Beta-adrenergic receptors and opposition of evoked sympathetic vasoconstriction: Effects of sex and exercise training

by

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Abstract

Stimulation of the sympathetic nervous system evokes the release of neurotransmitters and produces vasoconstriction that is primarily mediated by the binding of norepinephrine (NE) to alpha-adrenergic receptors. However, NE may also bind to beta (β)-adrenergic receptors and produce vasodilation that may oppose vasoconstriction. Exercise training has been shown to enhance contraction-mediated inhibition of sympathetic vasoconstriction (sympatholysis), however the underlying mechanism(s) have not been fully established. It is possible that exercise training may up-regulate β-adrenergic receptor mediated vasodilation and blunt vasoconstrictor responses. Furthermore, β-adrenergic receptor function in the peripheral vasculature may be sex-specific with beta-receptor mediated vasodilation being more important in the regulation of peripheral vascular resistance in females compared to males. Therefore the present study sought to determine if β-adrenergic receptor mediated vasodilation inhibits sympathetic vasoconstrictor responses in resting and contracting skeletal muscle. Additionally the present study investigated the effects of exercise training and sex on β-adrenergic receptor mediated opposition of evoked sympathetic vasoconstriction. It was hypothesized that β-adrenergic receptors would oppose evoked sympathetic vasoconstriction at rest and during exercise in females, but would not oppose sympathetic vasoconstriction in males. Additionally it was hypothesized that exercise training would augment β-adrenergic receptor mediated opposition to evoked sympathetic vasoconstriction in females. Male (n=18) and Female (n=17) Sprague-Dawley rats were randomized into Sedentary (S) or Exercise Trained (ET) groups. ET rats completed a 4-week treadmill training regimen (5 days/week, 15 min at 40m/min). At the completion of

ET or S, rats were anaesthetized and instrumented for the measurement of femoral vascular conductance (FVC), stimulation of the lumbar sympathetic chain, and contraction of the triceps surae muscle group. The percentage change of femoral vascular conductance (FVC) in response to sympathetic chain stimulation delivered at 2 and 5 Hz was determined at rest and during triceps surae muscle contraction (60% maximal contractile force) before (Control) and after β-adrenergic blockade (propranolol; 0.075mg/kg body mass, I.V.). Contrary to the hypothesis, β-adrenergic blockade decreased evoked sympathetic vasoconstriction ($P < 0.05$), and was not different between males and females at rest ($P > 0.05$). During skeletal muscle contraction, female rats had lower responses to evoked sympathetic vasoconstriction than males ($P < 0.05$), but it was not altered by β-adrenergic blockade ($P > 0.05$). In contrast to previous research from our laboratory, exercise training did not alter ($P > 0.05$) sympathetic vasoconstrictor responsiveness or inhibition of sympathetic vasoconstriction. The present study demonstrates that β-adrenergic receptors do not oppose sympathetic vasoconstriction at rest or during skeletal muscle contraction in the hindlimb of rats and at rest are not different between males and females. However during skeletal muscle contraction, females demonstrate a greater attenuation of evoked sympathetic vasoconstriction that was not altered by β-adrenergic receptor antagonism. Collectively, the results from this study suggest that β-adrenergic receptors do not oppose evoked vasoconstrictor responses in resting and contracting skeletal muscle. This study also demonstrated that contraction-mediated inhibition of sympathetic vasoconstriction is greater in female compared to rats. The enhanced blunting of vasoconstriction in female rats was not altered by exercise training and does not appear to be mediated by β-adrenergic receptors.

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List of Abbreviations

ANS – autonomic nervous system
SNS – sympathetic nervous system
PNS – parasympathetic nervous system
ACh – acetylcholine
RVLM – rostral-ventrolateral medulla
SNA – sympathetic nerve activity
MSNA – muscle sympathetic nerve activity
NE – norepinephrine
ATP – adenosine triphosphate
NPY – neuropeptide-Y
NO – nitric oxide
COX – cyclooxygenase
NOS – nitric oxide synthase

Chapter 1: Introduction

Introduction

Sympathetic Vascular Control

The autonomic nervous system (ANS) is comprised of both the sympathetic (SNS) and parasympathetic (PNS) branches and is an integral regulator of several physiological systems, including the cardiovascular system (22). Communication in the ANS is achieved by the transmission of neurotransmitters between neurons and end-organs. The pre-ganglionic neurons of both the SNS and PNS release acetylcholine (ACh), which binds to nicotinic receptors on post-ganglionic neurons. In the PNS post-ganglionic neurons release ACh that binds to muscarinic receptors on end-organs that are innervated by relatively short post-ganglionic neurons; however, in contrast, the SNS is composed of relatively long-post-ganglionic neurons and the primary neurotransmitter norepinephrine (NE) binds to adrenergic and non-adrenergic receptors on target tissues.

Sympathetic efferent nerve activity (SNA) is generated in the cardiovascular control center of the brain stem. Efferent sympathetic nerve impulses arise from the rostral-ventrolateral medulla (RVLM). The frequency and amplitude of impulses generated by the RVLM is influenced by the integration of signaling from a complex network of neural centers, receptors, signal molecules (e.g. glutamate, angiotensin II, nitric oxide), neurotransmitters, and peripheral reflexes (e.g. baroreceptors & chemoreceptors) (40).

In the cardiovascular system, the SNS is an important regulator of arterial blood pressure, vascular resistance and tissue blood flow (22). The skeletal muscle vascular bed is the largest vascular bed in the body and is therefore a major site of vascular

resistance and is integral to the control of blood pressure both at rest and during exercise (22). Sympathetic nerve activity directed to skeletal muscle (MSNA) causes vasoconstriction in the skeletal muscle vascular bed. Changes in the frequency and/or amplitude of efferent SNA result in changes to the quantity and type of neurotransmitter released which influences the magnitude of vasoconstriction (16).

The primary neurotransmitters involved in the control of skeletal muscle vascular resistance include norepinephrine (NE), adenosine-5'-triphosphate (ATP), neuropeptide Y (NPY) (See Figure 1). Vasoconstriction is produced by the binding of NE, ATP, NPY to post-synaptic alpha (α)-adrenergic (α_1 and α_2), purinergic (P2X) and NPY Y^1 receptors on vascular smooth muscle of skeletal muscle arterioles (65). Interestingly, NE can also bind to beta (β)-adrenergic receptors on the vascular smooth muscle and endothelium that cause vasodilation, demonstrating that the same ligand can produce opposing vascular responses. Therefore, the vascular smooth muscle must integrate signals from multiple ligands and receptors to determine the prevailing level of vascular resistance and therefore blood flow (34). Furthermore, post-synaptic receptors are expressed and distributed throughout the vasculature of the skeletal muscle and their differential distribution influences the precise control of blood flow between and within muscles (1). Recent evidence has demonstrated that proximal arterioles (1A-3A) are primarily under adrenergic control, whereas more distal vessels (4A & 5A) in the peripheral vasculature are controlled through NPY and ATP, in an *in vivo* rat gluteus maximus preparation (1). This research is in agreement with previous studies in the rat cremaster muscle that suggested that large arterioles (1A) were largely under control of α_1-adrenoreceptors, while the smaller arterioles (3A) were under α_2-adrenoreceptor

control (49). In contrast, a murine gluteus maximus model demonstrated that although heterogeneously distributed, 1A arterioles showed the highest level of vasoconstriction by an α_2-adrenergic receptor specific agonist, while 3A arterioles had greater constriction elicited by an α_1-adrenergic receptors specific agonist (42). Collectively, these data while inconclusive, suggest that the proximal vasculature is largely under α-adrenergic control, yet may differ between α-adrenoreceptor subtypes, and the distal vasculature is largely under non-adrenergic control (1, 42, 49).

Sympathetic Vascular Control During Exercise

During exercise the metabolic demands of active tissues markedly increase. To meet the elevated demands of skeletal muscle, cardiac output increases and skeletal muscle blood vessels dilate to increase muscle blood flow and oxygen delivery to meet the increased demand (13). At the onset of activity there is an almost immediate increase in flow to the exercising tissues achieved through the contraction of the muscle itself and the skeletal muscle pump, followed by sustained dilation and increased flow through other by-products of contraction (64). However, if the robust vasodilation were to occur systemically, blood flow required would far outstrip maximal cardiac output and a there would be a substantive drop in arterial pressure (6). Therefore SNA is increased to the skeletal muscle vascular bed, (8, 55).

Indeed, during exercise both the frequency and amplitude of SNA directed to skeletal muscle increases in an exercise intensity-dependent manner (16) suggesting that vasoconstriction in active muscle may actually increase during exercise. However, in contracting skeletal muscle, the responsiveness of □-adrenergic receptors is decreased in an exercise intensity-dependent manner (7, 10, 12, 44, 54, 55, 61). This attenuation of

sympathetic vasoconstriction during exercise is termed functional sympatholysis (6, 52). It is believed that this blunting of vasoconstriction allows for a precise matching of blood flow to metabolic demand within active skeletal muscles, while still maintaining blood pressure (55). In humans, there are multiple signaling mechanisms that have been implicated in the attenuation of sympathetic vasoconstriction during exercise. Although ATP, NO and prostaglandins have all been suggested to be involved in sympatholysis, none appear individually to be required for the attenuation of sympathetic vasoconstriction, and the mechanism through which sympatholysis occurs is not known (44, 47, 48, 55).

β-adrenergic Receptor Expression and Function

β-adrenergic receptors located on the endothelium have been shown to cause vasodilation in animal and human models (14, 15, 51). However, when the endothelium is removed, rat mesenteric arteries and canine pulmonary arteries dilate in response to the β-adrenergic receptor specific agonist isoproterenol, indicating that β-adrenergic receptors are present and functional on the vascular smooth muscle of these vascular beds (18, 57).

β-adrenergic receptors located on vascular smooth muscle appear to cause vasodilation through activation of adenylyl cyclase pathways, whereas β-adrenergic receptors in the endothelium cause vasodilation through release of nitric oxide (NO) (20). However, the signaling pathway through which β-adrenergic receptors cause vasodilation is poorly understood, may vary based on species and vascular bed examined, and has been mostly investigated in isolated vessel preparations. Both nitric oxide (NO) (3, 9, 11, 19, 21, 33) and cyclooxygenase (COX) (38, 53, 56) have been suggested to play a role in

β-adrenergic receptor vasodilation although neither appears to be requisite for vasodilation. Recent evidence suggests that there may be an interaction between NO and COX molecules, with COX products restraining the NO-mediated vasodilation of β-adrenergic receptors in the human forearm vasculature (36). Additional signaling may occur through p38 mitogen-activated protein kinases from β-adrenergic receptors in both vascular smooth muscle cells and endothelial cells (45). β-adrenergic receptors may also modulate phosphorylation of ERK1/2, with differing effects in the vascular smooth muscle and endothelium (51). The p38 and ERK1/2 signaling pathways in the vascular smooth muscle are thought to play a role in the vasodilation of the vasculature and to be responsible for angiogenesis in the endothelium (51). Although there are many pathways that may be activated by β-adrenergic receptors, the functional outcomes of the activated pathways *in vivo* are not necessarily understood.

Stimulation of β-adrenergic receptors with exogenous agonists causes vasodilation in the skeletal muscle vasculature (9, 11, 33, 37, 63). Historically there was little data to support a role for β-adrenergic receptors in the control of the peripheral vasculature, however a few recent studies (23, 36, 50) have investigated the role of β-adrenergic receptors in the sympathetic control of the peripheral vasculature. Whether β-adrenergic receptor mediated vasodilation opposes sympathetic vasoconstriction in the skeletal muscle vasculature has not been clearly established. A recent study in isolated arterioles has shown that NO and COX products may interact in response to exogenous infusion of a β-adrenergic specific agonist isoproterenol to produce vasodilation (37). This may be of importance to functional sympatholysis as sympatholysis occurs through

a NO-mediated mechanism in sedentary rats (62), and is increased via a NO-mediated mechanism through exercise training (26, 28, 30, 41).

Following selective or non-selective β-adrenergic blockade, exogenous phenylephrine infusion has been shown to increase forearm vascular conductance in human, suggesting that stimulation of β-receptors causes vasodilation in humans (63). In response to a variety of sympatho-excitatory maneuvers (neck pressure, thigh occlusion and hand-grip) a β-adrenergic receptor specific antagonist (propranolol) did not alter sympathetic vasoconstrictor responses in men and women (50). Sympathetic vasoconstrictor responses were not different between men and women in this study; however, post hand-grip hyperemia was larger in women compared to men and the larger hyperemia in women was abolished by β-adrenergic blockade (50). Taken together, these data suggest that β-adrenergic receptors contribute to vasodilation of the skeletal muscle vasculature, but do not modulate sympathetic vasoconstrictor responsiveness in sedentary individuals at rest. Furthermore the contribution of β-adrenergic receptors to vasodilation may differ between males and females.

Exercise Training & Sympathetic Vascular Control

In rodent models, sympatholysis appears to be mediated by an NO-dependent mechanism. Following exercise training our laboratory, and others, have shown an increase in the magnitude of sympatholysis occurs through a NO-mediated mechanism and that exercise training also augments the responsiveness of the adrenergic receptors (26, 28, 30, 41). At rest, the β-adrenergic receptors are more responsive to sympathetic stimulation after being exercise trained (26, 29-31). However, with exercise training, the response differs between β-adrenergic receptors, as functional sympatholysis occurs

through different pathways than it does in a sedentary state (30, 32, 44). Exercise training augments the inhibition of α_1-adrenergic receptors, and following blockade of α_1-adrenergic receptors, improvements in sympathoysis seen following exercise training were abolished (32). While these findings have advanced our understanding of the mechanisms involved in sympatholysis and plasticity in sympathetic vascular control, evoked vasoconstrictor responses remain blunted in contracting muscle in the presence of pharmacological inhibition of NO production and selective adrenergic receptor blockade (29-32, 41) in both sedentary and exercise trained rats. This suggests that other mechanisms contribute to the blunting of vasoconstriction in contracting muscle and remain to be identified.

Sex Differences and Sympathetic Vascular Control

The relationship between MSNA, cardiac output and peripheral resistance also appears to differ between males and females (24). In young males, MSNA is positively correlated with peripheral vascular resistance at rest, whereas this relationship is absent in females. (24). Consistent with the lack of a relationship between MSNA and vascular resistance in women, young females have been shown to have a blunted vasoconstrictor response to exogenous noradrenaline compared to males and post-menopausal females (23). Selective blockade of β-adrenergic receptors abolished the differences in noradrenaline evoked vasoconstrictor responses between young females and males and post-menopausal women, suggesting that β-adrenergic receptor mediated vasodilation may blunt vasoconstriction in young females. (23). β-adrenergic receptor blockade did not alter noradrenaline evoked vasoconstriction in post-menopausal women suggesting that estrogen status may influence β-adrenergic receptor function (4, 23, 46). Indeed,

isolated vessels from ovariectomized rats that underwent estrogen replacement showed endothelium-independent β-adrenergic vasodilation that was improved as compared to ovariectomized rats with no estrogen replacement in the mesenteric vascular bed (18). Although there has been a significant expansion in the body of knowledge surrounding β-adrenergic receptors contribution to sympathetic vascular control, the research to date has mostly been correlational (23, 24), isolated vessel preparations (18), or application of exogenous neurotransmitters (63), and tend to be in conflict with the one study done in an integrative model (50), therefore not allowing for a definitive conclusion that these receptors play a role in the integrative sympathetic control of the skeletal muscle vasculature.

In aged rats, there is a decreased responsiveness to infusion of a β-adrenergic receptor specific agonist isoproterenol was reversed with exercise training in isolated preparations from a rat aorta (35). To date, there has been no work evaluating sex differences or the effects of exercise training on the responsiveness of β-adrenergic receptors to direct sympathetic stimulation at rest or their possible contribution to functional sympatholysis during exercise.

Although our laboratory (27-32) and others (41, 59, 60, 62) have established some of the mechanisms responsible for the inhibition of sympathetic vasoconstriction in resting and contracting skeletal muscle in sedentary and exercise-trained male rats, the mechanistic basis for sympatholysis has not been fully elucidated. The potential role for β-adrenergic receptors in the opposition of sympathetic vasoconstriction is intriguing, as β-adrenergic receptors could be a potential contributor to integrative sympathetic vascular control. It is the aim of the thesis to elucidate sex differences in sympathetic

vascular control, as well as the changes in sympathetic vascular control that occurs after exercise training.

Purpose

With this background the following purposes were addressed in the present study:

I. To elucidate whether β-adrenergic receptors oppose evoked sympathetic vasoconstrictor responses at rest and during skeletal muscle contraction.

II. To elucidate the effects of exercise training on β-adrenergic receptor opposition of sympathetic vasoconstriction at rest and during skeletal muscle contraction.

III. To determine whether β-adrenergic receptor opposition of sympathetic vasoconstriction at rest and during skeletal muscle contraction is dependent on sex.

Hypotheses

I. Evoked vasoconstrictor responses would be unchanged at rest and during skeletal muscle contraction following β-adrenergic receptor blockade, demonstrating that β-adrenergic receptor do not oppose evoked sympathetic vasoconstriction.

II. Exercise training increases in evoked vasoconstrictor responses have been previously shown in male rats (27, 29, 30), and will also be demonstrated in females at rest and during skeletal muscle contraction.

III. Following β-adrengergic blockade, evoked sympathetic vasoconstriction will be raised in females, and unaltered in males at rest and during skeletal muscle contraction. Inhibition of evoked vasoconstrictor responses (sympatholysis)

during skeletal muscle contraction will be increased following exercise training through a β-adrenergic mediated mechanism in female rats that will not be present in male rats, suggesting that β-adrenergic receptor responsiveness is improved following exercise training in female rats only.

Significance

This thesis investigated β-adrenergic receptor mediated opposition of sympathetic vasoconstriction in resting and contracting skeletal muscle. To my knowledge this is the first study to investigate the role the β-adrenergic receptors play in the evoked vasoconstrictor response in contracting muscle and following exercise training in males and females. Regular exercise has been shown to reduce the risk of cardiovascular disease and exercise training is commonly prescribed for the treatment of cardiovascular disease, however the underlying physiological mechanisms that mediate the cardio-protective effects of exercise training have not been established. Whether exercise training modulates the function of β-adrenergic receptors and enhances vascular function and blood pressure control has not been investigated. Additionally, this study investigated whether sex differences are present in β-adrenergic receptor function. If sex differences are present, this information could provide knowledge of sex-specific therapeutic targets for the treatment of hypertension and cardiovascular disease. Prior to menopause, females are at a markedly lower risk for cardiovascular disease and then experience a marked increase in cardiovascular disease risk coincident with the onset of menopause (25). The mechanisms responsible for decreased cardiovascular disease risk in women prior to menopause have not been definitively established, but are thought to be mediated by estrogen and β-adrenergic receptors. Recent studies have shown potential for β-

adrenergic receptors to be involved in these sex differences as β-adrenergic receptors oppose vasoconstriction in response to pharmacological agonists in young females (23). However, this data only describes pharmacological infusion of sympathetic agonists and antagonists at rest and does not demonstrate an *in vivo* integrative mechanism (23). The present study addresses the possibility of sex differences in the role of β-adrenergic receptors in the *in vivo* integrative control of the peripheral vasculature in response to evoked sympathetic vasoconstriction at rest and during skeletal muscle contraction. These differences between males and females may have potential implications for pharmacological or other treatment targets in people with cardiovascular disease, as well as may further our mechanistic understanding of vascular function and the integrative control of blood pressure.

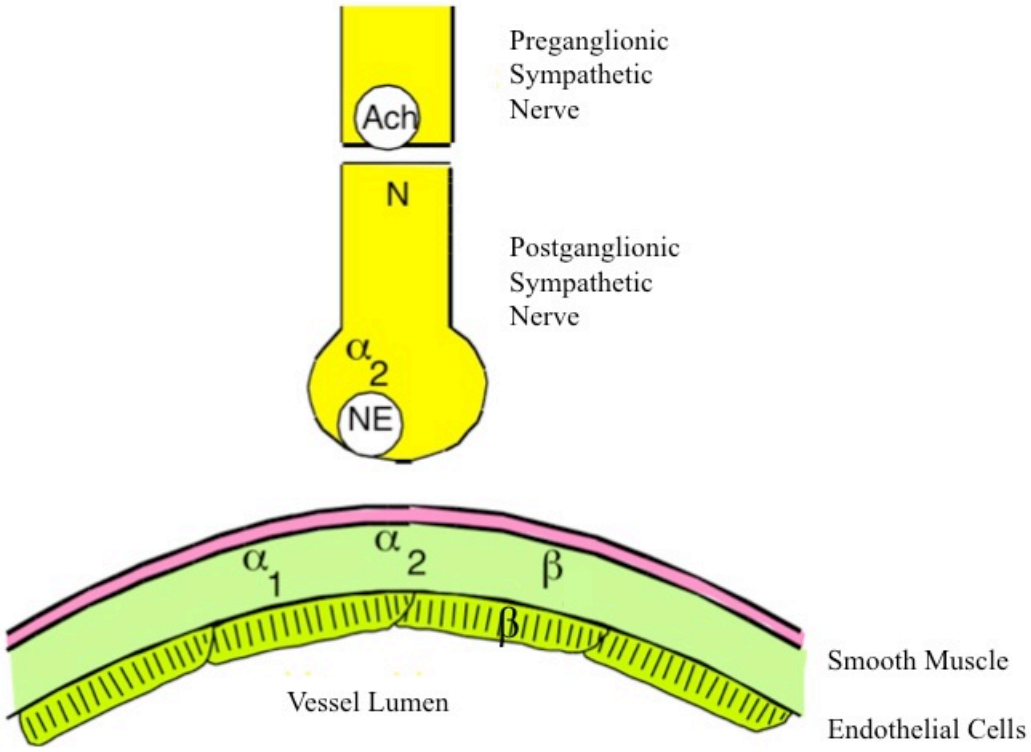

Figure 1. A conceptual figure showing the sympathetic vasoconstriction pathway occurring in skeletal muscle vascular beds through the release of the neurotransmitter norepinephrine (NE). NE released from sympathetic nerve fibres acts on α_1- and α_2-adrenergic receptors on the vascular smooth muscle causing vasoconstriction. NE also acts on β-adrenergic receptors on the vascular smooth muscle and endothelium causing vasodilation of the vasculature.

Chapter 2: β-adrenergic receptors and evoked sympathetic vasoconstriction: Sex and exercise training effects

Introduction

The sympathetic nervous system (SNS) plays an integral role in the control of the cardiovascular system (22). A predominant function of the SNS is the control of blood pressure, peripheral vascular resistance and tissue blood flow (22). Sympathetic nerve activity (SNA) generated in the RVLM travels down sympathetic nerve fibres to the vasculature, causing the release of the neurotransmitters norepinephrine (NE), neuropeptide-Y (NPY) and adenosine-triphosphate (ATP) (22). The primary neurotransmitter NE acts on alpha (α)-adrenergic receptors on the vascular smooth muscle (VSM) to cause vasoconstriction. This same neurotransmitter may also bind with beta (β)-adrenergic receptors and causes vasodilation of the vasculature (63). When α-adrenergic receptors are blocked, an adrenergic agonist (phenylephrine) causes vasodilation of the forearm vasculature (63). At rest, the peripheral vasculature is tonically vasoconstricted to maintain vascular resistance and blood pressure (22). As the same neurotransmitter acts on both types of adrenergic receptors, it is possible that β-adrenergic receptor mediated vasodilation could oppose α-adrenergic receptor mediated sympathetic vasoconstriction in response to neurotransmitter release and blunt vasoconstrictor responsiveness.

During skeletal muscle contraction evoked sympathetic vasoconstrictor responsiveness is attenuated, and this contraction-mediated inhibition of sympathetic

vasoconstriction is termed functional sympatholysis (52). Recent studies from our laboratory, and others have established that sympatholysis occurs partly through a NO-mediated mechanism and inhibition of α-adrenergic receptors that is improved following exercise training (5, 27-32, 41, 44, 45). Although partly NO-mediated, the exact mechanism(s) for sympatholysis is not fully understood, therefore β-adrenergic receptor mediated vasodilation could potentially play a role in the inhibition of sympathetic vasoconstriction during skeletal muscle exercise, as well as the response to endurance exercise training. In humans, sympathetic vasoconstrictor responses to sympatho-excitatory maneuvers including neck pressure, thigh cuff occlusion, and isometric hand-grip were not altered in the resting leg of males or females following β-adrenergic receptor blockade (propranolol), suggesting that β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction in resting skeletal muscle (50). However β-adrenergic receptor-mediated opposition of sympathetic vasoconstriction during exercise was not investigated, and studies have not addressed this in contracting skeletal muscle.

Recent research has shown that the regulation of blood pressure may differ between males and females, as the way in which SNA, cardiac output, and peripheral resistance interact to regulate blood pressure is different, with males exhibiting a strong relationship between MSNA and total peripheral resistance (TPR) that is absent in females (23, 24). Recently, it has been suggested that β-adrenergic receptor mediated vasodilation may explain the lack of a relationship between MSNA and TPR in females, as young females demonstrated blunted forearm vasoconstriction to noradrenaline that was abolished with β-adrenergic receptor blockade (propranolol) (23). However, a recent study reported that forearm vasodilation to exogenous infusion of the β-adrenergic

specific agonist isoproterenol was not different between males and females (36). Overall, the role that β-adrenergic receptors play in the opposition of evoked sympathetic vasoconstriction has not been well established at rest, and has yet to be investigated in contracting skeletal muscle. Furthermore, the effect of exercise training on the *in vivo* function of β-adrenergic receptors has not been established, and there is not conclusive evidence that these receptors and their function are mediated by sex.

Therefore, the purpose of the study was to: 1) examine whether β-adrenergic mediated vasodilation opposes evoked sympathetic vasoconstriction in resting and contracting skeletal muscle; 2) determine if sex alters β-adrenergic receptor mediated vasodilation and the opposition of evoked sympathetic vasoconstriction at rest or contracting skeletal muscle; 3) to examine the exercise training effects on β-adrenergic receptor mediate vasodilation and the opposition of evoked sympathetic vasoconstriction at rest or during skeletal muscle contraction. It was hypothesized that β-adrenergic receptors would not augment evoked sympathetic vasoconstriction in resting skeletal muscle, but would augment sympathetic vasoconstriction during contracting skeletal muscle in female rats. Additionally, exercise training would increase β-adrenergic receptor mediated opposition to evoked sympathetic vasoconstriction in females, while males would not be altered.

Methods

All experiments were conducted in accordance with the guidelines of the Canadian Council for Animal Care and under protocols approved by the University of Alberta Health Sciences Animal Care and Use Committee (AUP #244 & #1493).

Animals and Animal Care

Sprague-Dawley (n=19 males and n=17 females) rats were housed in a 12:12h light-dark cycle, environmentally controlled (22-24^{O}C, 40-70% humidity) room. Water and rat chow was provided ad libitum (Lab diet 5001, PMI Nutrition, Brentwood, MO). Animals were obtained from the institutional breeding colony at the University of Alberta at ~150 g body weight. The 19 males were randomized into sedentary (n=10) and exercise trained (n=9) groups and the 17 females were also randomized into sedentary (n=8) and exercise trained (n=9) groups (See Figure 2 for overview of overall sample size and randomization). All rats underwent the same training paradigm and surgical procedures as described below.

Endurance Exercise Training

Following randomization, all rats were habituated to the lab and exercise by walking 10 minutes a day for 5 days at 10m·min^{-1}, 0° incline. Following familiarization, the rats began the sedentary cage behaviour or exercise training for four weeks, with the training intensity (40m·min^{-1} 15% grade) corresponding to ~80% VO_{2max} as calculated from rodent studies utilizing direct metabolic measurements (2, 17). Our laboratory has previously shown that this training paradigm augments both the evoked sympathetic vasoconstrictor response (27, 29-32), and improves the inhibition of sympathetic vasoconstriction during skeletal muscle contraction (sympatholysis) (29-32) in an exercise training intensity-dependent manner. From our laboratories previous experience, exercise trained rats initially ran at the prescribed speed for 15 intervals of 1 minute running: 1 minute rest, with the running interval time increased each day until rats were able to run continuously for 15 minutes. Once trained rats were able to complete 15

minute of high-intensity treadmill exercise (within 2 weeks), the rats continued at this intensity for 15-minute bouts for the remaining two weeks.

Instrumentation

Anaesthesia was induced with inhalation of isoflurane (3.5% in balance oxygen). The right jugular vein was cannulated for continued intravenous administration of anaesthesia with alpha-chloralose (8-16 mg kg^{-1} hr^{-1}) and urethane (50-100 mg kg^{-1} hr^{-1}. A tracheotomy was performed to allow spontaneous breathing and maintenance of blood gases. The right carotid artery was cannulated and attached to a pressure transducer (Abbott, North Chicago, IL) for the measurement of arterial blood pressure (ABP), and from which the heart rate (HR) signal was attained. The depth of anaesthesia was assessed by the stability of hemodynamic variables and the absence of a withdrawal reflex in response to painful stimulus (i.e. paw-pinch). Core temperature were monitored by rectal probe and maintained at 37^{o}C by external heating pad (Physitemp, TCAT-2, Clifton, NJ). Agonist and antagonist drugs were infused through cannulae inserted into the right femoral artery and vein with the tip of the cannula positioned proximal to the iliac bifurcation allowing for injection of agonists and antagonist agents into the experimental left femoral artery and vein. The femoral blood flow was measured using a transit time flow probe (7V; Transonic Systems, Ithaca, NY) connected to a flow-meter (T106, Transonic Systems, Ithaca, NY) around the left femoral artery. At the completion of surgical preparation the tracheal tube was connected to a small animal ventilator (Model 683**,** Harvard Apparatus, Holliston, MA) for the maintenance of blood gasses throughout the experiment. Heparinized saline (10 units/mL;0.5-1.0 mL/hour) was continuously infused through the femoral vein via a syringe pump (K.D. Scientific) to

prevent blood clotting. Femoral artery and carotid artery cannulae were flushed regularly with heparinized saline to maintain patency. At the conclusion of the experiments, rats were euthanized by anaesthetic overdose. Following euthanization the trieps surae muscle group was dissected and weighed, as well as the heart was dissected and weighed.

Hind-limb Muscle Contractions

The right sciatic nerve was exposed and instrumented with a cuff electrode. The triceps surae muscle group was then dissected free of all skin and connective tissue and attached to a force transducer (Model FT03, Grass Technologies, Warwick, RI) via the calcaneal tendon. Hind-limb contractions were produced by electrical stimulation of the sciatic nerve with Chart 7.2™ software (AD Instruments, Colorado Springs, CO). Maximal contractile force (MCF) was determined by stimulation of the triceps surae muscle group with 25, 1ms impulses delivered at 100Hz, 10× motor threshold (MT). The optimal muscle length for tension development was determined by progressively lengthening the muscle and repeating the nerve stimulation until a plateau in tension (peak – base- line) is observed. The triceps surae muscles was stimulated (40 Hz, 0.1 ms pulses in 250 ms trains, at a rate of 60 trains min^{-1} at ~3× MT) to contract rhythmically at 60% MCF.

Lumbar Sympathetic Chain Stimulation

A laparotomy was performed and the aorta and vena cava were temporarily retracted to attach a bipolar silver-wire-stimulating electrode to the lumbar sympathetic chain at the L3/L4 level. The electrode was embedded and electrically isolated in a rapidly curing nontoxic silicone elastomer (Kwiksil, WPI, Sarasota, FL). The electrode was used to

deliver constant current stimulations through an isolated stimulator (Digitimer DS3, Welwyn City, UK). Following a 20-min stabilization period the following experiments were conducted.

Experimental Protocol

Within 48 hours of completing their last training session, the rats were anaesthetized and instrumented for study as described above. The vascular response to sympathetic stimulation delivered in random order at 2 and 5Hz were examined at first in resting and then contracting muscle of rodents from each group. Following 20 minutes of recovery time, pharmacological infusions (left femoral vein) of the β-adrenergic receptor antagonist were performed: propranolol (0.075 mg kg^{-1}), based on pilot data collected. Complete blockade of β-adrenergic receptors was determined by infusion of a β-agonist bolus of isoproterenol (0.05 μg, left femoral artery). Sympathetic stimulation was then repeated in resting and contracting muscles in the presence of the β-adrenergic blockade.

Pharmacology

All drugs were obtained from Sigma-Aldrich (Oakville, ON, Canada) and dissolved in 0.9% physiological saline.

Data Analysis

Arterial blood pressure and femoral artery blood flow (FBF) were sampled at 100 Hz and femoral vascular conductance (FVC) was calculated as FBF ÷ MAP (mL min^{-1} $mmHg^{-1}$). Muscle force production was measured continuously and from this, peak force development was determined for each contractile bout. To compare force production between groups and experimental conditions, average contractile force was

calculated from minutes 3 to 7 (the time period encompassing the sympathetic stimulations) of each contractile bout. The average of one minute changes in HR, MAP, FBF, and FVC in response to sympathetic stimulation were calculated as an absolute change and as a percentage change from the minute preceding the sympathetic stimulation in Control and propranolol conditions. The percentage change in FVC is the accepted metric to assess the magnitude of sympathetic vasoconstrictor responses as percentage changes in FVC accurately reflects percentage changes in resistance vessel radius across different baseline levels of vascular conductance (6, 61). The magnitude of sympatholysis was calculated as the difference between the percentage change in FVC in response to sympathetic stimulation at rest and the percentage change in FVC in response to sympathetic stimulation during muscular contraction for control and propranolol conditions. All data are expressed as mean ± standard deviation.

Statistical Analysis

Sympathetic vasoconstrictor responsiveness before and after propranolol infusion was compared using a four-way repeated measures ANOVA (Sex x Training Status x Drug Condition x Contractile State). When significant F-ratios are found, interactions were assessed using Student-Newman-Keuls post-hoc analysis. The effect of the drug condition on baseline hemodynamic variables, as well as the hyperemic response to exercise was compared using a three-way repeated measures ANOVA (Sex x Training Status x Drug Condition). When significant F-ratios are found, interactions will be assessed using Student-Newman-Keuls post-hoc analysis. Finally, anthropometric data was compared between groups to determine the effect of training on body mass, muscle mass, heart mass and heart mass:body mass ratio using a two-way between group

ANOVA (Sex x Training Status). When significant F-ratios are found, interactions were assessed using Student-Newman-Keuls post-hoc analysis. A p-value of < 0.05 was considered statistically significant.

Delimitations

Power analysis based on rodent training studies in our laboratory (27, 29-32) suggest that an expected change of femoral vascular conductance would be ~15% in response t sympathetic stimuli. Assuming $\beta=0.2$ and $\alpha<0.05$, and an effect size of 0.8, a sample of n=10-12 rats per condition would yield a statistically significant difference. With these sample sizes, the number of rats was determined to be 40 (10 sedentary time-control & 10 exercise trained for both males and females). It is important to note that although rat and murine models typically provide good mechanistic basis for humans, the experiments contained herein are limited in that they are not performed in a human model.

Results

Anthropometrics and Indices of Training Efficacy

Body mass was greater ($P < 0.05$) in males compared to females. Exercise trained (ET) male rats had a lower ($P < 0.05$) body mass than sedentary (S) males, whereas body mass was not different ($P > 0.05$) between S and ET female rats. Heart mass was larger ($P < 0.05$) in males compared to females, but was not altered ($P > 0.05$) by ET in either male or female rats. Heart to body mass ratio was greater ($P < 0.05$) in females compared to males, and was increased ($P <0.05$) by ET rats in male and female rats (Table 1). ($P < 0.05$) Soleus, medial gastrocnemius, and lateral gastrocnemius muscle mass was larger (P

<0.05) in male compared to female rats. Exercise training did not alter (P > 0.05) skeletal muscle masses in male or female rats (Table1).

Effect of β-adrenergic Blockade

Resting Hemodynamics & Exercise Hyperemia

Baseline HR and MAP were lower at rest (P < 0.05) following β-adrenergic blockade. However, there was no difference (P > 0.05) in baseline FBF and FVC following administration of propranolol (Table 2). The increase in FVC in response to infusion of the β-adrenergic specific agonist isoproterenol was abolished (Main effect of drug; P < 0.05) following administration of propranolol (Figure 5).

The pressor response in response to contraction was not altered (P > 0.05) following β-adrenergic blockade. The absolute and percentage increases in FBF and FVC in response to contraction were greater (Main effect of drug; P < 0.05) in the propranolol compared to the control condition in both male and female rats (Table 4). Peak force was lower (P < 0.05) and fatigue index was higher (P < 0.05) following β-adrenergic blockade, however average forces were not different (P > 0.05) (Table 5).

Sympathetic Vasoconstrictor Responses

Following β-adrenergic blockade, sympathetic vasoconstrictor responsiveness was decreased (Main effect of drug; P < 0.05) at both 2 Hz and 5 Hz (Figure 4). The MAP response to sympathetic stimulation delivered at both 2 and 5 Hz was increased (Main effect of drug; P < 0.05) following propranolol administration (Table 3).

Effect of Exercise Training

Resting Hemodynamics & Exercise Hyperemia

Heart rate (HR) at baseline was lower ($P < 0.05$) in ET rats compared to S rats, while all other resting hemodynamic variables (MAP, FBF, FVC) were unaltered ($P > 0.05$) by exercise training (Table 2). There was an increased FVC response ($P < 0.05$) to isoproterenol infusion following exercise training (Figure 5).

The hyperemic response to exercise (FBF and FVC) was increased ($P < 0.05$) following exercise training, while the MAP response to exercise was not altered ($P > 0.05$) by exercise training (Table 4). Peak force, average force, and fatigue index were not altered ($P > 0.05$) by exercise training (Table 5).

Sympathetic Vasoconstrictor Responses

Tracings showing evoked sympathetic vasoconstriction at rest and during skeletal muscle contraction from a representative rat are found in Figure 3.

Exercise training did not alter ($P > 0.05$) evoked vasoconstrictor responses (FVC) to sympathetic stimulation at 2 and 5 Hz (Figure 4) in resting and contracting skeletal muscle. During skeletal muscle contraction, evoked vasoconstrictor responses at both 2 Hz and 5 Hz were lower ($P < 0.05$) than at rest (Figure 4). The pressor response (MAP) to evoked sympathetic vasoconstriction was not altered ($P > 0.05$) by exercise training (Table 3).

Effect of Sex

Resting Hemodynamics & Exercise Hyperemia

Male rats had a lower ($P < 0.05$) resting HR than female rats. MAP was not different ($P > 0.05$) between males and females. Female rats had lower ($P < 0.05$) resting FBF and FVC than male rats (Table 2).

There was no difference ($P > 0.05$) in MAP response to contraction between male and female rats (Table 4). The absolute increase in FBF and FVC in response to skeletal muscle contraction was smaller ($P < 0.05$) in females compared to males, however the percentage increase as compared to baseline in FBF and FVC was not different ($P > 0.05$) between the sexes (Table 4). Peak force and average force were lower ($P < 0.05$) in females, but fatigue index was not different ($P > 0.05$) between males and females (Table 5).

Sympathetic Vasoconstrictor Responses

There was no difference ($P < 0.05$) between evoked sympathetic vasoconstriction in male and female rats at 2 Hz and 5 Hz during rest (Figure 4). Females had smaller responses ($P < 0.05$) to evoked sympathetic vasoconstriction during skeletal muscle contraction than males at both 2 Hz and 5 Hz (Figure 4). There was no difference ($P > 0.05$) in pressor response to evoked constriction at 2 Hz, but an increased ($P < 0.05$) pressor response in female rats at 5 Hz stimulation frequency (Table 3).

Discussion

The primary purpose of the present study was to determine whether β-adrenergic receptor mediated vasodilation opposes evoked sympathetic vasoconstrictor responsiveness. The secondary purposes were to examine the effect of sex and exercise

training on β-adrenergic receptor function and the opposition of sympathetic vasoconstriction. Previous data has shown that there is no β-adrenergic mediated opposition of evoked sympathetic vasoconstriction in resting skeletal muscle in humans (50). However, whether β-adrenergic receptor mediated vasodilation opposes sympathetic vasoconstriction in contracting skeletal muscle has not been investigated. Previous studies from our laboratory have demonstrated plasticity in the adrenergic control of the vasculature and the contraction-mediated inhibition of sympathetic vasoconstriction (27, 29-32), however no studies to date have looked at the effect of exercise training of the β-adrenergic mediated opposition of evoked sympathetic vasoconstriction. In young women there is a blunted response to infusion of exogenous noradrenaline not present in males, that is abolished following β-adrenergic blockade (23). This potential for a β-adrenergic mechanism opposing sympathetic vasoconstriction could explain the lack of a relationship between MSNA and peripheral resistance in young females, while males exhibit a correlation between MSNA and peripheral resistance (24).

The findings from the present study demonstrate that β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction at rest or during skeletal muscle contraction sedentary and exercise-trained male and female rats. Additionally β-adrenergic receptor function does not appear to differ between male and female rats. The present findings demonstrate that in females there is a greater contraction mediated inhibition of evoked sympathetic vasoconstriction, but this sex difference in function is not mediated by β-adrenergic receptors.

Effect of β-adrenergic Blockade on Evoked Vasoconstriction and Vascular Control

Following β-adrenergic blockade, there was a decrease in evoked sympathetic vasoconstriction at both rest and during skeletal muscle contraction (Figure 4). This result is contrary to what was expected as previous research showed no change to evoked vasoconstrictor responses at rest in humans (50), and blunted exogenous infusion of noradrenaline by β-adrenergic receptors in the forearm vasculature of young females was abolished following β-adrenergic blockade (23). Infusion of the β-adrenergic receptor agonist isoproterenol caused vasodilation in the hindlimb vasculature (Figure 5) indicating that there are β-adrenergic receptors located on the endothelium of the hindlimb vasculature that are functional in both male and female rats. Following infusion of the β-adrenergic receptor antagonist propranolol, the vasodilatory response to isoproterenol was abolished in the hindlimb vasculature (Figure 5), demonstrating that β-adrenergic receptors were blocked in the propranolol condition.

This data demonstrates that although there are functional β-adrenergic receptors in the hindlimb vasculature, β-adrenergic receptor mediated vasodilation does not inhibit evoked sympathetic vasoconstriction. In fact, when β-adrenergic receptors were blocked, sympathetic vasoconstriction was decreased indicating that there is a mechanism during systemic blockade of β-adrenergic receptors in a rodent model that increases flow to the hindlimb during evoked sympathetic vasoconstriction. In response to evoked sympathetic vasoconstriction, β-adrenergic blockade does increase MAP at both stimulation frequencies indicating there is an increase in vasoconstriction in another vascular bed that is not present in the hindlimb vasculature (Table 3), as there is a smaller decrease in constriction (FVC). The increase in MAP, but decreased FVC responses to

evoked vasoconstriction following propranolol infusion suggests that increased pressure in another vascular bed that could shunt blood flow to the hindlimb where β-adrenergic receptors are not opposing sympathetic vasoconstriction. The hyperemic response in both FBF and FVC to exercise is also increased following infusion of propranolol (Table 4). The increase in hyperemic response to the same contractile stimulus suggests that there is more blood flow being directed to the contracting hindlimb, consistent with the notion that propranolol may be constricting another vascular bed in the body and shunting blood flow to the hindlimb vasculature. Although β-adrenergic receptors may be present and functional in the hindlimb vasculature, they may not necessarily be acting to inhibit evoked sympathetic vasoconstriction.

The findings from the present study are in contrast to previous research that has demonstrated that sympathetic vasoconstriction in response to sympathetic maneuvers at rest is not altered following local infusion of propranolol in the human leg (50). Although significant, the magnitude of decrease in evoked sympathetic vasoconstriction was small, however the effect was seen in every group as compared to its control condition. Additionally, this effect is not a time or order of condition effect as reproducibility pilot data did not demonstrate a decrease in evoked sympathetic vasoconstriction in the second control condition.

Although other research has suggested that β-adrenergic receptors may oppose sympathetic vasoconstriction (4, 23, 24), they draw this conclusion from infusion of exogenous noradrenaline in the human forearm (23). The current data confirms that there are β-adrenergic receptors in the hindlimb vasculature of both male and female rats through exogenous application of isoproterenol, and previous work has demonstrated that

β-adrenergic receptors are present in the human forearm vasculature and respond not only to β-specific agonist isoproterenol but to Phenylephrine (63) and Noradrenaline (23), agonists which are more similar to the neurotransmitters that cause *in vivo* sympathetic vasoconstriction.

Taken together the previous research and our current research demonstrate that there are functional β-adrenergic receptors in the skeletal muscle vasculature (23, 63), but that they do not oppose evoked sympathetic vasoconstriction at rest (50) or during skeletal muscle contraction.

Effects of Exercise Training on Evoked Vasoconstrictor Responses and Vascular Control

The second purpose of the study was to investigate the effects of exercise training on β-adrenergic receptor mediated vasodilation and the inhibition of sympathetic vasoconstriction. This is the first study to my knowledge that examines the effect of exercise training on *in vivo* β-adrenergic receptor function in resting and contracting skeletal muscle. In contrast with previous exercise training studies in a rodent model from our laboratory (27, 29-32), exercise training did not increase evoked sympathetic vasoconstrictor responsiveness in resting and contracting skeletal muscle (Figure 4). Additionally, exercise training also increased vascular responsiveness to infusion of the β-adrenergic specific agonist isoproterenol (Figure 4). This finding is consistent with data from an isolated vessel preparation that demonstrated exercise training maintained β-adrenergic receptor response in aged animals, where the sedentary aged animals showed decreased β-adrenergic mediated vasodilation (35). The increased β-adrenergic mediated vasodilation following exercise training did not increase β-adrenergic mediated

opposition of sympathetic vasoconstriction. This data demonstrates that although the responsiveness of β-adrenergic receptors is increased following exercise training, these receptors do not play a role in the inhibition of sympathetic vasoconstriction. Additionally, in contrast to our previous studies, inhibition of sympathetic vasoconstriction (sympatholysis) was not altered following exercise training.

Effects of Sex on Evoked Vasoconstrictor Responses and Vascular Control

The final purpose of the present study was to investigate the effect that sex plays on β-adrenergic receptors and evoked sympathetic vasoconstrictor responses. At rest, sympathetic vasoconstriction was not different between male and female rodents (Figure 4). This finding in this rat model is consistent with data in human subjects that evoked vasoconstrictor responses at rest are not different between sexes (50). The data is however in contrast to other research that suggests that at rest females have blunted responses to sympathetic vasoconstriction (23). There is a methodological difference between the present study and the latter study mentioned (23) as the previous study uses exogenous Noradrenaline to elicit vasoconstrictor responses, whereas our study and the other previous research (50) evoke sympathetic vasoconstriction in a more integrative model. Although responses to exogenous Noradrenaline were blunted through β-adrenergic mediated vasodilation in females (23), the present study and others (50) have demonstrated that evoked sympathetic vasoconstriction is not different between males and females at rest.

Another recent study, demonstrated similar results to the present study, showing that there is no sex difference between β-adrenergic receptor-mediated vasodilation in the human forearm in response to isoproterenol infusions (36). Although demonstrating

contrasting results to the dominant theory in the literature (23), the present study in conjunction with previous studies (36, 50) has clearly demonstrated that β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction at rest or during skeletal muscle contraction. Although β-adrenergic receptors may blunt vasoconstriction to the exogenous infusion of Noradrenaline in the female human forearm (23), they do not play a role in the integrative *in vivo* control of the peripheral vasculature and therefore the control blood pressure.

However, during skeletal muscle contraction, female rats have a lower sympathetic vasoconstriction than males, and exhibit a greater contraction-mediated inhibition of sympathetic vasoconstriction (sympatholysis) than males (Figure 6). This novel finding demonstrates that there is a difference in the contraction-mediated inhibition of sympathetic vasoconstriction between sexes. Evoked sympathetic vasoconstriction during contraction was not different in the presence of β-adrenergic blockade indicating that the mechanism through which this sex difference occurs is not mediated by β-adrenergic receptors. These results suggest that there may be a mechanistic difference in contraction-mediated inhibition of sympathetic vasoconstriction between males and females.

Contraction-mediated inhibition of sympathetic vasoconstriction may be dependent upon on the type of muscle fiber recruited during contraction. Indeed, previous data from female Sprague-Dawley rats has demonstrated that contraction of glycolytic, not oxidative, skeletal muscle fibres attenuates α_2-adrenergic receptor mediated vasoconstriction (58). If female rats had a higher proportion of contracting glycolytic fibres in the hindlimb than male rats, this could potentially explain the

enhanced sympatholysis in female compared to male rats in the present study. However, fibre type distribution and recruitment was not quantified in the present study and sex differences in the fibre type distribution of the hindlimb in Sprague-Dawley rats have not been reported. Although speculative, fibre type distribution may play a role in the sex differences seen in the present study.

Perspectives & Significance

The present study has demonstrated that β-adrenergic mediated vasodilation does not inhibit evoked sympathetic vasoconstrictor responses in either male or female rats, and is unaltered by exercise training. Although previous research has suggested that β-adrenergic mediated vasodilation may inhibit sympathetic vasoconstriction in females through the application of exogenous neurotransmitters (4, 23), our current study in combination with previous research in humans (50) demonstrates that evoked sympathetic vasoconstriction is not inhibited by β-adrenergic receptors. In fact, in exercise trained animals that demonstrated increased β-adrenergic mediated vasodilation, there was no β-adrenergic mediated opposition of evoked sympathetic vasoconstriction. This suggests that there may be another mechanism that is responsible for blunted peripheral resistance responses to SNA that have been demonstrated in young females (24, 46).

There is however a difference in contraction-mediated inhibition of sympathetic vasoconstriction between male and female rats, that does not occur through β-adrenergic receptors. This novel finding demonstrates that there may be another mechanism present that alters sympatholysis in female rats that is not present in male rats. This finding is

significant in that this altered mechanism of sympatholysis in females may be responsible for the blunted peripheral resistance to SNA seen in young human females (24, 46). Understanding of this mechanism is important, as following menopause females lose this ability to blunt MAP responses to SNA (24, 46), and therefore the mechanism through which this occurs could be an important therapeutic target in a clinical cardiovascular setting.

Table 1. Anthropometrics

Group	Body Mass (g)	Heart Mass (g)	Heart mass: Body mass ($mg \bullet g^{-1}$ Body Mass)	Soleus (g)	Medial Gastrocnemius (g)	Lateral Gastrocnemius (g)
Sedentary Males	478 ± 44	1.5 ± 0.15	3.1 ± 0.29	0.22 ± 0.03	1.6 ± 0.20	0.83 ± 0.08
Trained Males	419 ± 25 [a]	1.4 ± 0.12	3.4 ± 0.18 *	0.21 ± 0.02	1.5 ± 0.11	0.79 ± 0.10
Sedentary Females	265 ± 28 ^	0.90 ± 0.07 ^	3.4 ± 0.25 ^	0.14 ± 0.02 ^	0.95 ± 0.08 ^	0.48 ± 0.06 ^
Trained Females	283 ± 33 ^	1.0 ± 0.13 ^[a]	3.7 ± 0.34 ^*	0.14 ± 0.02 ^	1.1 ± 0.08 ^	0.53 ± 0.08 ^

Values are mean ± standard deviation. ^ indicates a significant main effect of Sex, * indicates a significant main effect of Exercise Training, [a] indicates a significant effect of exercise training within a sex. A p-value of 0.05 was considered statistically significant.

Table 2. Basal Hemodynamics

Group	Drug Condition	HR (beats•min^{-1})	MAP (mmHg)	FBF (mL•min^{-1})	FVC (mL•min^{-1}• mmHg^{-1})
Sedentary Males	Control	409 ± 34	98 ± 9	4.8 ± 0.76	0.048 ± 0.0078
	Propranolol	388 ± 18 ^{Y}	94 ± 7 ^{Y}	4.6 ± 1.25	0.049 ± 0.0134
Trained Males	Control	388 ± 21 *	101 ± 9	4.5 ± 0.83	0.043 ± 0.0083
	Propranolol	364 ± 19 *^{Y}	97 ± 12 ^{Y}	3.8 ± 0.87 ^{b}	0.040 ± 0.0081
Sedentary Females	Control	425 ± 26 ^	99 ± 15	2.6 ± 1.0 ^	0.026 ± 0.0075 ^
	Propranolol	405 ± 30 ^^{Y}	93 ± 14 ^{Y}	3.0 ± 1.2 ^	0.032 ± 0.0102 ^
Trained Females	Control	405 ± 20 ^*	106 ± 19	3.2 ± 0.7 ^	0.031 ± 0.0061 ^
	Propranolol	388 ± 22 ^*^{Y}	103 ± 16 ^{Y}	3.1 ± 0.7 ^	0.029 ± 0.0058 ^

Heart rate (HR), mean arterial blood pressure (MAP), femoral blood flow (FBF), and femoral vascular conductance (FVC) at rest. Values are mean ± standard deviation. ^ indicates a significant main effect of Sex, * indicates a significant main effect of Exercise Training, ^{Y} indicates a significant main effect of propranolol, ^{b} indicates a significant effect of propranolol within a group. A p-value of < 0.05 was considered statistically significant.

Table 3. Sympathetic Stimulation

Group	Drug	Rest				Contraction			
		2 Hz		5 Hz		2 Hz		5 Hz	
		FBF ($mL \bullet min^{-1}$)	MAP (mmHg)	FBF ($mL \bullet min^{-1}$)	MAP (mmHg)	FBF ($mL \bullet min^{-1}$)	MAP (mmHg)	FBF ($mL \bullet min^{-1}$)	MAP (mmHg)
Sedentary Males	Control	-1.4 ± 0.59	2.5 ± 8.7	-2.0 ± 0.67	15.4 ± 12.2	-1.7 ± 1.2	1.7 ± 8.7	-3.2 ± 1.7	11.4 ± 10.4
	Propranolol	-1.2 ± 0.73	5.5 ± 9.1 *	-1.8 ± 0.87 *	19.2 ± 14.6*	-1.4 ± 0.69	4.0 ± 5.9 *	-3.2 ± 1.6 *	11.3 ± 11.0 *
Trained Males	Control	-1.6 ± 0.59	4.9 ± 6.3	-2.1 ± 0.63	14.3 ± 9.1	-1.6 ± 1.2	4.6 ± 6.6	-3.9 ± 1.4	9.9 ± 6.5
	Propranolol	-1.1 ± 0.59	9.6 ± 4.6 *	-1.8 ± 0.86 *	17.1 ± 11.4 *	-1.0 ± 0.79	5.9 ± 3.7 *	-2.8 ± 1.6 *	12.1 ± 8.6 *
Sedentary Females	Control	-0.8 ± 0.46 ^	6.7 ± 7.4	-1.1 ± 0.50 ^	20.8 ± 8.2	-0.03 ± 0.64 ^[a]	8.2 ± 8.3	-0.59 ± 1.2 ^[a]	16.7 ± 8.9
	Propranolol	-0.7 ± 0.36 ^	11.1 ± 9.3 *	-1.0 ± 0.71 ^*	27.9 ± 14.1 *	-0.02 ± 0.68 ^[a]	9.0 ± 6.2 *	-0.47 ± 1.7 ^*[a]	18.8 ± 11.3 *
Trained Females	Control	-1.2 ± 0.59 ^	1.5 ± 8.8	-1.6 ± 0.49 ^	14.3 ± 12.2	-0.7 ± 0.91 ^[a]	4.4 ± 3.6	-1.9 ± 1.6 ^	11.9 ± 7.1
	Propranolol	-0.8 ± 0.42 ^	10.2 ± 11.8 *	-1.4 ± 0.61 ^*	19.8 ± 14.3 *	-0.7 ± 1.0 ^[a]	5.1 ± 7.6 *	-1.6 ± 1.9 ^*	14.1 ± 7.9 *

Absolute changes of femoral artery blood flow (FBF) and mean arterial pressure (MAP) in response to sympathetic stimulation delivered at 2 and 5 Hz at rest and during skeletal muscle contraction at 60% maximal contractile force in Control and propranolol conditions. Values are mean ± SD. ^ indicates a significant main effect of sex, * indicates a significant main drug effect of propranolol, [a] indicates a significant effect of muscle contraction within group. A p-value of 0.05 was considered statistically significant.

Table 4. Hyperemic Response to Exercise

Group	Drug Condition	MAP (mmHg)	FBF ($mL \bullet min^{-1}$)	FVC ($mL \bullet min^{-1} \bullet mmHg^{-1}$)	MAP (%)	FBF (%)	FVC (%)
Sedentary Males	Control	6.8 ± 11.0	7.3 ± 2.2	0.07 ± 0.02	7.3 ± 11.0	157 ± 56	138 ± 44
	Propranolol	11.7 ± 7.2	8.8 ± 2.0 ^{Y}	0.08 ± 0.02 ^{Y}	13.2 ± 8.8	203 ± 77^{Y}	168 ± 71 ^{Y}
Trained Males	Control	3.3 ± 6.0	7.4 ± 1.5 *	0.07 ± 0.01 *	3.5 ± 6.3	162 ± 43 *	153 ± 40 *
	Propranolol	7.2 ± 7.7	8.7 ± 2.6 *^{Y}	0.08 ± 0.02 *^{Y}	7.9 ± 8.6	254 ± 84 *^{Y}	227 ± 71 *^{Y}
Sedentary Females	Control	5.8 ± 11.1	3.5 ± 1.4 ^	0.03 ± 0.01 ^	7.9 ± 15.5	140 ± 53	121 ± 33
	Propranolol	7.0 ± 10.9	4.7 ± 2.1 ^^{Y}	0.04 ± 0.01 ^^{Y}	8.4 ± 12.3	183 ± 46 ^{Y}	161 ± 32 ^{Y}
Trained Females	Control	6.6 ± 14.1	5.9 ± 1.4 ^*	0.05 ± 0.01 ^*	8.6 ± 16.8	175 ± 40 *	158 ± 54 *
	Propranolol	7.9 ± 11.1	7.1 ± 1.3 ^*^{Y}	0.06 ± 0.01 ^*^{Y}	9.4 ± 14.3	247 ± 56 *^{Y}	217 ± 32 *^{Y}

Absolute and percentage increases in mean arterial pressure (MAP), femoral blood flow (FBF), and femoral vascular conductance (FVC). Values are mean ± standard deviation. ^ indicates a significant main effect of Sex, * indicates a significant main effect of Exercise Training, ^{Y} indicates a significant main effect of propranolol. A p-value of < 0.05 was considered statistically significant.

Table 5. Contractile Forces

Group	Drug Condition	Peak Force (g)	Average Force (g)	Fatigue Index (%)
Sedentary Males	Control	978 ± 215	536 ± 48	48.7 ± 13.8
	Propranolol	844 ± 138 *	526 ± 43	42.6 ± 12.8 *
Trained Males	Control	956 ± 177	510 ± 73	52.3 ± 5.2
	Propranolol	891 ± 122 *	544 ± 95	46.3 ± 6.5 *
Sedentary Females	Control	751 ± 201 ^	443 ± 48 ^	45.7 ± 12.1
	Propranolol	730 ± 111 ^*	461 ± 82 ^	44.5 ± 7.5 *
Trained Females	Control	832 ± 161 ^	466 ± 40 ^	49.0 ± 11.9
	Propranolol	753 ± 70 ^*	466 ± 53 ^	47.3 ± 5.3 *

Peak force, average force, and fatigue index. Values are mean ± standard deviation. ^ indicates a significant main effect of Sex, * indicates a significant main effect of Exercise Training, $^{\Upsilon}$ indicates a significant main effect of propranolol. A p-value of < 0.05 was considered statistically significant.

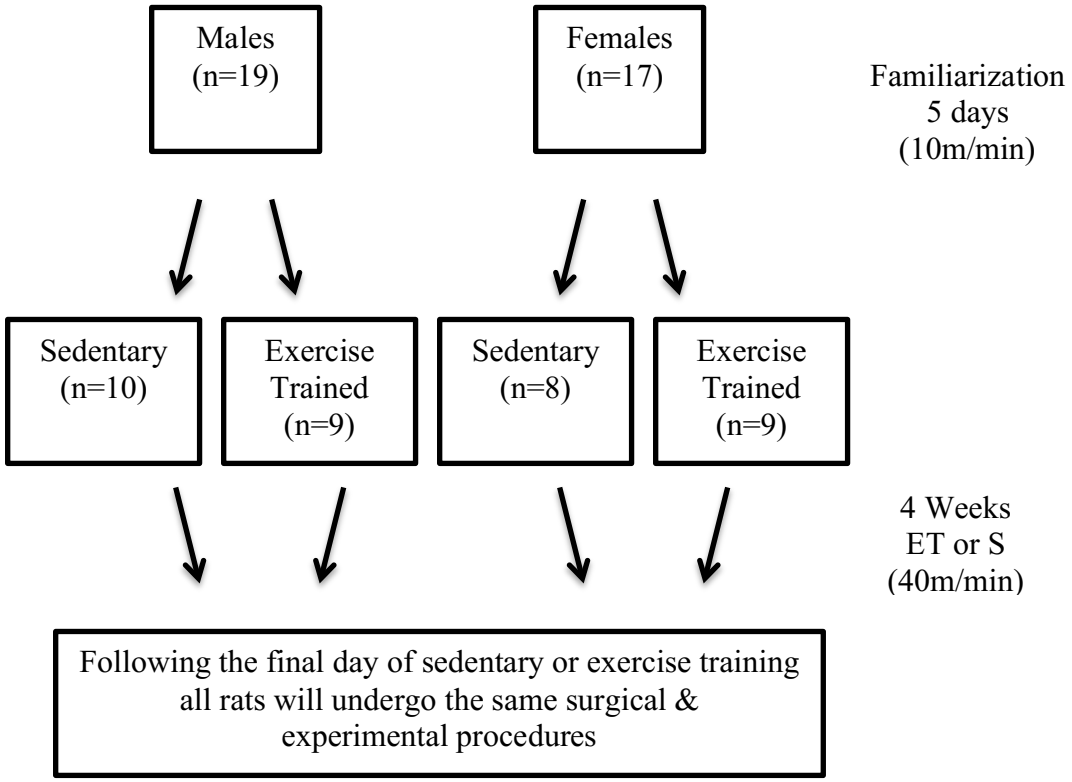

Figure 2. Figure demonstrating the randomization and timeline of rats in the present study.

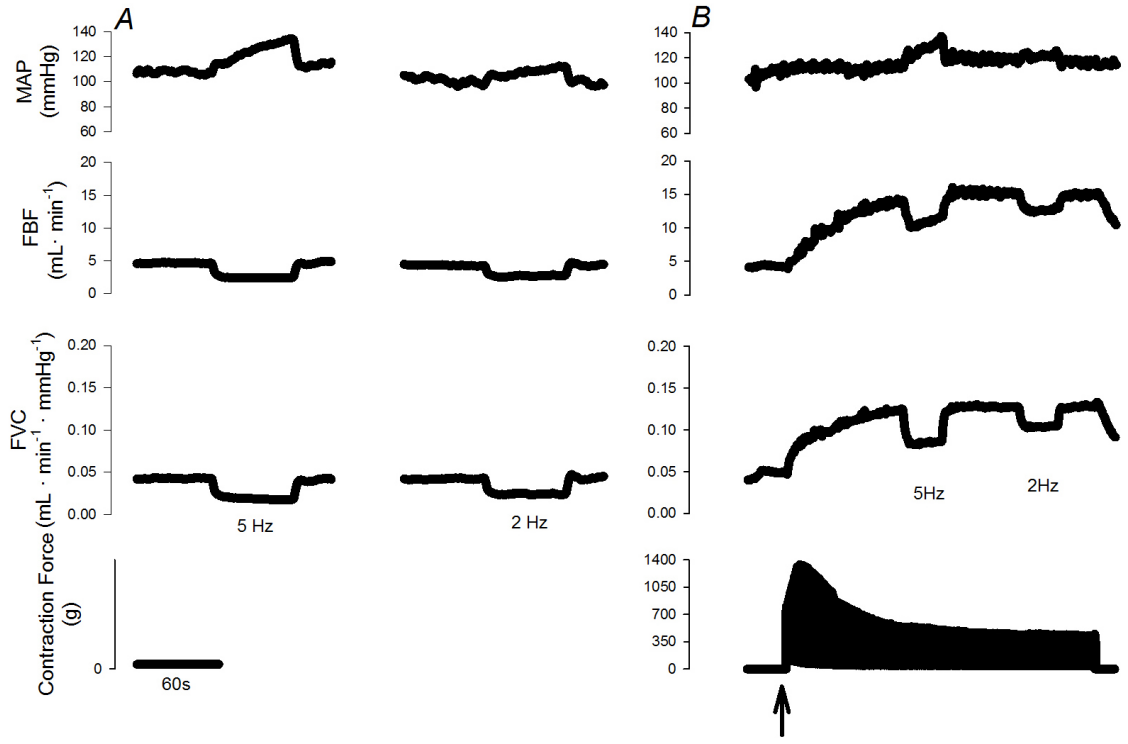

Figure 3. Original data from a representative sedentary male rat, illustrating the response of mean arterial blood pressure (MAP), femoral blood flow (FBF), femoral vascular conductance (FVC) and contractile force to lumbar sympathetic chain stimulation delivered at 2 Hz and 5 Hz in resting skeletal muscle (left) and during skeletal muscle contraction at 60% of maximal contractile force (right).

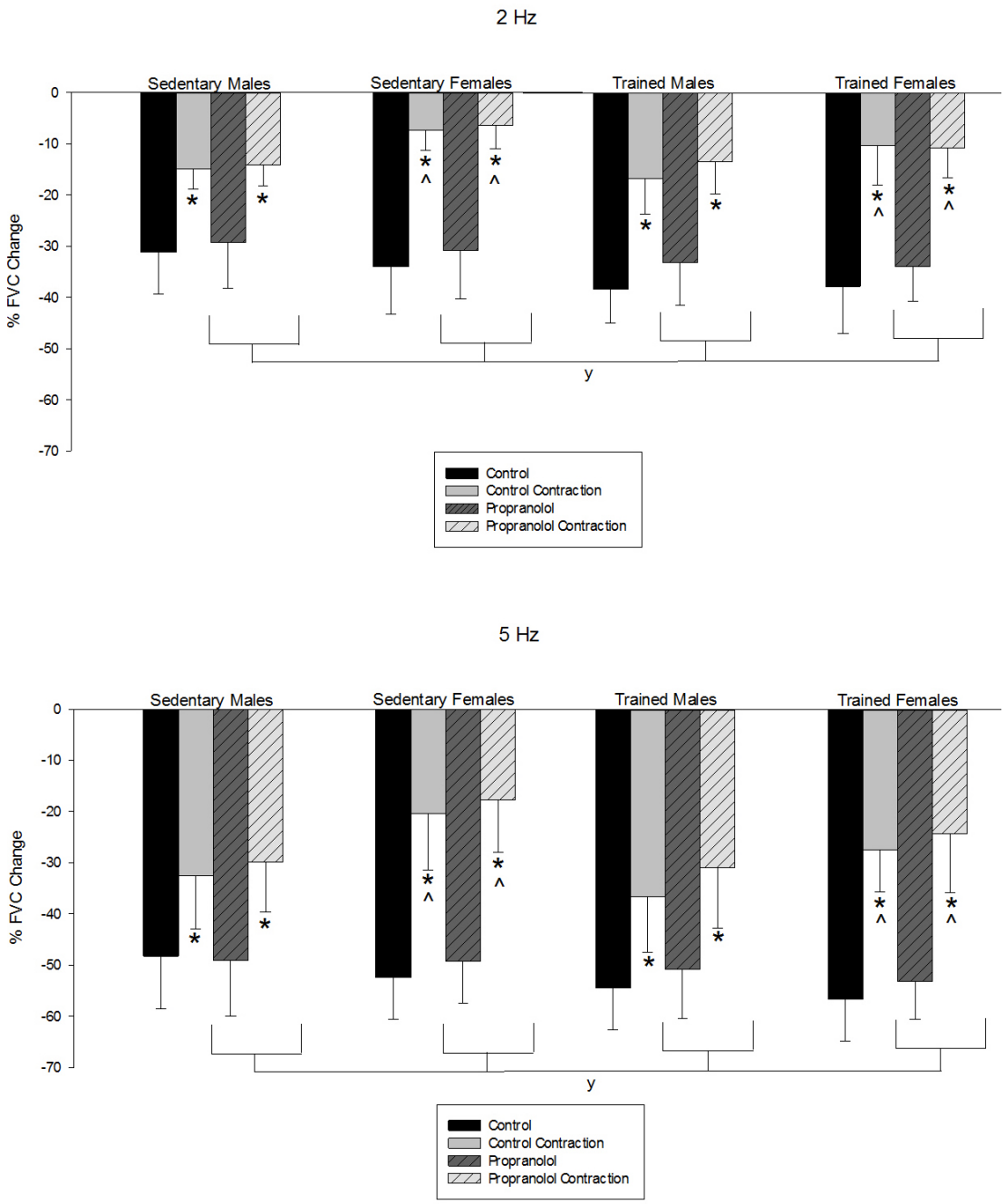

Figure 4. The percentage change in femoral vascular conductance (% FVC) in response to sympathetic stimulation at 2 Hz (top) and 5 Hz (bottom) delivered at rest and during hindlimb contraction at 60% of maximal contractile force in sedentary males, sedentary females, trained males, and trained females during Control (solid bars) and Propranolol (0.075 mg/kg IV; hatched bars) conditions. Values are mean ± SD. * indicates a main effect of muscle contraction, ^{y} indicates a main effect of drug condition, ^ indicates a difference between males and females during muscle contraction. A $P < 0.05$ was considered statistically significant.

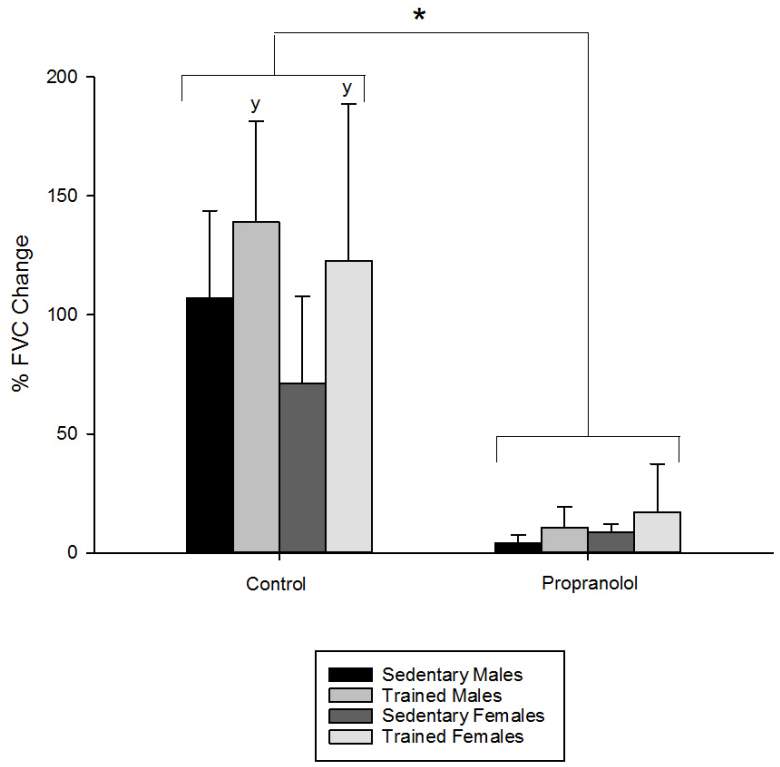

Figure 5. The percentage change in femoral vascular conductance (% FVC) to infusion of isoproterenol (0.75 ug bolus IA) in sedentary males (black), trained males (light gray), sedentary females (dark gray) and trained females (white) during Control and propranolol (0.075 mg/kg IV) conditions. * indicates a main effect of propranolol, ^{y} indicates an effect of exercise training during control conditions. A $P < 0.05$ was considered statistically significant.

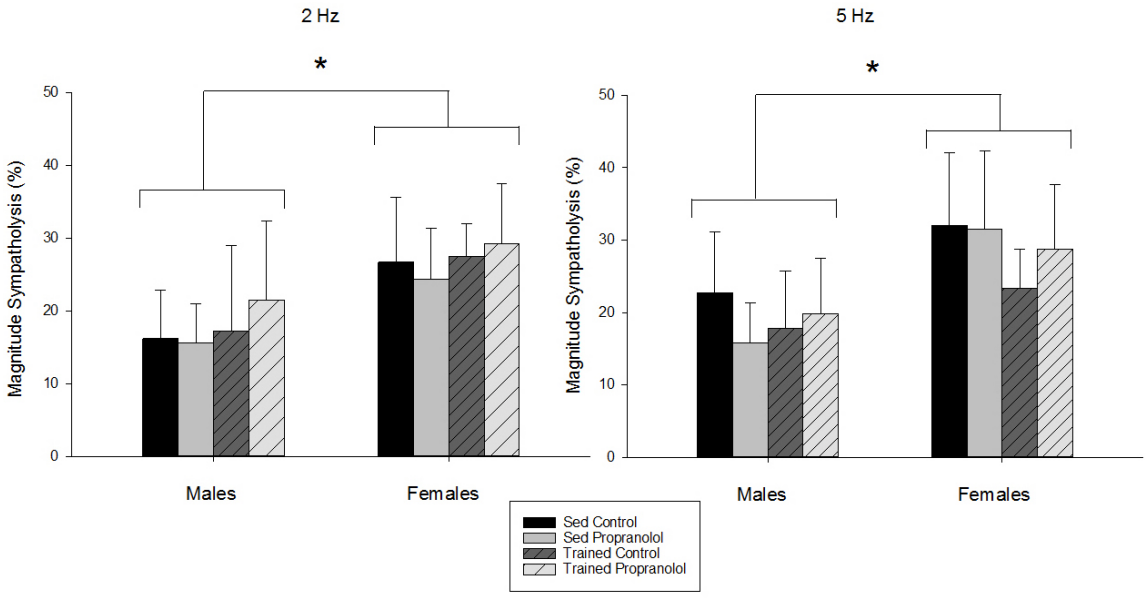

Figure 6. Magnitude of Sympatholysis (% FVC Rest - % FVC Contraction) at 2 Hz (left) and 5 Hz (right) in sedentary males, trained males, sedentary females, trained females during Control (solid bars) and propranolol (0.075 mg/kg IV; hatched bars) conditions. * indicates a main effect of sex. A $P < 0.05$ was considered statistically significant.

Chapter 3: General Discussion

The main purpose of the present study was to determine if β-adrenergic receptor mediated vasodilation opposed evoked sympathetic vasoconstriction in resting or contracting skeletal muscle of sedentary and exercise trained male and female rats. The current findings demonstrated that β-adrenergic receptors did not oppose sympathetic vasoconstriction, and in fact during β-adrenergic blockade sympathetic vasoconstriction was decreased. There were no sex differences in evoked sympathetic vasoconstriction at rest, however during skeletal muscle contraction females exhibited lower evoked vasoconstrictor responses and a higher sympatholysis. This greater sympatholysis was not mediated by β-adrenergic receptors as β-adrenergic blockade did not alter sympatholysis. Contrary to previous research from our laboratory (27, 29-32) and others (41, 44, 45) exercise training did not alter evoked vasoconstrictor responses or functional sympatholysis in the present study.

Effects of Sex on Control of Blood Pressure

Previous studies have established that there are differences in the relationship between cardiac output, MSNA and peripheral resistance between males and females (23, 24, 46), and were suggested to occur through a β-adrenergic mediated mechanism (23). The present study demonstrates that β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction in either males or females. The current findings do however demonstrate for the first time that females have lower evoked vasoconstrictor responses during skeletal muscle contraction (and therefore greater sympatholysis) than male rats. This sex difference in sympatholysis is important to blood pressure control

research, as the present study demonstrates that it does not occur through a β-adrenergic mediated mechanism as previously suggested (23). β-adrenergic receptors were a reasonable candidate to provide sex differences, as the sex differences are absent following menopause in females (46), and the responses to exogenous Noradrenaline were blunted in young females with functional β-adrenergic receptors (23). This blunted vasoconstriction response in young females, but not post-menopausal females, to noradrenaline (23) is consistent with previous research in isolated vessels demonstrating that β-adrenergic receptor function is dependent upon estrogen status, and β-adrenergic mediated vasodilation was significantly reduced in vessels of ovariectomized rats (18). Although these studies provide good theoretical support for β-adrenergic receptors opposing evoked sympathetic vasoconstriction, the present study and previous research (50) shows that in an integrative preparation, β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction at rest, and the current findings also demonstrate that they do not oppose sympathetic vasoconstriction during skeletal muscle contraction.

Effects of Exercise Training on Sympathetic Vasoconstriction

Contrary to previous studies from our laboratory (27, 29-32), exercise training did not increase evoked sympathetic vasoconstrictor responsiveness or augment functional sympatholysis. This was however, the first study to examine the effects of exercise training in female rats as well as males in an *in vivo* integrative preparation. Although not altering sympathetic vasoconstrictor responses, exercise training did increase β-adrenergic mediated vasodilation through exogenous infusion of β-adrenergic specific agonist isoproterenol into the hindlimb vasculature. This finding is consistent with

previous research demonstrating that exercise training restores β-adrenergic mediated vasodilation in isolated rat aorta preparations from aged rats (35).

Demonstrating that exercise training is able to augment β-adrenergic mediated vasodilation may be important to understanding improvements seen in cardiovascular control following exercise training. However the present study also demonstrates that these receptors do not oppose evoked sympathetic vasoconstriction at rest or during contraction. Although potentially important as an improved mechanism following exercise training, β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction and therefore likely play a minor role in sympathetic vascular control.

Limitations

Although the present study provides novel insight into β-adrenergic receptor function and the opposition to evoked sympathetic vasoconstriction, the current findings are limited in some respects. It must be noted that although rodent models provide an excellent basis for mechanistic research, the translation of the roles for β-adrenergic receptors in vascular control in humans must also be established. The current results differ from our previous research demonstrating exercise training effects on evoked sympathetic vasoconstriction, as well as functional sympatholysis. The previous research from our laboratory (27, 29-32) was however conducted only in male rats, and the current study is the first to examine the effects of exercise training in female rats, which may have a different response to exercise training and effect size as compared to male rats. Finally, we are limited in our knowledge of the distribution and function of β-adrenergic receptors in different vascular beds, as changes in the distribution and function dependent on vascular bed have the potential to impact the findings from the current study.

Future Directions and Perspectives

β-adrenergic receptors cause vasodilation in the vasculature in response to infusion of exogenous agonists noradrenaline (23), phenylephrine (63), and β-adrenergic specific agonist isoproterenol. However the mechanism through which β-adrenergic receptors cause vasodilation in the vasculature is unclear. A recent study has suggested that β-adrenergic receptor vasodilation is influenced by cyclooxygenase suppression of nitric oxide synthase (36). Although being the first study to link NO and cyclooxygenase, the study still relies on infusion of exogenous isoproterenol (36) and does not address β-adrenergic receptor function in an integrative context. The present study and previous research (50) have demonstrated that β-adrenergic receptors do not oppose evoked sympathetic vasoconstriction in the skeletal muscle vascular bed at rest and therefore the role of β-adrenergic receptors in an integrative cardiovascular physiology setting is poorly understood.

The current study did demonstrate that females have a lower evoked vasoconstrictor response during skeletal muscle contraction (therefore a greater magnitude of sympatholysis) than males. Although they demonstrated greater sympatholysis, the mechanism for this result is unclear, as β-adrenergic blockade did not reduce the greater sympatholysis. The mechanism through which a greater sympatholysis occurs in females may be integral to understanding the relationship between MSNA and peripheral resistance that has been previously reported in females (24, 46), and also to understanding the cardio-protective effects of estrogen.

The present study has demonstrated that there are no sex differences between males and females in β-adrenergic receptor mediated opposition of sympathetic

vasoconstriction at rest, previously shown in humans (50), and for the first time during skeletal muscle contraction. Based on these current and previous findings from an *in vivo* preparation in humans (50), β-adrenergic receptors may not be therapeutic targets for either pharmacological, or exercise training interventions. Although exercise training in the present study did augment β-adrenergic mediated vasodilation in the hindlimb vasculature of rats, this increase in vasodilation did not oppose evoked *in vivo* sympathetic vasoconstrictor responses. This data together with previous studies suggest that β-adrenergic receptors respond *in vivo* to exogenous agonist infusion (23, 63), but do not alter the integrative sympathetic control of the skeletal muscle vasculature.

There was a clear sex difference in the opposition of sympathetic vasoconstriction during skeletal muscle exercise demonstrated in the present study. Although not mediated by β-adrenergic receptors, the previous research demonstrating a correlation in females between SNA and TPR following menopause (24, 46) is suggestive that estrogen does play a role in the regulation of the peripheral vasculature. Estrogen has been shown to influence NO production through its effects on endothelial NO synthase and have been previously reviewed (39). Given that previous studies have also demonstrated that NO in part mediates functional sympatholysis (28, 30, 41, 43) it is possible that estrogen status could indirectly be contributing to the sex differences in sympatholysis by having more bioavailable NO to inhibit sympathetic vasoconstriction. The exact pathway through which sympathetic vasoconstriction is inhibited during skeletal muscle exercise is still unknown, and although having potential for playing an indirect role, estrogen status may also directly effect the pathway through which the inhibition of sympathetic vasoconstriction occurs.

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