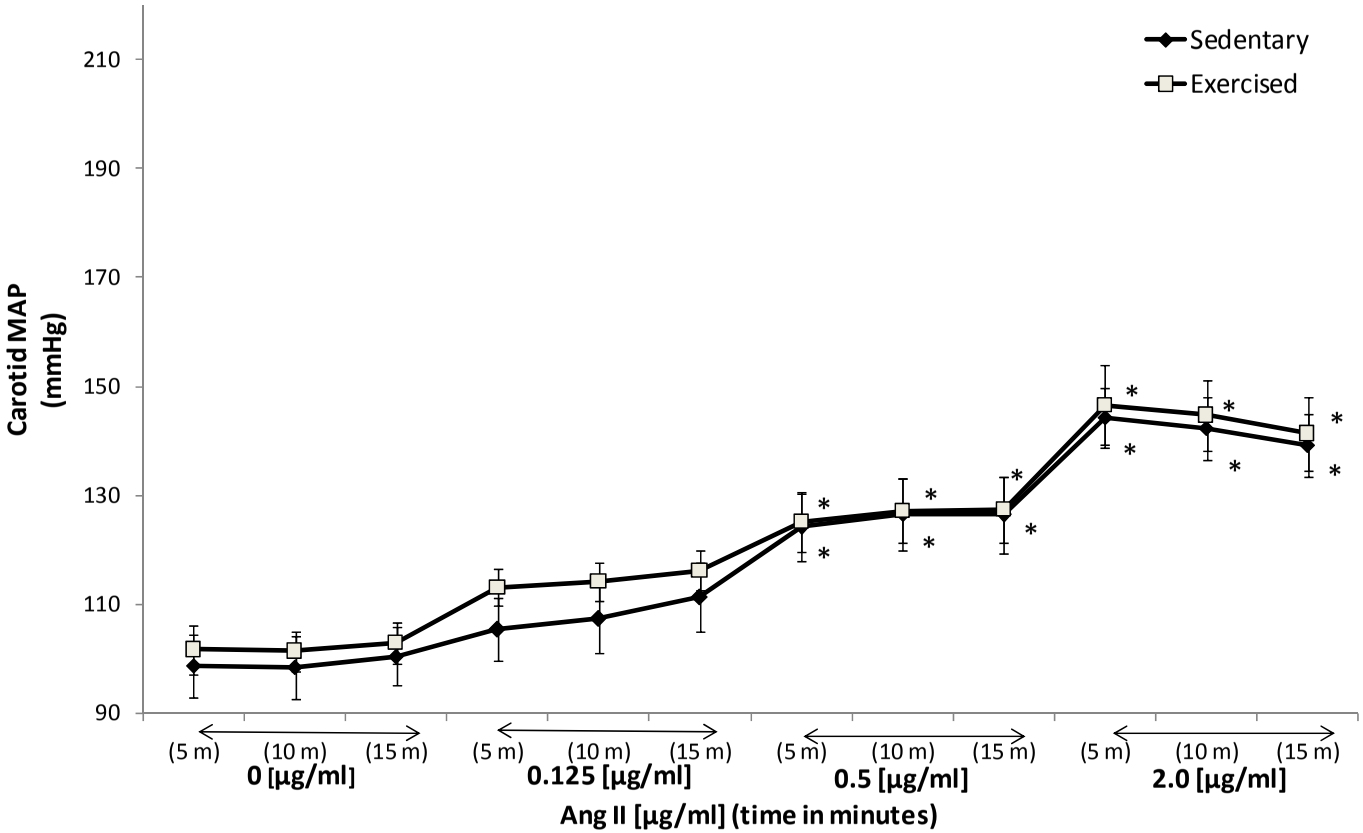

Figure 2: Mean arterial pressure in WKY rats, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

* $p < 0.05$ when compared to baseline

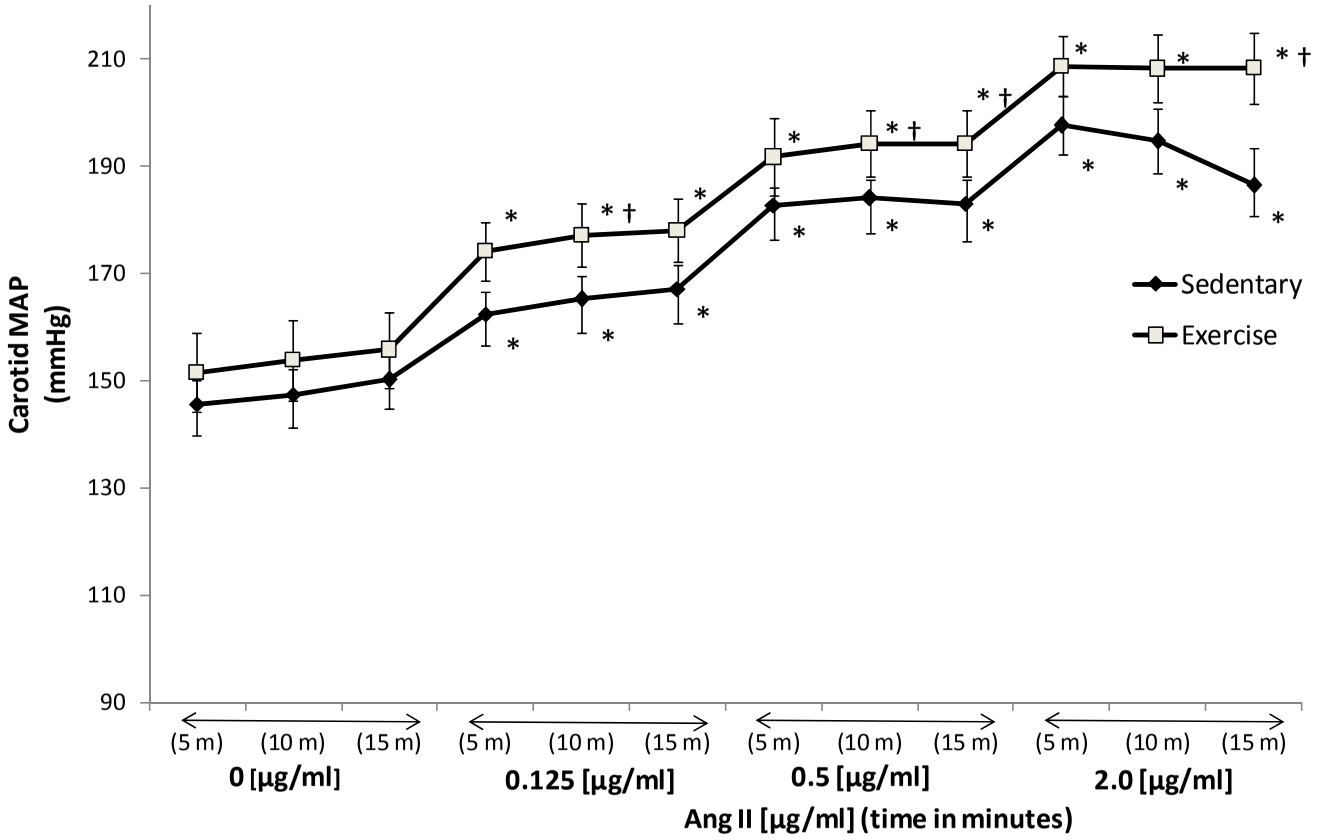

Figure 3: Mean arterial pressure in SHR, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

* $p < 0.05$ when compared to baseline

† $p < 0.05$ when compared to sedentary

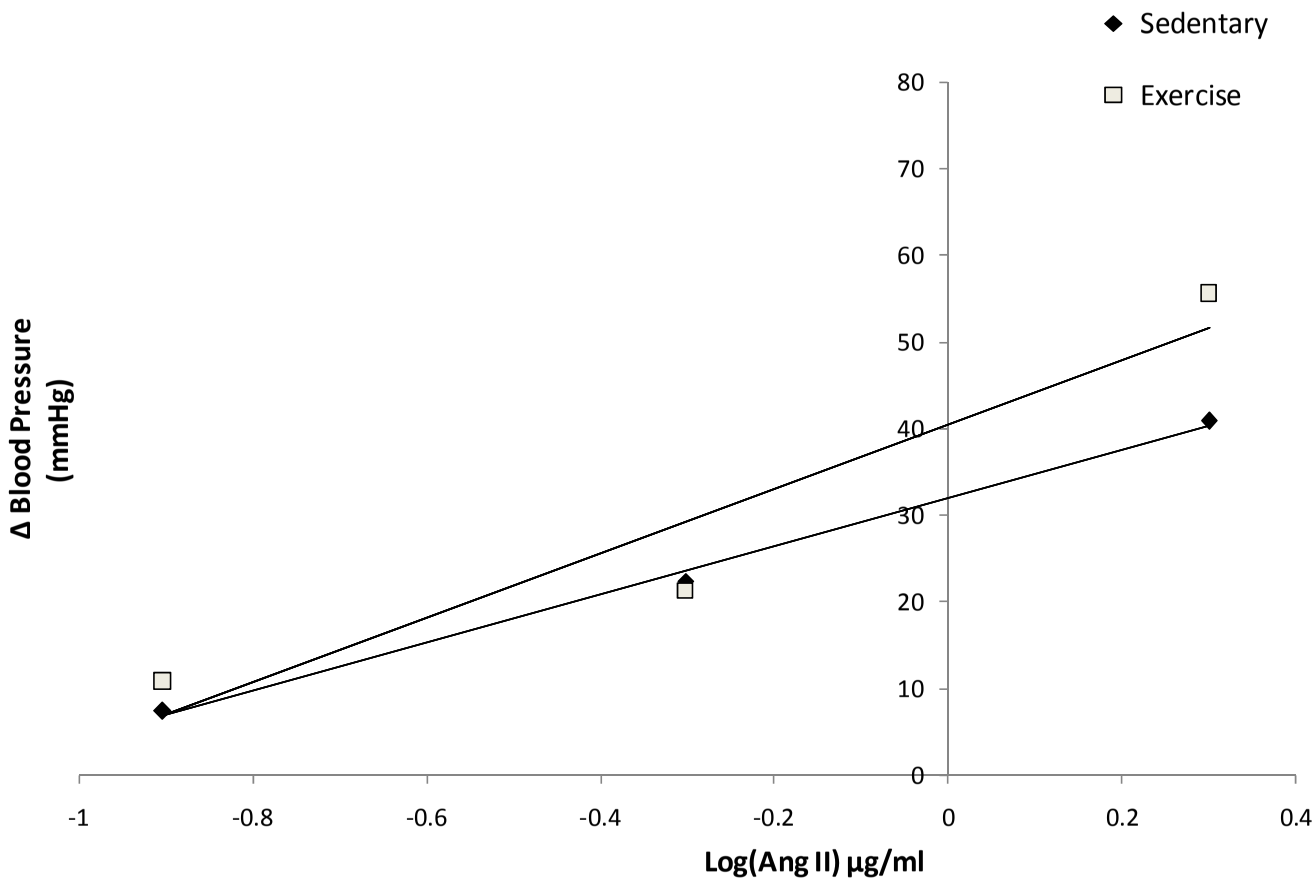

Figure 4: Mean peak change in blood pressure from baseline versus the log of each Ang II concentration in WKY rats. The slope correlates to the vascular sensitivity to Ang II.

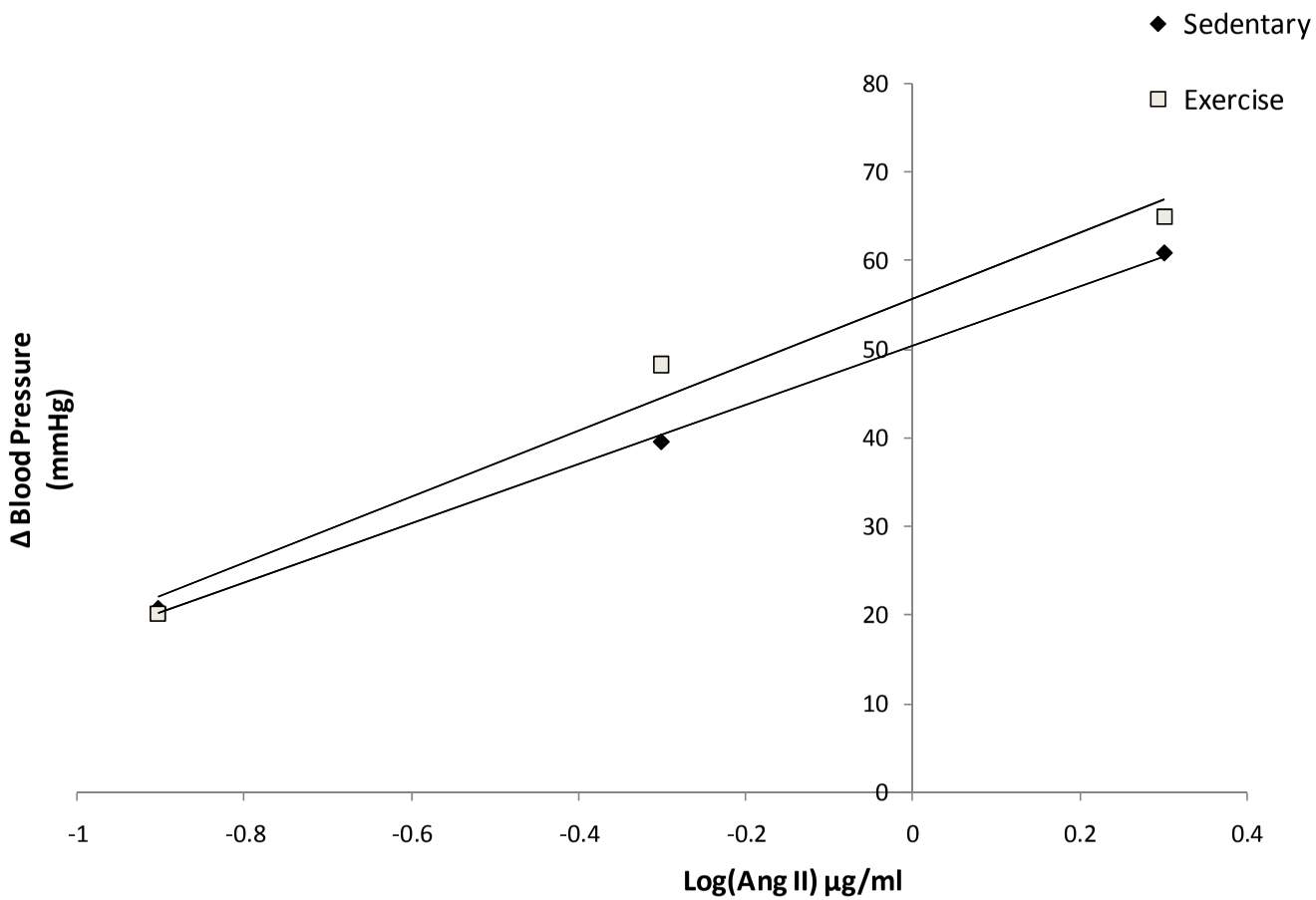

Figure 5: Mean peak change in blood pressure from baseline versus the log of each Ang II concentration in SHR. The slope correlates to the vascular sensitivity to Ang II.

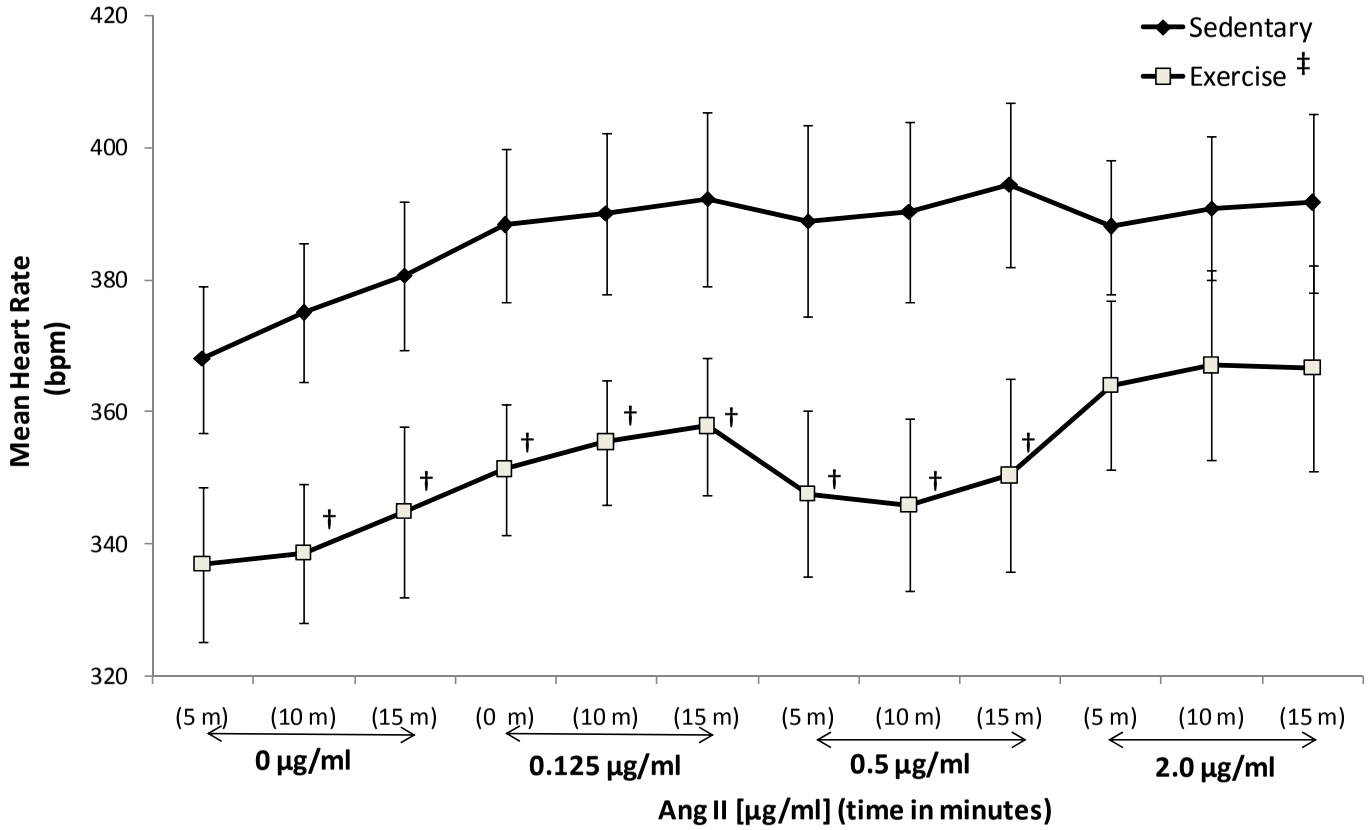

Figure 6: Heart rate in WKY rats, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

† $p < 0.05$ when compared to the sedentary

‡ $p < 0.05$ when overall heart rates were compared to sedentary

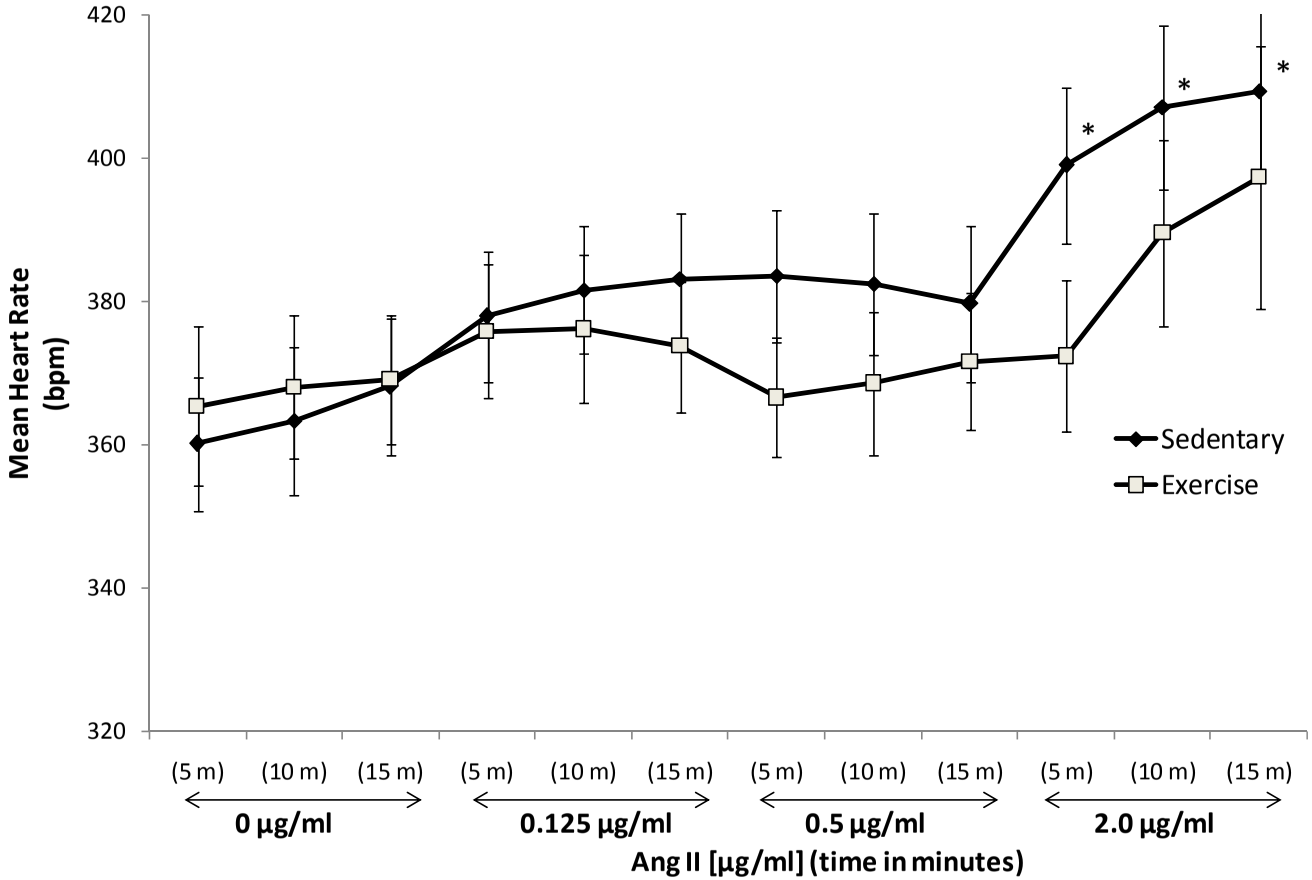

Figure 7: Heart rate in SHR, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

* $p < 0.05$ when compared to baseline

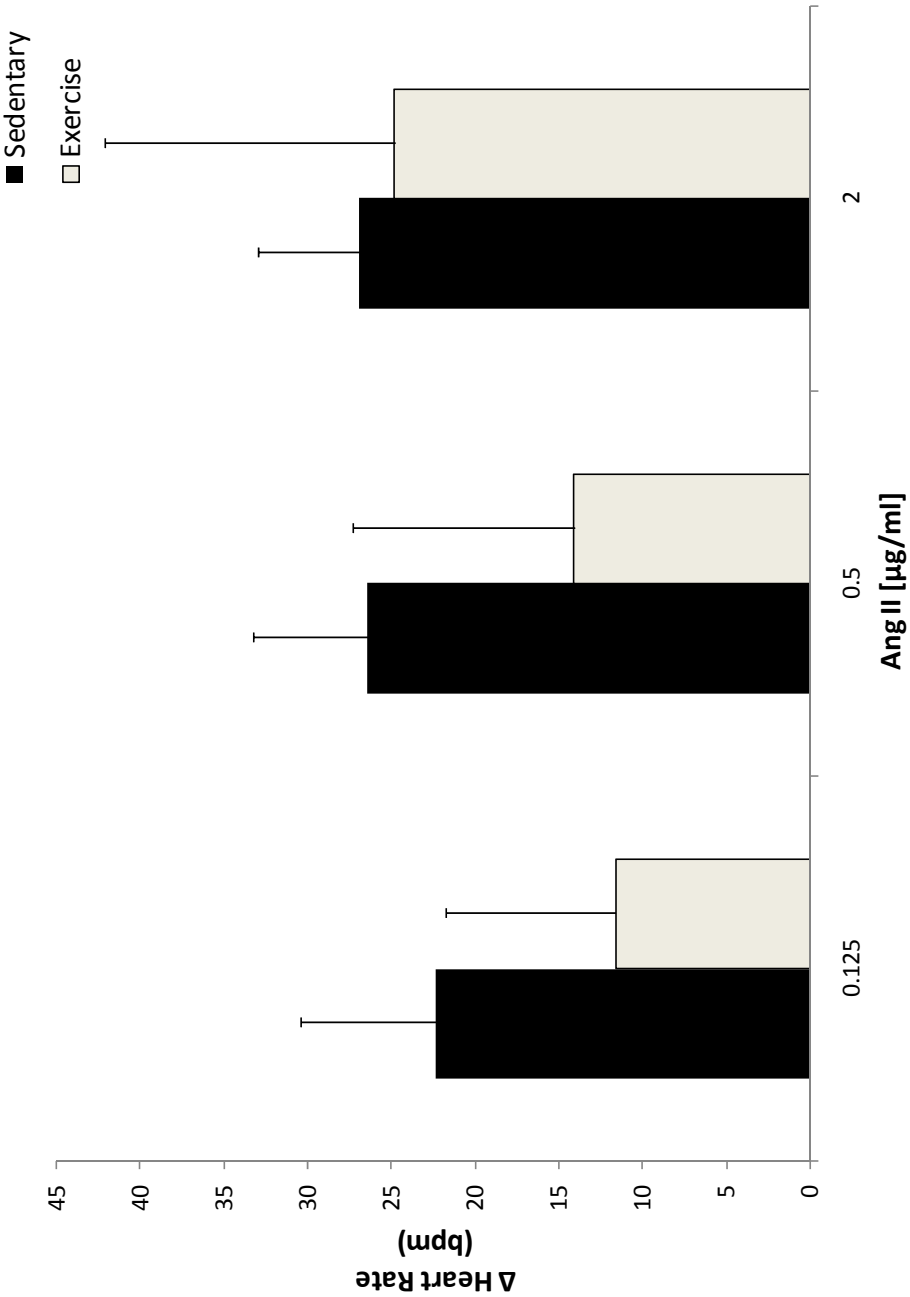

Figure 8: Change in heart rate from baseline in WKY rats, measured at peak mean arterial pressure during each Ang II infusion. Values are reported as mean ± SE.

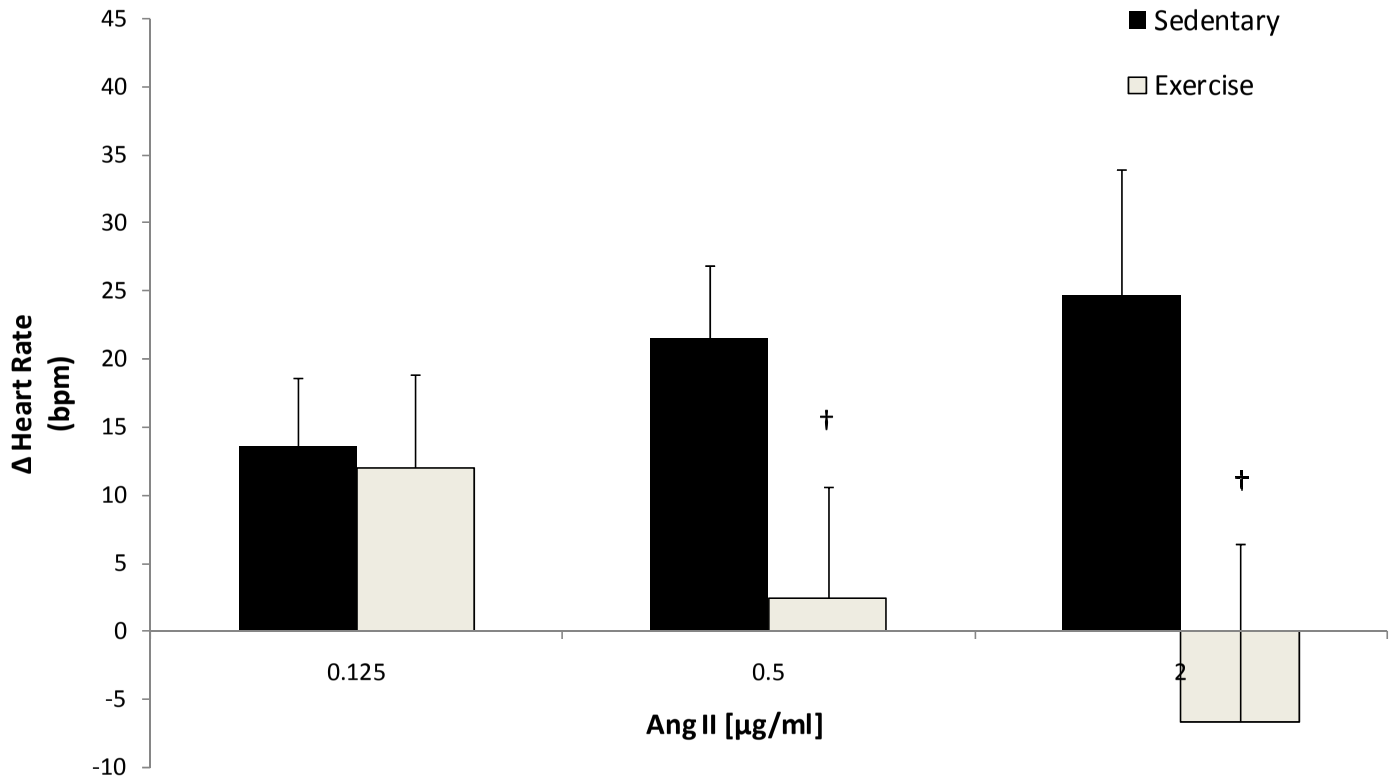

Figure 9: Change in heart rate from baseline in SHR, measured at peak mean arterial pressure during each Ang II infusion. Values are reported as mean ± SE.

† $p < 0.05$ when compared to the sedentary.

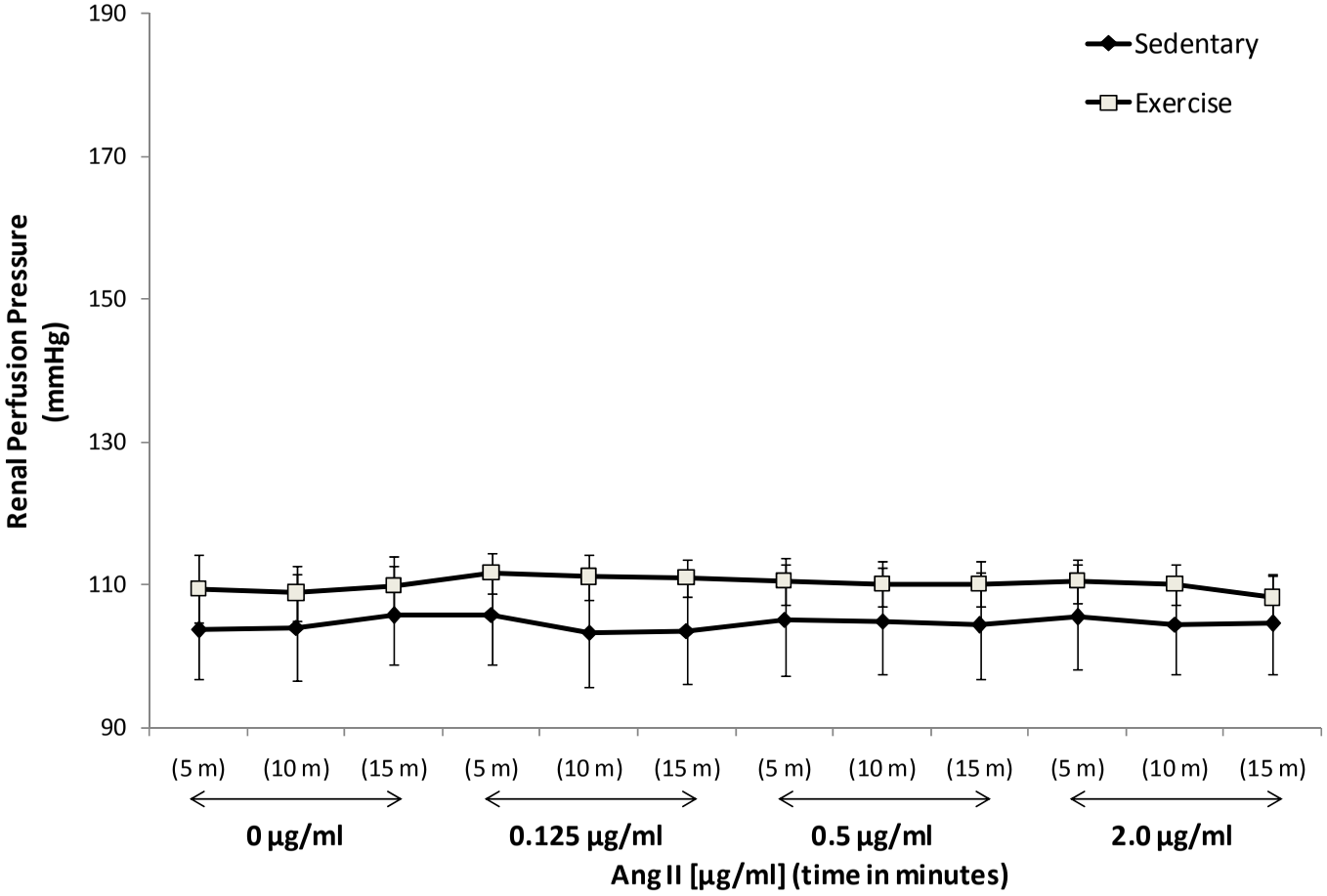

Figure 10: Renal perfusion pressure in WKY rats, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

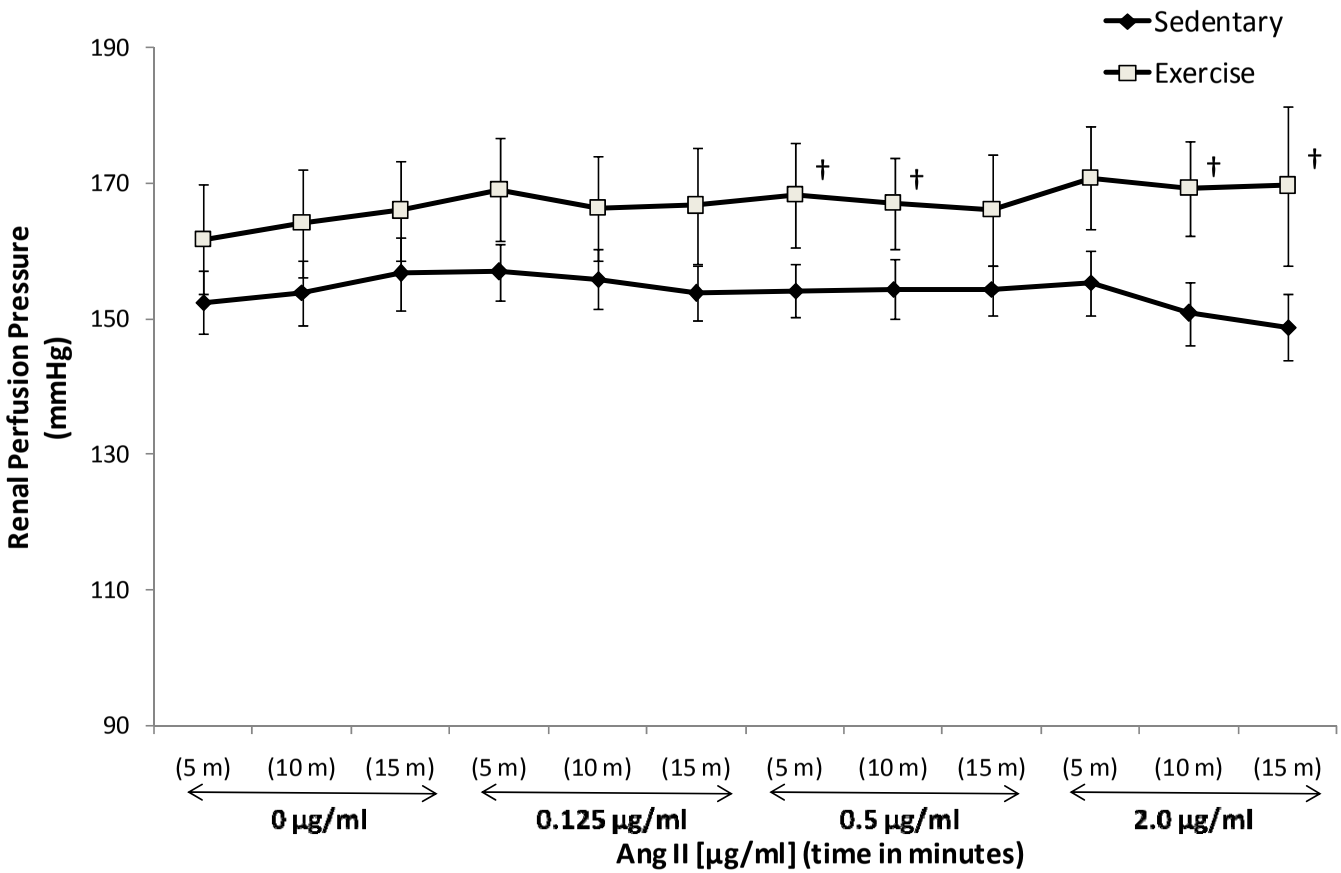

Figure 11: Renal perfusion pressure in SHR, averaged every 5 minutes during each Ang II infusion. Values are reported as mean ± SE.

† $p < 0.05$ when compared to the sedentary

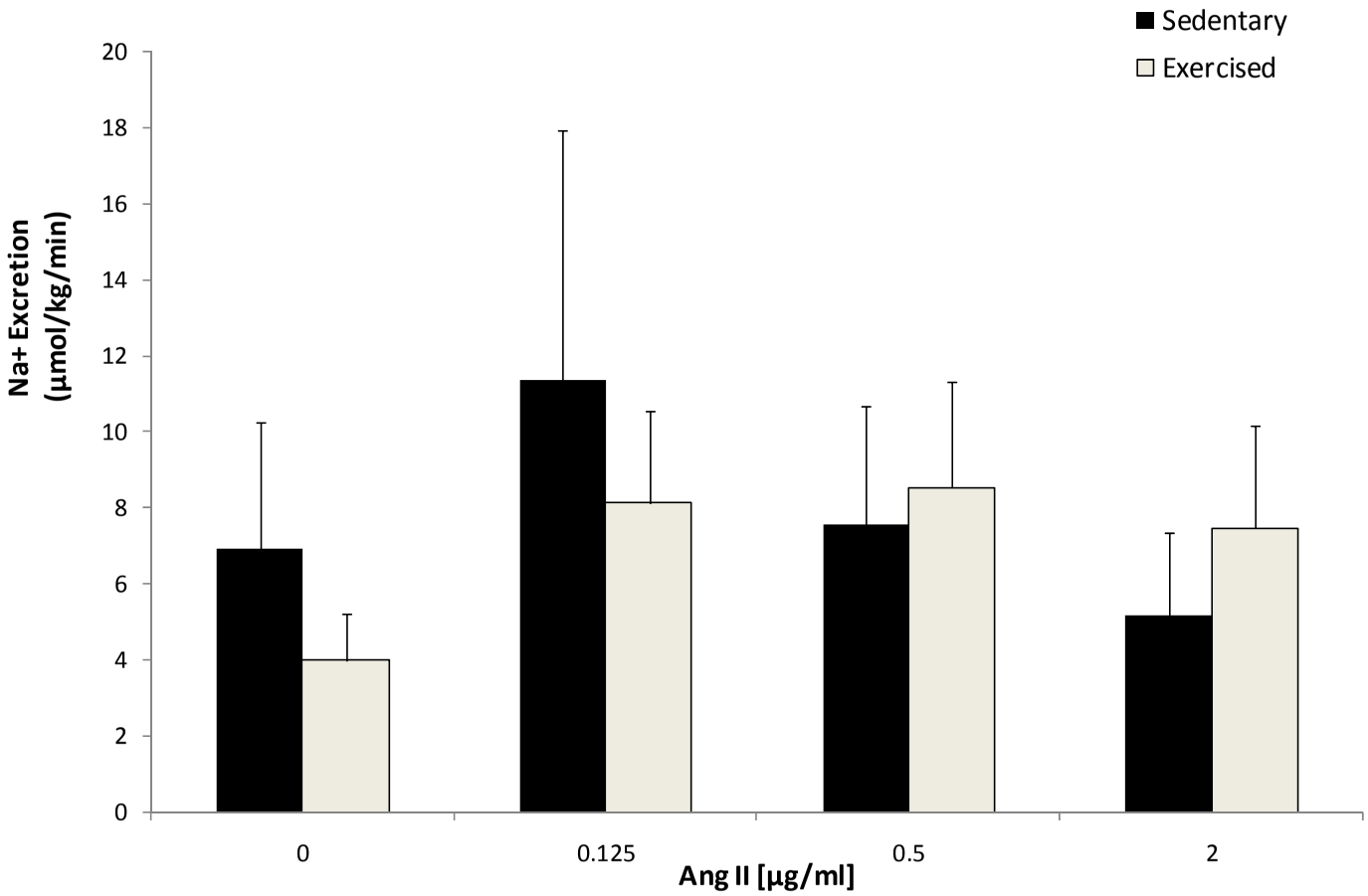

Figure 12: Urinary sodium excretion in WKY rats during each Ang II infusion. Valus are reported as mean ± SE.

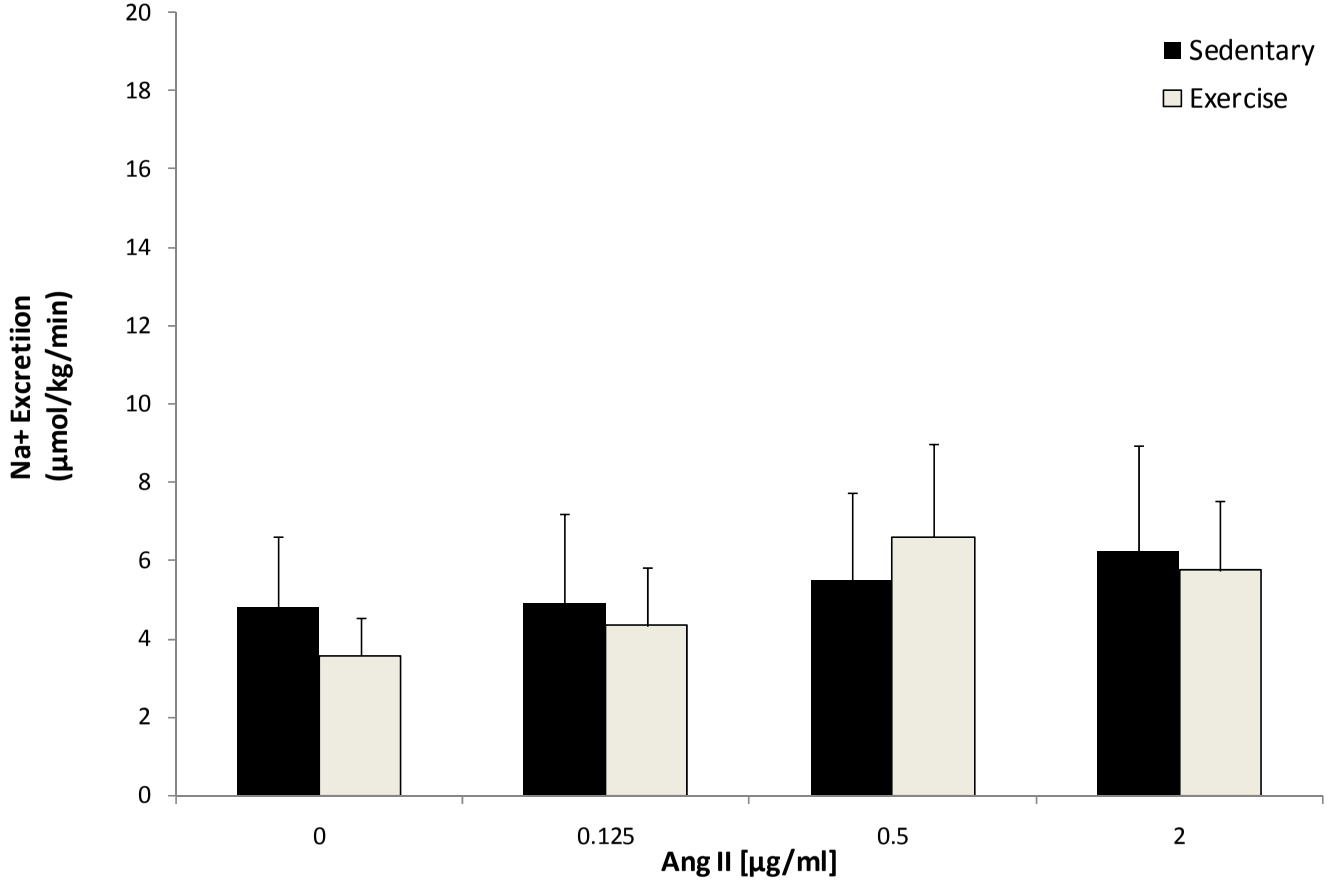

Figure 13: Urinary sodium excretion in SHR during each Ang II infusion. Values are reported as mean ± SE.

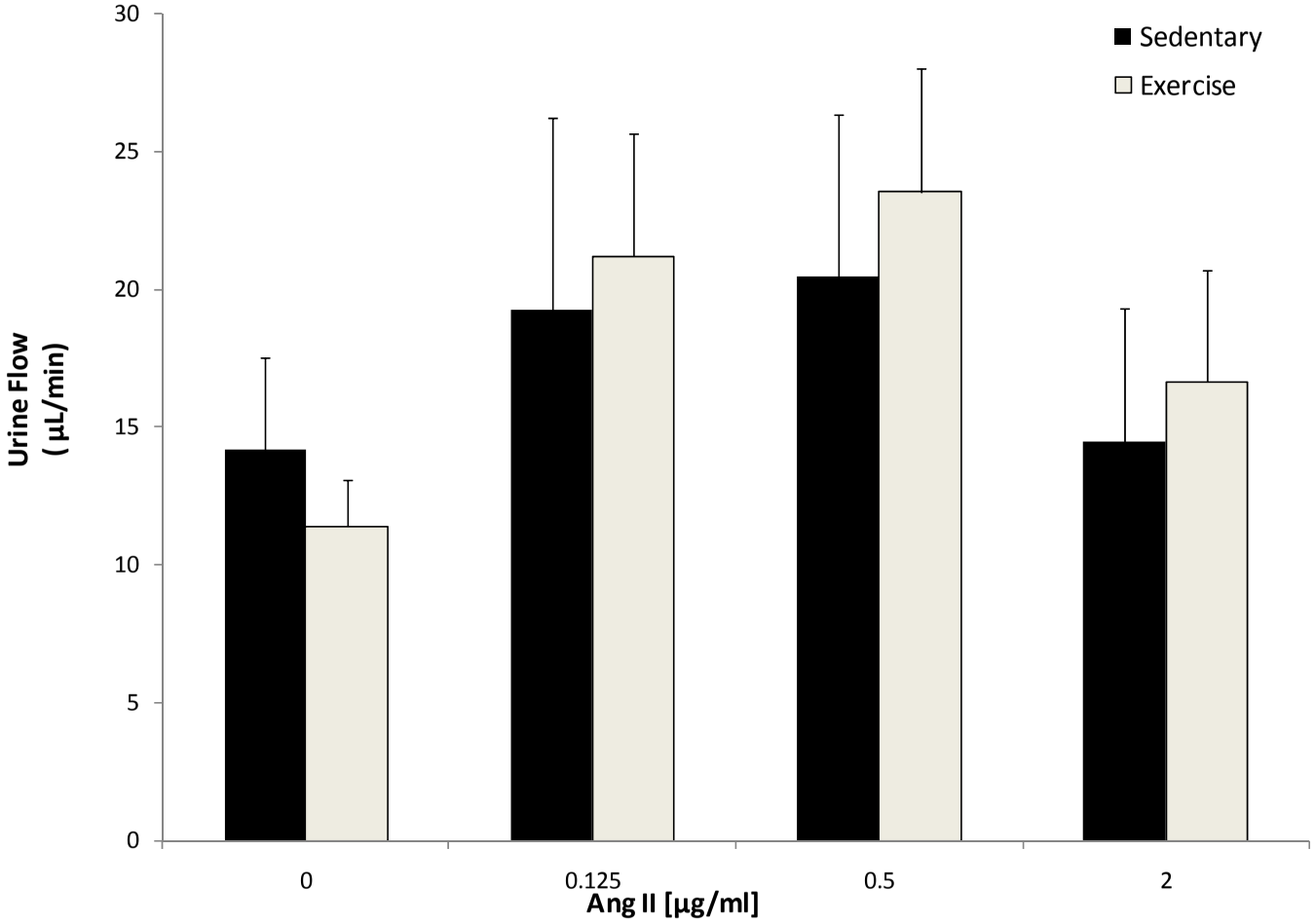

Figure 14: Urine flow rate in WKY rats during each Ang II infusion. Values are reported as mean ± SE.

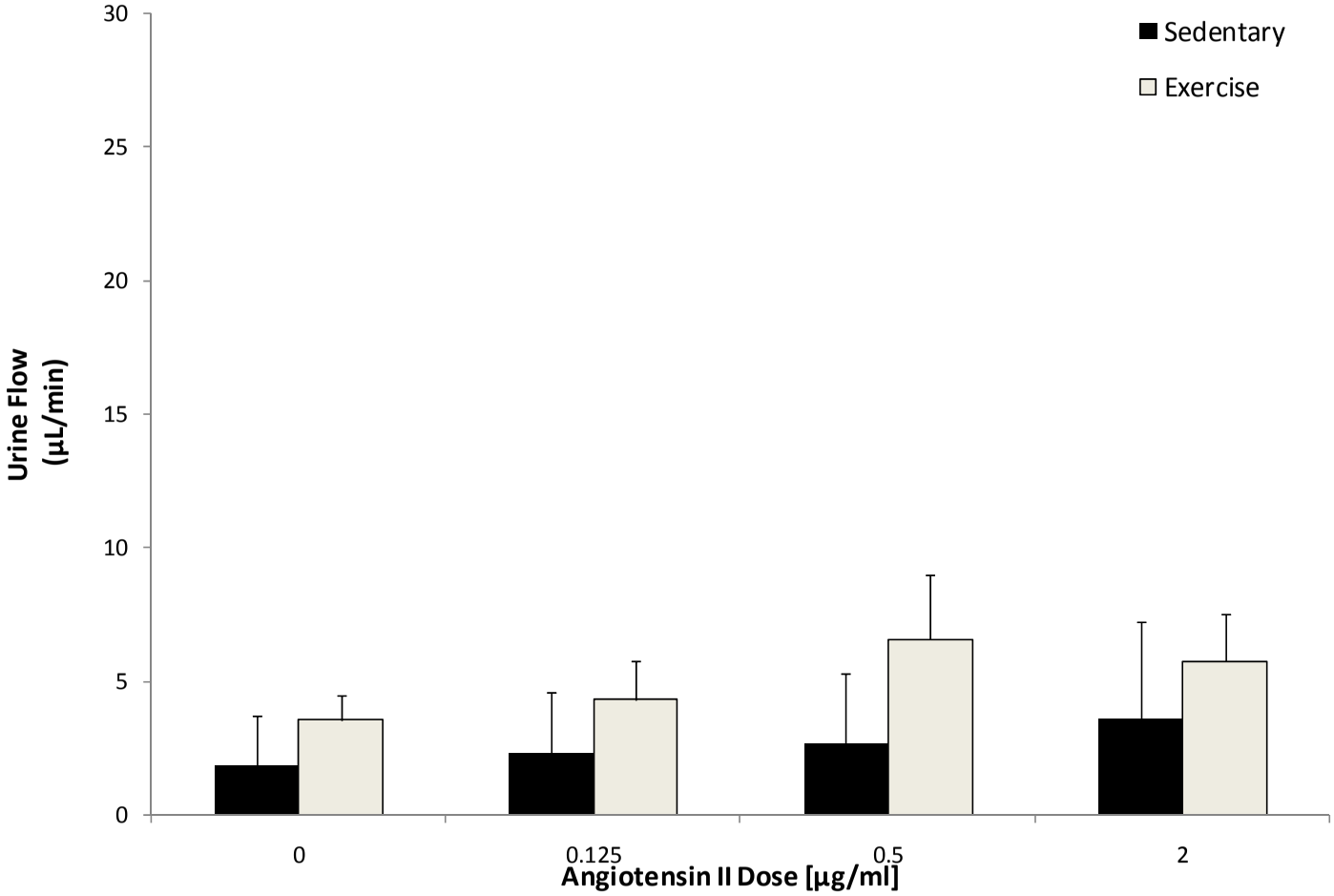

Figure 15: Urine flow rate in SHR during each Ang II infusion. Values are reported as mean ± SE.

DISCUSSION

In the present study when renal perfusion pressure (RPP) was held constant, no significant difference was found in sodium excretion or urine flow rates when compared to baseline, or between the exercised or sedentary groups in either WKY or SHR. However, during the study there were significant differences observed in running distances, mean arterial pressure (MAP), and heart rate (HR).

The sodium excretion and urine flow rate in response to angiotensin II (Ang II) infusion in the present study showed no significant differences when the exercised rats were compared to sedentary. This finding indicates that the exercise-induced difference in sodium excretion in response to Ang II infusion, observed in a prior study, was due to a change in the renal sodium response to an increase in renal perfusion pressure, and not an altered response to Ang II. Ang II has been shown to have a natriuresis and diuresis effect (16). This effect is associated with high rates of Ang II infusion and is dependent on an increase in RPP. If RPP is held constant, the natriuresis and diuresis does not occur. The natriuresis and diuresis may be due to a decreased fractional reabsorption of sodium in the proximal and distal tubules, or perhaps due to higher sodium delivery to tubules when RPP is not controlled (16, 33).

The mechanism by which exercise altered the renal response to a rise in perfusion pressure is unclear. Pressure natriuresis plays a role in the long term regulation of blood pressure, however, the mechanisms behind this response are not fully understood. Because renal blood flow and glomerular filtration rate are efficiently autoregulated, and

thus remain constant during increases in renal perfusion pressure, pressure natriuresis is mediated by the inhibition of tubular sodium reabsorption (12). Studies have shown that the pressure-diuretic and natriuretic response is associated with significant changes in renal interstitial hydrostatic pressure (RIHP) (12, 13). Increased RIHP has been proposed to inhibit tubular sodium reabsorption by reducing the passive diffusion of sodium through leaky tubular elements (12). Blood flow in the medulla appears to be poorly autoregulated, therefore when renal artery pressure increases, renal medullary blood flow increases, causing a greater renal interstitial hydrostatic pressure (12, 13). It is conceivable that exercise could alter the distribution of blood flow in the kidney when RPP is elevated, thus increasing medullary blood flow even further, resulting in a greater pressure natriuresis.

Another proposed mechanism for pressure natriuresis is an increased production of nitric oxide (NO) by the renal vascular endothelium. When renal artery pressure increases, the autoregulatory responses vasoconstrict the preglomerular vasculature. Although blood flow remains constant as a result of this response, the velocity of flow within each vessel increases, inducing shear stress in the vessel wall (12). Shear stress stimulates the production of NO. Nitric oxide increases urinary sodium excretion by directly inhibiting tubular transport and altering the intrarenal haemodynamic environment (12). Endothelial dysfunction is a common finding in hypertensive individuals, and decreased production of nitric oxide aggravates the hypertension. Regular exercise is known to improve endothelial function, and thus could result in

increases in nitric oxide production during elevations in RPP. The increase in NO could explain the difference in sodium excretion between exercise and sedentary rats (12).

A remaining question regarding the mechanism behind the pressure natriuresis response involves the kidney's internal baroreceptors or pressure sensors. It is unknown how the increase in renal artery pressure is sensed by the kidney. Improvements in the kidney's sensitivity to changes in renal artery pressure could be initiated by exercise. Exercise is known to improve the sensitivity of the systemic baroreceptor response to a rise in blood pressure, and thus has the potential to alter the baroreceptor response in the kidney (12). A more sensitive renal baroreceptor could theoretically improve the pressure natriuresis response.

The altered sodium excretion in the prior study occurred only in female and not male rats. It is unclear why the males failed to show a significant effect, but other sex differences are well described in the area of hypertension. The incidence and progression rate of cardiovascular disease and hypertension is higher in men when compared to same aged premenopausal females (25). After menopause the sex difference is eliminated and the progression rate is similar (25). It has been suggested that the mechanism behind the sex differences may include the role of sex hormones in regulating the activity of many regulatory systems such as the renin angiotensin system (25).

In the present study, female WKY and SHR voluntarily ran a minimum of 10 weeks before the acute study. In the WKY rats, running distance peaked at week 6 and remained fairly constant throughout the rest of the study, whereas the SHR running

distance peaked at week 5 then declined. Other studies looking at the effects of voluntary exercise in rats found similar average weekly running distances as those observed in the present study, (WKY 48.59 km/week and SHR 45.65 km/week) (8). Kingwell also reported a decline in running distance in SHR, but not in the WKY (19). They suggested that improved aortic compliance in exercised WKY rats positively affected exercise ability. An increase in aortic compliance permits the aorta walls to further expand during blood ejection from the left ventricle, allowing the aorta to more easily accommodate the increase in arterial blood volume during exercise. The authors proposed that the decrease in physical activity of SHR could be due to their high blood pressure and lack of ability to change their aortic compliance (19,18).

The present study also found that exercised SHR rats had a trend of higher MAP throughout the acute study, however significant between group differences in the exercised versus sedentary SHR were observed only at a few points. Other studies have found that exercise causes a decrease in MAP and resting heart rate in both normotensive and hypertensive rats, although these findings were observed in conscious rats (4, 14, 28). Brum reported that during anesthetized conditions, exercised SHR had a higher MAP compared to sedentary SHR, a similar finding to the present study (4). This may be due to the fact that SHR are known to have a decreased blood volume, and exercise training is known to increase blood volume. During surgical procedures, blood volume decreases further due to minor blood loss and fluid loss from exposed tissue. Therefore exercised SHR, with an improved blood volume, may be better able to maintain their normal blood pressure under these conditions.

Exercised WKY had a significantly lower heart rate than sedentary for the majority of the acute study. Exercised SHR had a lower heart rate, only when MAP increased, but this was not significant. Exercise has been shown in other studies to decrease resting and exercise heart rate in male WKY and SHR rats, as well as in humans (17, 38). The mechanism by which the decrease in heart rate occurs has been proposed to be an increase in efferent parasympathetic nervous system regulation of heart rate, and a simultaneous decrease in efferent sympathetic nervous system regulation of heart rate. The decrease in sympathetic nervous system activity also leads to a decrease in vascular tone and a reduction in MAP (17).

When the change in heart rate at peak MAP was examined in the present study, the exercised SHR had a significantly smaller increase in HR during infusions at Ang II 0.5μg/ml and showed a reduction in HR at 2.0 μg/ml Ang II. These results suggest an exercised-induced improvement in the SHR baroreceptor sensitivity. Other studies have found exercise training increased the sensitivity of the baroreflex control of HR in male WKY and SHR (4, 14). The authors proposed two possible mechanisms for baroreflex bradycardia in SHR: 1) a maintained tachycardia and hypertension during exercise could increase baroreflex sensitivity post-exercise, 2) exercise can increase the magnitude and frequency of the shear stress acting on the endothelial cells, causing release of endothelial factors (ie. NO), that could enhance the baroreceptor sensitivity after exercise (14). The current study did not show a baroreceptor effect in the exercised WKY. This may be explained by the use of females, rather than males, in the current study, or by differences

in the type of drug used to increase mean arterial pressure (Ang II versus sodium nitroprusside and phenylephrine) (4, 14).

CONCLUSION

In conclusion, chronic voluntary exercise in female SHR and WKY rats increased urinary sodium excretion in response to angiotensin II infusion, but the effect disappeared when renal perfusion pressure was held constant. Thus, the exercise-induced effect on urinary sodium excretion was likely due to a change in the renal response to a rise in blood pressure. These are the first studies to show that exercise improves renal sodium excretion in response to a rise in renal perfusion pressure, and to suggest that exercise may increase the sensitivity of the pressure natriuresis relationship. Future studies are needed to investigate the pressure natriuresis relationship in exercised and sedentary rats using non-pharmaceutical means to raise blood pressure. Also, additional studies are needed to elucidate the mechanisms by which exercise affects the pressure natriuresis relationship.

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