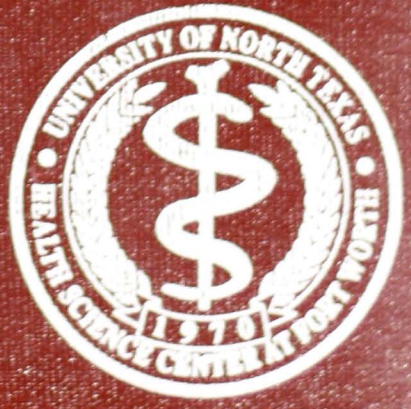
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Control of the vasculature during exercise is balance between sympathetic vasoconstriction and metabolic vasodilation. There is an exercise intensity dependent reduction in vasoconstriction resulting in a shift towards vasodilation within "metabolically active" muscle and tissues, a phenomenon known as "functional sympatholysis". Previous studies investigating the alpha-receptors during exercise have used intra-arterial infusions of alpha-agonists. These studies indicate that alpha-receptor vasoconstriction is completely attenuated during mild intensity exercise. When the alpha receptors are pharmacologically blocked the magnitude and onset of "functional sympatholysis" is not as drastic when compared to the agonist infusion studies.

Intense exercise also activates the renin-angiotensin- system leading to production of angiotensin II (AngII), which increases exponentially at approximately 55% maximal oxygen uptake (55% VO_{2max}). While the mechanisms of "functional sympatholysis" have been extensively studied less is known about the role of AngII in the control of the vasculature during exercise. Therefore, the purpose of the investigations within this dissertation was to: i) determine if alpha-1- blockade in an exercising human model will identify a greater maintenance of alpha-1 mediated vasoconstriction when compared to agonist infusion studies; ii) to determine if the metabolites produced within the active

skeletal muscle will attenuate angiotensin II vasoconstriction; and iii) to determine if AngII vasoconstriction provides a greater percentage contribution to vascular tone as exercise intensity increases. We demonstrated that i) pharmacologic alpha-1-blockade identified a greater maintenance of alpha-1 vasoconstriction during moderately heavy exercise; and ii) this effect decreased as intensity increased in the exercising leg and increased with intensity in the non-exercising leg. In the second investigation we demonstrated that AngII and phenylephrine (PE) mediated vasoconstriction were attenuated to a similar degree during low and mild intensity exercise. In the third investigation we observed that AT_1-receptor blockade; i) attenuated the increases in MAP that occur during high-intensity exercise; ii) did not affect the vasculature in the exercising leg but; iii) we identified that AngII does partially control the vasculature in a "non-metabolically active" muscle group.

CONTROL OF THE PERIPHERAL VASCULATURE DURING EXERCISE: ANGIOTENSIN II

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Original Articles

- *Brothers, R. M.,* S. Ogoh, W.L. Eubank, M. Jenschke, and P. B. Raven. *Effect of Prazosin on the Control of the Leg Vasculature during One Legged Knee Extension Exercise.* In submission, A. J. Physiol, 2007.

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Presentations

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- Oral presentation at the Texas chapter of the American College of Sports Medicine (TACSM) annual meeting, 2006. *Metabolic Inhibition of Phenylepherine and Angiotensin-II Induced Vasoconstriction during Low and Mild Exercise Workloads.* 2006.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

ABP	arterial blood pressure
MAP	mean arterial pressure
HR	heart rate
EL	exercising leg
NEL	non-exercising leg
AngII	angiotensin II
PE	phenylephrine
TYR	tyramine
FAD	femoral artery diameter
FBV	femoral blood velocity
FBF	femoral blood flow
FVC	femoral vascular conductance
LVC	leg vascular conductance
α_1	alpha-1-receptor
α_2	alpha-2-receptor
NP	neck pressure
NS	neck suction
Q	cardiac output
SE	standard error
SV	stroke volume
MSNA	muscle sympathetic nerve activity

WL_{max}	workload max
VO_{2max}	Maximal oxygen uptake
Yr	year
%	percent
SVR	systemic vascular resistance

CHAPTER I

INTRODUCTION

REVIEW OF RELATED LITERATURE

The autonomic nervous system and the hemodynamic response to exercise.

It is widely accepted that upon the onset of exercise there is an intensity dependent decrease in parasympathetic autonomic activity (PSNA) with a subsequent increase in sympathetic autonomic activity (SNA) (12, 20, 33). These autonomic adjustments are mediated by two different mechanisms of neural control, one mechanism being initiated from the central nervous system ("central command") and the other involving a feedback mechanism from type III and type IV skeletal muscle afferents ("exercise pressor reflex") (1, 9, 20). The feedback from these two mechanisms of neural control are responsible for the cardiovascular adjustments necessary to perform and maintain exercise performance, such as increases in heart rate (HR), stroke volume (SV), cardiac output (Q), arterial blood pressure (ABP), and a redistribution of Q to the "metabolically active" tissues. In addition to sympathoexcitatory signals originating from central command and the exercise pressor reflex, it has been shown that the arterial baroreflexes (carotid and aortic) and cardiopulmonary baroreflexes modulate parasympathetic and sympathetic drive, thus increasing the complexity of the neural control mechanisms engaged during exercise (25, 28, 38, 41).

In order to sustain exercise it is necessary to increase blood flow and thus oxygen delivery to the active skeletal muscle to meet the increased metabolic demands of the exercising muscles. Several investigations have found that during intense dynamic exercise the vasculature of the active muscles are able to vasodilate to a point where skeletal muscle blood flow can increase up to 100-fold thus receiving up to 85-90% of the cardiac output (2, 33, 36). Thus during intense, whole body exercise a marked hemodynamic challenge to the control of the cardiovascular system occurs as it attempts to maintain mean arterial blood pressure, while at the same time providing adequate perfusion to the exercising muscles (2, 33, 36). In competition with the increasing local metabolic vasodilation as exercise intensity and duration increases there is a progressively greater activation of the sympathetic nervous system (14), which has been shown to induce vasoconstriction in the vasculature of the resting skeletal muscles (13) and exercising skeletal muscles (6, 23). However, sympathetically induced vasoconstriction is attenuated by the metabolic by-products of the exercising muscles (3, 6, 10, 13, 30, 44, 48). In 1962 Remensynder et al. (30), introduced the term "functional sympatholysis", to describe the phenomenon that sympathetic vasoconstriction in the vasculature of an exercising muscle was attenuated by metabolites produced during skeletal muscle contractions, thereby blunting the sympathetically induced vasoconstriction (30). Functional sympatholysis has been observed in numerous human and animal exercise models, thus supporting the idea of metabolic inhibition of sympathetically induced vasoconstriction (5, 6, 10, 13, 30, 34, 48).

Although the exact mechanisms resulting in functional sympatholysis remain incompletely understood, it is believed that the metabolites produced during exercise somehow inhibit postjunctional adrenergic signaling of both α_1 and α_2 adrenoreceptors (3, 48). Both animal and human studies (3, 19, 48) have demonstrated that the sensitivity of alpha-2 adrenoreceptors, which are believed to populate the majority of the adrenoreceptors located in the small nutrient arterioles, appears to be greater than the alpha-1 adrenoreceptors which are primarily located on the larger resistance arterioles (3, 48). A wide array of factors have been proposed to be involved in the altered vascular responses during exercise including adenosine (18, 36), prostaglandins and thromboxanes (15), increased muscle temperature (8), hypoxia (13), acidosis (19), nitric oxide (37, 45), and activation of K_{ATP} channels (17). Therefore, it is likely that because the alpha-2 adrenoreceptors are located more distally, in the nutrient arterioles, they are more accessible to metabolites that are produced in the exercising tissue and, therefore, at lower intensities of exercise are inhibited before the more proximal alpha-1 adrenoreceptors (48). Another possibility for the heterogenous response to metabolites could be in the G-protein signaling pathways utilized by each receptor resulting in an increase in intracellular calcium concentrations and thus vasoconstriction. The alpha-1 adrenoreceptor binds to a G_q membrane bound protein which cleaves phospholipase C (PIP_2) resulting in a diacylglycerol (DAG) mediated phosphorylation of protein kinase C (PKC) and an IP_3 mediated increase in calcium release from the sarcoplasmic reticulum (SR), both of which enhance vasoconstriction. The alpha-2 adrenoreceptor binds to a Gi membrane bound protein which exerts its physiological actions by phosphorylation of

membrane calcium channels which increases the influx of calcium into the cell and thus results in calcium induced calcium release from the SR. Additionally activation of the Gi protein prevents the adenylate cyclase mediated formation of cAMP, and prevents the cAMP mediated inhibition of myosin light chain kinase (MLCK) (26). Thus the stimulation of the α_2 adrenoreceptor leads to an increase in intracellular calcium concentration and stimulates vasoconstriction. This heterogeneity in the G-protein coupled signaling pathways leading to α_1 and α_2 adrenoreceptor mediated vasoconstriction may be an underlying intra-cellular mechanism explaining the greater sensitivity of the α_2 receptors to "functional sympatholysis" than the α_1 receptors.

Exercise and the Renin – Angiotensin System (RAS)

During exercise the increased activity of the sympathetic nervous system activates the renin-angiotensin system (RAS), which ultimately leads to an increase in circulating concentrations of the powerful vasoconstrictor hormone angiotensin II (AngII) (4, 39, 46). Several investigations have found that during dynamic exercise there is a significant increase in plasma concentrations of AngII, which occurs in a work intensity dependent manner (4, 39, 46). Previous investigations examining the effects of AngII during exercise have focused on the effects of various angiotensin type 1 (AT_1) receptor antagonists and angiotensin converting enzyme (ACE) inhibitors in both humans (42, 47) and miniature swine (40) during moderate (~60% maximal heart rate reserve) and intense (~80% maximal heart rate reserve) cycle ergometry and treadmill running exercise. These investigations reported that during intense dynamic exercise there was a significant

increase in plasma concentrations of AngII (40, 42, 47), and that administration of either an AT_1 receptor blocker or an ACE inhibitor during exercise significantly attenuated the increase in MAP associated with intense, dynamic exercise. Furthermore these drug interventions during exercise resulted in a reduction in vascular resistance to "less metabolically active tissues" thereby enabling a redistribution of cardiac output to these tissues (40, 42). The fact that circulating concentrations of AngII are significantly elevated during exercise and that both ACE inhibition and AT_1 receptor blockade significantly attenuated the exercise dependent increase in MAP and resulted in a redistribution of cardiac output to "less metabolically active tissues" suggests that similar to adrenergic catecholamines, AngII plays a role in the pressor response and the redistribution of cardiac output associated with exercise, and that this effect becomes more pronounced as exercise intensity increases. Therefore, it is possible that a percentage amount of the exercise induced vasoconstriction is mediated not only by sympathetic activation of adrenergic receptors on vascular smooth muscle, but also by actions of circulating AngII on the AT_1 receptors located on the vascular smooth muscle.

Strange et al. (41) demonstrated that during upright, dynamic, cycling exercise at intensities eliciting 40%-70% VO_{2max}, R-wave gated pulses of -50 Torr NS significantly increased LVC. However at a higher workload, 88% VO_{2max}, this exercise induced attenuation of sympathetic vasoconstriction to the same NS stimulus was abolished, suggesting that the hypertensive stimulus created by NS failed to withdraw any of the sympathetically induced vasoconstriction. Buckwalter et al. (6, 7) and O'Leary et al. (24)

observed that intra-arterial infusion of the selective α_1 adrenoreceptor antagonist prazosine resulted in a significant increase in absolute LVC in both resting and exercising conditions. However when the changes in LVC in both investigations were plotted as a percent change, an attenuation of the prazosine induced increases in LVC with increasing exercising intensities was observed. In other words, similar to the findings of Strange et al. (41), Buckwalter (6, 7) and O'Leary (24) observed an inverse relationship between increases in LVC and exercise intensity when sympathetic vasoconstriction was functionally removed. The interpretations of the investigators included suggestions that the high sympathetic activity at or near maximal exercise may not originate from the arterial baroreflexes (41), that during high intensity exercise functional sympatholysis has already maximally inhibited sympathetic vasoconstriction, and that with the high flow rates achieved in these protocols there is less of an impact of α adrenoreceptor mediated vasoconstriction in the maintenance of arterial blood pressure (7). Interestingly work by Stebbins et al. (40) in miniswine performing dynamic treadmill running exercise at 80% heart rate reserve (HRR) and by Symons et al. (43) in a rat model performing dynamic treadmill exercise at 50% VO_{2max} have both indicated that AT_1 receptor blockade using Losartan resulted in a significant attenuation of the exercise mediated rise in arterial blood pressure as well as a distribution of cardiac output to "less metabolically active" tissues, however neither study reported a change in vascular conductance or vascular resistance in exercising muscles. Additionally in a dynamic cycling exercise human model Warren et al. (47) demonstrated that as exercise intensity increased there was a significant attenuation of arterial blood pressure following oral ingestion of Valsartan

when compared to unblocked conditions. This attenuation of MAP was more pronounced as exercise intensity increased thus resulting in a reduction of ~20mmHg with Valsartan during exercise at 80% HRR when compared to unblocked conditions (47). Unfortunately peripheral blood flow was not assessed in this investigation. Although there was no significant changes in vascular conductance in the animal studies despite significant reductions in MAP it is possible that in the study performed by Symons where vascular conductance in control rats increased by ~10% (non-significant) that the 50% exercise intensity and the short exercise duration of only 5 minutes was of sufficient duration and / or intensity to elicit maximal effects of AngII. In this regard it is well documented that there is an increase in circulating plasma concentrations of AngII and furthermore that this increase is proportional to exercise intensity. As previously mentioned the human study performed by Warren et al. (47) demonstrated a significant attenuation of the MAP response (~20mmHg) to exercise following Valsartan treatment when compared to controls. Although they did not assess peripheral blood flow in this study it has been previously documented (2, 33, 36) that during intense, i.e. 80-85% HRR, exercise that roughly 85-90% of Q is directed towards exercising muscles. Therefore it seems plausible to speculate that a decrease in MAP of ~20mmHg (~15%) following oral ingestion of Valsartan reflects a vasomodulatory role of AngII in the control of the peripheral vasculature in the exercising muscles with increasing exercise intensities. In this regard it is also interesting to note that the findings of Strange et al. (41), Buckwalter et al. (6, 7), and O'Leary et al. (24) identifying a decreased vascular response to functional withdrawal of sympathetic vasoconstriction with increasing

exercise intensities all occurred at approximately the same intensities of exercise that Stebbins et al. (40), Symons et al. (42, 43), and Warren et al. (47) had demonstrated the greatest effect of Valsartan. Additionally, it is well documented (39, 46) that at exercise intensities of roughly 55% VO_{2max} there is a significant spike in plasma concentrations of AngII. Therefore, another possibility is that as the exercise intensity was gradually increased and approached VO_{2max}, there was a progressively greater sympathetic activation, thus leading to an increased stimulus for renin release and subsequent increased concentrations of the potent vasoconstrictor Ang II (29). Therefore potentially providing another mechanism for the maintenance of systemic arterial blood pressure in the face of sympathetic withdrawal, or attenuation associated with the progressive increase in the effects of functional sympatholysis.

SPECIFIC AIMS

A number of studies investigating the role of the alpha-receptors during exercise have infused alpha-agonists intra-arterially to activate these receptors. In these infusion studies it has been demonstrated in humans exercising at relatively low work intensities that alpha-2 (α_2) mediated vasoconstriction appears to be almost 100% functionally attenuated (10, 31, 48) and α_1 mediated vasoconstriction is 60-70% attenuated and furthermore, during mild intensity exercise intra-arterial infusion of the alpha-1-receptor agonist PE mediated vasoconstriction is completely attenuated. In other words in these agonist infusion models the very mechanism which is designed to maintain our blood pressure regulation during exercise appears to be completely absent.

In contrast to the agonist infusion studies (5, 10, 32, 48), when the role of the alpha-receptors is probed via pharmacological blockade of the physiological activation of the α_1-adrenoreceptor the presence of a functional vasoconstrictor response at moderate to heavy intensity exercise is identified. For example in dogs exercising on a treadmill at near maximal intensities, α_1 blockade resulted in an increased iliac conductance of approximately 20% when compared to control exercise (6, 7, 24). In other words, in these exercising models, physiological blockade of the α_1-adrenoreceptor "unmasked" the maintenance of 20% α_1 mediated vasoconstriction during heavy intensity exercise. Therefore, specific aim 1 of the projects was aimed to determine if there is a disconnect between the degree and onset of sympatholysis between agonist infusion studies and pharmacological blockade studies.

Exercise mediated sympatho-excitation results in an increased stimulation of renal beta I adrenergic receptors leading to increased plasma renin activity and plasma concentrations of Angiotensin II (AngII) (4, 39, 46). In the limited number of studies investigating the role of AngII using Angiotensin Type 1 (AT_1) receptor blockade during exercise a significant role of AngII activity in modifying the control of the peripheral vasculature was identified, by decreases in MAP (22, 47), systemic vascular resistance (SVR), and increases in blood flow and vascular conductance in "non-active" tissues and organs (no change was observed in "active" tissues and organs) (40, 43). Furthermore the effects of AT_1 receptor blockade using Valsartan are dependent on exercise intensity with the greatest effects occurring during higher intensities of exercise.

Additionally, the α_1-receptor and AT_1 receptor share very similar G-Protein coupling signaling mechanisms. Therefore, it is possible that the lack of effect of AT1 receptor blockade in the exercising limb in the above mentioned studies could be the result of the mechanisms leading to "functional sympatholysis" inhibiting vasoconstriction elicited by both adrenergic and non-adrenergic vasoconstrictors.

Therefore, we suggest that intra-arterial alpha-receptor agonist infusion overestimates the degree and onset of "functional sympatholysis". Furthermore we aimed to determine if the mechanisms of functional sympatholysis that lead to attenuation of alpha-1 and alpha-2 receptor mediated vasoconstriction also attenuates the vasoconstrictive properties of the non-adrenergic vasoconstrictor AngII. Lastly we aimed to determine if there is a shift from adrenergic to non-adrenergic control of the vasculature during relatively high intensity exercise when alpha-adrenergic mediated vasoconstriction is functionally attenuated. To test these fundamental hypotheses the following specific aims will be accomplished.

I. To test the hypothesis that alpha-1-adrenoreceptor blockade in an exercising human model will identify a greater maintenance of alpha-1 mediated vasoconstriction despite higher exercise intensities when compared to agonist infusion studies.

II. To test the hypothesis that the metabolites produced within the active skeletal muscle will not attenuate the vasoconstrictive actions of

angiotensin II to the same degree as the alpha-1-receptor specific agonist phenylephrine.

III. To test the hypothesis that AngII mediated vasoconstriction provides a greater percentage contribution to vascular tone of active and inactive skeletal muscle as exercise intensity increases.

The experimental techniques and methodology designed to explore specific aims I, II, and III are specifically explained in chapters 2, 3, and 4. However, we propose below a general description of the experimental design, experimental protocols and methods used to address the specific aims.

EXPERIMENTAL DESIGN

Ultrasound Doppler measurements of common femoral arterial diameter (FAD) and common femoral arterial blood velocity (FBV) allowed for assessment of common femoral artery blood flow (FBF) during rest and during various intensities dynamic one-legged knee extension exercise. Beat-to-beat arterial blood pressure was monitored invasively via intra-arterial catheterization and subsequent calculation of femoral vascular conductance (FVC). To offset any vasomodulatory and thus blood pressure effects of local and systemic drug treatment in these studies, FVC was selected as the primary variable of interest when identifying control of blood flow through the femoral arteries. In all studies, by measuring and comparing the changes in FVC in the EL we were able to determine to role of the alpha-1-adrenoreceptor and the AT_1-receptor during rest and one-legged kicking exercise. In the studies addressed in specific aims 1 and 2 we examined FVC in the non-exercising leg (NEL) in addition to the EL in order to assess the role of the alpha-1-adrenoreceptor and the AT_1-receptor in the control of the peripheral vasculature in a "non-metabolically active" muscle bed.

The Acute Effect of Oral Prazosin and Valsartan Administration on the Control FVC

In specific aims 1 and 3 we assessed the effect of oral blockade of the alpha-1-receptor using Prazosin (1mg / kg body weight) and the AT_1-receptor using Valsartan (80 mg). Again FVC was the variable of choice to probe the effects on the femoral artery because of the blood pressure reducing effects of systemic blockade of these receptors.

The validity of these results requires that the doses of both Prazosin and Valsartan efficiently block alpha-1 and AT_1 receptors respectively. Therefore in the Prazosin studies three separate bolus challenge infusions of a pressor dose of 1.0 μg/kg body weight of Phenylephrine (PE) was injected intra-venously (35). The first challange was performed in the morning before the control protocol began. This exact same bolus dose of PE was injected at 2 hours post Prazosin ingestion as well as immediately following completion of the study. In the Valsartan studies we performed three separate challenge infusions a bolus dose of 1.5 μg of AngII was injected intra-venously (21). The first challange was performed in the morning before the control protocol began. This exact same bolus dose of AngII was injected at 2 hours post Valsartan ingestion as well as immediately following completion of the study. During all PE and AngII injections arterial blood pressure was closely monitored to determine that the Prazosin and Valsartan dose sufficiently antagonized the pressor response to PE and AngII, respectively.

METHODS

A brief description of the methods used to assess FBF and FVC in these investigations, is provided in this section of the chapter. More detailed descriptions are provided in chapters 2, 3, and 4 of this dissertation.

Doppler ultrasound technology is commonly used in these types of investigations to determine leg blood flow during rest and during exercise (11, 16, 27, 48) . In order to determine FBF it is first necessary to determine FAD and FBV in the desired femoral

artery. Ultrasound imaging of femoral artery diameter and Doppler measurement of FBV was measured using a transducer probe positioned at a site approximately 2 cm distal to the inguinal ligament where the best spatial resolution was achieved and where the diameter of the femoral artery is not affected by the skeletal muscle contractions that are associated with exercise. FBF is then calculated using the formula: FBF = FBV * π * radius^2.

The reproducibility and validity (when compared to thermodilution) of Doppler ultrasound measurements of FBF during one-legged kicking exercise has been previously described (27, 48) during exercise intensities up to 70W. The average FBF measurements obtained from all exercise intensities in the current study were in agreement with previously described values.

As previously mentioned beat-to-beat MAP was measured invasively in all studies of this dissertation. In order to do so a catheter was inserted into the radial artery (specific aims 1 and 3) and into the femoral artery (specific aim 2) using sterile techniques under local anesthesia and aseptic conditions, and was connected to a pressure transducer positioned at the level of the heart. Measurements of FBF and MAP allowed for calculations of FVC as calculated by the formula: FVC = FBF/MAP.

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CHAPTER II

Effect of Prazosin on the Control of the Leg Vasculature during One Legged Knee Extension Exercise

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Abstract

A majority of the studies investigating the role of α_1-adrenoreceptors have infused exogenous agonists intra-arterially to activate these receptors and identified a marked attenuation of α_1-adrenoreceptor vasoconstriction during mild and moderate intensity exercise. Little is known about the maintenance of adrenergic receptor mediated vasoconstriction and the role of physiological activation of these receptors during moderately heavy intensity exercise in humans. We tested the hypothesis that oral blockade of the alpha-1 receptor with Prazosin will identify a maintained vasoconstriction during moderately heavy intensity exercise and that the effect of Prazosin will decrease as exercise intensity increases. In seven subjects (5men 2 women), femoral blood flow (FBF) using Doppler Ultrasound and blood pressure using radial artery catheterization were measured in the exercising (EL) and non-exercising (NEl) during rest and three varying intensities (~40, 60 & 75% maximum workload intensity, WL_{max}) of dynamic one-legged kicking exercise with and without Prazosin. After oral ingestion of Prazosin significant increases in FBF (+ 39±6, 25±6, 17±3, and 9±2%) and FVC (52±11, 31±8, 27±5, and 17±6 %) were observed during rest and 40, 60, and 75% WL_{max} exercise respectively. In the NEL FBF (37±6, 44±6, 51±7, and 65±4%) and FVC (50±11, 52±4, 63±6, and 79±6%) increased during rest and 40, 60, and 75% exercise, respectively. These findings indicate that there is maintenance of α_1 mediated vasoconstriction during moderately heavy exercise in humans. Furthermore, the onset and magnitude of the "functional sympatholysis" appears to differ between intra-arterially mediated pharmacological activation and endogenous activation of the α_1-receptors.

INTRODUCTION

During exercise it is well established that the transduction of the sympathetic nerve activity at the blood vessel wall operates via the α-adrenergic receptor signaling pathway to effectively produce vasoconstriction. However, a number of recent studies have demonstrated that the degree of alpha-1 (α_1) adrenergic receptor mediated vasoconstriction progressively decreases in active skeletal muscle as the intensity of exercise increases, (i.e. "functional sympatholysis" is occurring) (4, 8, 12, 42). Somewhat surprising to us is the fact that in humans exercising at relatively low work intensities (at HRs of < 100 bpm) alpha-2 (α_2) mediated vasoconstriction appears to be almost 100% functionally attenuated (12, 29, 42) and α_1 mediated vasoconstriction is up to 60-70% attenuated. Dinenno et al. (12) and Rosenmeier et al. (29) both demonstrated that dynamic forearm exercise at intensities equivalent to ~10 – 15% maximal voluntary contraction (MVC) resulted in an approximate 70% attenuation of the decreased forearm vasoconstrictor response to pharmacological α_1-receptor activation with intra-arterial Phenylephrine (PE), when compared to the vasoconstrictor effects of PE during an intra-arterial adenosine infusion produced vasodilation at rest (control). Although neither study reported heart rate (HR) values a review of the literature indicated that the intensity and mode of forearm exercise results in an approximate increase of 10% in HR (39). Additionally, it has been demonstrated that mild intensity (27W) one-legged kicking exercise, which results in a 35-40% increase in HR (from a resting HR of approximately 60 bpm), results in a 60% attenuation of the vasoconstrictor effect of intra-arterial PE infusion when compared to resting control (4, 42).

In contrast to the agonist infusion studies (4, 12, 30, 42), when the phenomenon of "functional sympatholysis" was probed using animal models by pharmacologically blocking the physiological activation of the α_1-adrenoreceptor the presence of a functional vasoconstrictor response at moderate to heavy intensity exercise was identified. For example when the α_1-adrenoreceptor antagonist Prazosin was administered to dogs exercising on a treadmill at 3 miles per hour (mph), an intensity that elicited a 60% increase in HR, hind limb iliac conductance increased by approximately 70% when compared to control exercise (5, 7, 26). Furthermore in dogs exercising at near maximal intensities and eliciting a 135% increase in HR (resting baseline approximately 75 bpm), α_1 blockade resulted in an increased iliac conductance of approximately 20% when compared to control exercise (5, 7, 26). In other words, in these exercising models, physiological blockade of the α_1-adrenoreceptor "unmasked" the maintenance of 70% and 20% α_1 mediated vasoconstriction during mild and heavy exercise, respectively. More recently, when physiologic activation of the exercise pressor reflex and its consequent sympathoexcitation was accomplished by using terminal aortic occlusion in dogs during mild, moderate, and heavy intensity exercise the presence of a maintained vasoconstriction was identified even at the highest exercise intensities (9).

While direct comparisons between the above mentioned studies are difficult because of the differences in mode of exercise, experimental limbs, and species, the striking inconsistencies of effect size between agonist and antagonist effects present the possibility that intra-arterial infusion of an "industrial" pharmacological agonist in

humans may overestimate the sympatholytic effects of exercise. Therefore in the present study, we aimed to test the hypothesis that oral administration of the α_1-adrenergic receptor antagonist Prazosin would result in significant increases in femoral blood flow (FBF) and femoral vascular conductance (FVC) and that the effect of Prazosin would be inversely proportional to exercise intensity. This was accomplished by measuring femoral blood flow (FBF) and femoral vascular conductance (FVC) in both the exercising leg (EL) and non-exercising leg (NEL) during rest and three different intensities of dynamic one-legged kicking exercise with and without oral administration of the α_1-adrenergic receptor antagonist Prazosin. In order to examine the relationship between physiological activation of the sympathetic nervous system and exercise intensity, subjects performed the exercise at workload intensities equivalent to ~40, 60, and 75% of their respected maximum workload intensity (WL_{max}).

METHODS

Subjects and general information

Five men and two women (age, 28±2 years; height, 176±4 cm; weight, 76±8 kg; mean ± s.e.m.), volunteered to participate in the present investigation. All subjects were healthy, non-smokers, free of known cardiovascular and respiratory disease, and were not using prescription or over-the-counter medications. Prior to the study all subjects provided written informed consent, were familiarized with all the testing protocols, and were asked to abstain from drinking alcohol and caffeine and to not exercise for the 24 hour time period prior to any scheduled experiments. All experimental procedures were approved by the Institutional Review Board at the University of North Texas Health Science Center and were in accordance with the guidelines of the *Declaration of Helsinki.*

All experimental protocols were performed with the subject seated in a semi-recumbent position (~45 degrees) in a modified car seat which allowed for optimal exercise performance while enabling Doppler ultrasound measurements of blood velocity and vessel diameter of the desired femoral artery to be obtained. One-legged kicking exercise was performed on a modified cycle ergometer as previously described (2) in the right leg at a rate of 60 kicks per minute. A standard four-lead electrocardiogram was used for HR monitoring and blood pressure was assessed invasively from a radial artery catheter.

Experimental Protocols

Protocol 1: Effect of Prazosin on the peripheral vasculature during different intensities of exercise.

Experimental day 1. Using a modified Monark cycle ergometer (Ergomedic 874 E, Monark) (34) with resistance against knee extension, each subject performed a graded, dynamic one-legged knee extension maximal exercise test to determine their respective maximal workload intensity (WL_{max}). The subjects began exercising at a 0.1 kg workload for 2 minutes, after this warm-up period an additional 0.1 kg of weight was added in one minute increments until the subject was no longer able to perform the exercise at a rate of 55 - 60 kicks per minute (kpm) despite verbal encourgament. The subject's individual maximum power was used to calculate the individual weight (Kg) required to achieve intensities equal to 40, 60, and 75% of their wL_{max}.

Experimental day 2.

Morning: The protocol for experimental day 2 is outlined in Figure 1. Heart rate (HR), blood pressure (BP), femoral arterial diameter (FAD), and femoral blood velocity (FBV) was measured in all subjects during rest and three different exercise intensities of ~40 (low), ~60 (moderate), and ~75% (high) of each subjects respective WL_{max}. The resting condition was always performed first which was immediately followed by the low, moderate, and high exercise intensities (i.e. there was no rest period between changes in intensities). The HRs and BPs were continuously measured throughout the duration of the protocol. During rest condition measurements of FAD and FBV were performed in

both the exercising (EL) and non-exercising leg (NEL) following a 5 minute seated rest steady-state period. During each of the exercise conditions FAD and FBV were assessed following a 5 minute exercising period to achieve steady state. Each rest and submaximal exercise workload lasted approximately 15 minutes (i.e. entire duration of the control protocol approximated one hour). The FAD and FBV were assessed in both the EL and the NEL at minutes 5, 10 15 of rest and each submaximal exercise intensity. Additionally, at the completion of each rest and each submaximal intensity (i.e. minutes 15, 30, 45, and 60 of protocol) a 4ml blood sample was drawn from the radial catheter for analysis of osmolality and plasma catecholamines.

Afternoon: The experimental protocol performed and measurements obtained in the afternoon were exactly the same as in the morning. The only difference between the two protocols was that the afternoon protocol was performed at least 2 hours following oral ingestion of the α_1-adrenoreceptor antagonist Prazosin. The 2 hour resting duration was chosen for two reasons; i) to allow for recovery from the exercise performed in the morning; and ii) it has been demonstrated that peak Prazosin activity is achieved approximately 2 hours post-ingestion (16).

Protocol 2. Validation of Prazosin mediated α_1-adrenoreceptor blockade.

The validity of these results requires that the dose of Prazosin was capable of significantly inhibiting the pressor effects of α_1-adrenoreceptor agonists throughout the duration of the afternoon protocol. Therefore in the morning following catheterization

(prior to any data collection) a bolus dose of 1.0 μg/kg body weight of Phenylephrine (PE) was injected intra-venously (33). This exact same bolus dose of PE was injected at 2 hours post Prazosin ingestion (immediately prior to the afternoon protocol) as well as immediately following completion of the study (Figure 1). The dose of PE was chosen because it has been demonstrated to evoke an increase in mean arterial blood pressure of approximately 15 - 20mmHg (33). During all PE injections arterial blood pressure was closely monitored to determine that the Prazosin dose sufficiently antagonized the pressor response to PE.

Catheterization

A 4.45 cm, 20 gauge catheter (Arrow) was inserted into the radial artery using sterile techniques under local anesthesia with ~2 ml of lidocaine and aseptic conditions, and was connected to a pressure transducer (Maxxim Medical, Athens, TX, USA) positioned at the level of the heart. The arterial catheter allowed for assessment of beat-to-beat arterial blood pressure (ABP). Additionally a 1.2 mm, 18 gauge venous catheter was inserted into the median antecubital vein and was used for bolus drug injections during the Phenylephrine (PE) challenge protocols.

Femoral Blood Flow

Measurements of femoral arterial diameter (FAD), and femoral blood velocity (FBV) and subsequent FBF measurements, were obtained using a doppler ultrasound machine (Phillips, model: HDI 5000 Sono CT; Bothell, WA, USA). Ultrasound imaging of

femoral artery diameter was measured using a linear array transducer operating at an imaging frequency of 7 - 8 MHz at a site approximately 2 cm distal to the inguinal ligament where the best spatial resolution was achieved. The average of 2 measurements of maximal (systolic) FAD was determined at rest and during one-legged knee extension exercise in the EL and NEL at minutes 5, 10 and 15 of the resting and each exercise trial. Two 20sec segments of FBV profiles were obtained in each the EL and NEL at minutes 5, 10 and 15 of the resting and each exercise trial using the same linear array transducer with a Doppler frequency of 5 MHz and a sample depth of 3.5 – 5 mm. All FBV measurements were taken with the transducer held at an insonation angle as close to 30 - 40° to the femoral artery as possible while optimizing the velocity spectra waveforms and the sample volume centered. The ultrasound and doppler data of FAD and angle-corrected, time-and space-averaged, and intensity-weighted mean FBVs were calculated using commercially available software (Phillips, model: HDI 5000 Sono CT; Bothell, WA, USA). The ultrasound recordings and 20sec segments of blood velocity spectra were saved to the HDI 5000 hard drive for offline analysis.

The reproducibility and validity (when compared to thermodilution) of Doppler ultrasound measurements of FBF during one-legged kicking exercise has been previously described (27, 42) during exercise intensities up to 70W. The average FBF measurements obtained from all exercise intensities in the current study were in agreement with previously described values.

Drugs

In the afternoon of day 2 each subject ingested an oral dose of the α_1-adrenoreceptor antagonist Prazosin (Mylan Pharmaceuticals, Morgantown, WV, USA). The dose of Prazosin ingested was equal to 1 mg /20 kg of body weight. Phenylephrine (Baxter Healthcare Corp., Deerfield, IL, USA) was used as a selective α_1-adrenoreceptor agonist in the challenge protocols to ensure the efficacy of Prazosin. Phenylephrine was diluted with physiological saline to yield a bolus dose equal to 1.0 μg/kg body weight.

Blood Sampling

Plasma samples were drawn from the arterial catheter immediately upon completion of each phase of the protocol (i.e. at the completion of rest, and at the completion of 40% WL_{max} intensity exercise, 60% WL_{max} intensity, and 75% WL_{max} intensity exercise for a total of 8 samples).

Plasma Catecholamines: Approximately 2.5 ml of plasma sample was centrifuged and stored at -70°C. Samples were then thawed and plasma concentrations of norepinephrine and epinephrine were separated and analyzed by high-performance liquid chromatography.

Plasma Osmolality: Approximately 1.5 ml of plasma sample was immediately transferred to a heparin treated tube and was centrifuged (4°C) for 10 minutes. The plasma osmolality was then immediately measured by freezing point depression (Advanced Instruments, model 3DII).

Data analysis and statistics

Mean arterial pressure (MAP) was derived from the arterial pressure waveform. Femoral blood flow (FBF) in the EL and NEL is represented as the average of measurements obtained at min 5, min 10, and min15 of each phase of the protocol and was calculated by the formula: FBF (ml/min) = $FBV_{mean} \cdot \pi \cdot (FAD/2)^2$. Femoral vascular conductance (FVC) is also represented as the average taken at the above mentioned time points and was calculated by the formula: FVC = FBF/MAP. Two-way analysis of variance and repeated measures (two-way RM-ANOVA) were used for comparisons of physiological variables between rest and the three exercise intensities and with and without Prazosin, as well as for comparisons of the effect of Prazosin between the EL and NEL and the three exercise intensities. In the absence of a significant interaction between drug and exercise intensity and limb and exercise intensity a *Post-hoc* analysis was performed by Tukey's procedure for further analysis when a significant main effect was determined. All data is expressed as means ± SE. Statistical significance was set at $p<0.05$.

RESULTS

Cardiovascular and Hemodynamic Responses to Seated Rest and Three Intensities of Dynamic One-Legged Kicking Exercise

The mean values for cardiovascular and hemodynamic variables obtained at rest and during the three intensities of exercise with and without Prazosin are presented in Table 1. Compared to rest the HR was increased during all three exercise intensities. Furthermore HR was significantly increased with Prazosin during rest and each exercise intensity when compared to the control condition. MAP was unchanged during the low intensity exercise condition when compared to rest, however, MAP increased significantly above rest during the moderate and high exercise intensities. Prazosin resulted in significant reductions in MAP during rest and all three exercise intensities. Moderate and high intensity exercise resulted in sympathetic activation as indicated by significant increases in plasma norepinephrine (NE) during moderate and high intensity exercise. Plasma NE was significantly increased during all conditions following Prazosin ingestion when compared to control.

Effectiveness of Prazosin mediated α_1-adrenoreceptor blockade.

The pressor responses to a 1.0 μg / Kg body weight bolus intra-venous infusion of PE are illustrated in Figure 2. During control rest PE yielded an increase in MAP of 14±0.4 mmHg. Two hours post Prazosin ingestion (i.e. immediately prior to the start of the afternoon experimental protocol) the same dose of PE yielded an increase in MAP of 3.6±0.2 mmHg (75±1.7% blockade). The same dose of PE administered at the

completion of the afternoon experimental protocol yielded an increase in MAP of 3.1±0.1 mmHg (78±1.0% blockade). These data identify the presence of significant blockade of the α_1-adrenergic receptor throughout the experimental protocol.

Effect of Prazosin on Femoral Blood Flow and Femoral Vascular Conductance during Seated Rest and three Intensities of Dynamic One-Legged Kicking Exercise

As expected α_1-adrenoreceptor blockade significantly increased absolute FBF and FVC in the EL (Figure 3) and NEL (Figure 4) during rest and the three exercise intensities. However, in the EL there was an exercise intensity dependent reduction in the relative effectiveness of Prazosin to increase FBF and FVC. For example, during rest FBF in the EL leg increased by 39±6% with Prazosin, whereas during the high intensity workload FBF only increased by 9±2% (Figure 3A and C). A similar pattern was observed for FVC in the EL such that during rest FVC increased by 52±11% whereas during high intensity exercise FVC only increased by 18±3% (Figure 3B and D). In contrast, Prazosin resulted in exercise intensity dependent increases in relative FBF and FVC in the NEL. During rest Prazosin mediated blockade resulted in a 37±6% increase in FBF while during high intensity exercise FBF was increased by 65±4% (Figure 4A and C). Similarily, during rest with Prazosin, FVC increased by 50±11% while during high intensity exercise FVC was increased by 79±6% (Figure 4B and D). The magnitude of absolute and relative increases in FBF and FVC during the three exercise intensities was greater in the NEL when compared to the EL (Figure 4C and D).

DISCUSSION

There are several primary findings in the present investigation. First the increases in FBF and FVC observed in the EL after Prazosin blockade of the α_1-adrenergic receptor identified a maintained role of the α_1 adrenergic receptor control of the vasculature in the human thigh during dynamic exercise at intensities of up to 75% $workload_{max}$. Second, the magnitude of increases in FBF and FVC during the three exercise intensities was greater in the NEL when compared to the EL. Third, the effect of Prazosin in the EL was inversely related to exercise intensity while the effect of Prazosin in the NEL was directly proportional to exercise intensity confirming the presence of an exercise mediated functional sympatholysis.

α-receptor mediated vasoconstriction in the EL during exercise

The finding of a negative relationship between the effect of Prazosin to increase FBF and FVC in the EL and exercise intensity is in agreement with reports from previous studies in exercising dogs.(5, 7, 26). This negative relationship is expected and is explained by the phenomenon of "functional sympatholysis", which describes the balance between neurally mediated vasoconstriction and locally mediated vasodilation in an attempt to maintain arterial blood pressure and to optimize blood flow and thus substrate delivery to metabolically active tissues, respectively (28). Although the exact mechanisms resulting in functional sympatholysis remain incompletely identified, it is thought that the metabolites produced during exercise inhibit postjunctional adrenergic signaling of both α_1 and α_2 adrenoreceptors (3, 6, 12). Both animal and human studies (3, 36, 42) have

demonstrated that the α_1-adrenoreceptors, which are primarily located on the larger upstream conduit arteries (3, 21, 42) appear to be less sensitive to the metabolic attenuation of the sympathetically mediated vasoconstriction than the α_2-adrenoreceptors. The α_2-adrenoreceptors populate the small nutrient arterioles (3, 21, 42).

Many of the previous studies that have examined the control of the peripheral vasculature during exercise in humans using intra-arterial agonist infusions have reported a 55% - 80% attenuation of intra-arterial pharmacologic α_1 mediated vasoconstriction during very light workload intensities (4, 12, 29, 42). Furthermore, it has been reported in humans (42) that α_1 mediated vasoconstriction is completely abolished during 37W one-legged kicking exercise (same exercise paradigm as used in the present study), an intensity of exercise that resulted in a 25 bpm increase in HR (i.e. a 30% increase; 57±4 at rest versus 82±2 bpm during 37W exercise). Additionally, several studies in humans have demonstrated an almost complete (12, 29) and a complete (36, 42) inhibition of α_2 mediated vasoconstriction during exercise intensities as low as 7W leg kicking (intensity that raised HR from 57±4 to 65±2 bpm; a 14% increase in HR) (42). Collectively it appears that during light or mild exercise intensities that have been shown to increase HR only 30%, i.e. exercising at roughly 80-85 bpm, there is a complete inhibition of α_1 and α_2 mediated vasoconstriction evoked by intra-arterial infusion of pharmacological agonists. A similar finding of an intensity dependent reduction in the effectiveness of intra-arterially infused PE in the hindlimb of exercising dogs has been reported (8). However the magnitude of attenuation and intensities of exercise required to produce this attenuation of sympathetic vasoconstrictor activity differ in degree, i.e. treadmill running

at 6 mph with a 10% grade (near maximal exercise ~ 155% increase in HR) resulted in an approximately 60% inhibition of intra-arterial PE mediated vasoconstriction (6, 8). Furthermore, it has been reported that when Prazosin is infused intra-arterially to probe the effects of α_1-adrenoreceptor blockade there is approximately a 65%, 35%, and an 18% increase in iliac conductance during mild, moderate, and high intensities of exercise, respectively (5, 7, 26). The "unmasking" of a significant α_1-adrenoreceptor mediated vasoconstriction following Prazosin treatment in the current study during the 2 lower intensities of exercise (similar results are observed for high intensity exercise) are not as drastic as the results observed by previously in exercising dogs (5, 7, 26). However, the data of the present study identify a greater maintenance of α_1-adrenoreceptor vasoconstriction at higher exercise intensities than was reported in the studies that used intra-arterial infusion of pharmacological agonist activation of the α_1-adrenergic receptors. One possible reason that Prazosin treatment in the dog studies during the lower exercise intensities resulted in much larger increases in exercising limb conductance is that anatomically dogs have a relatively bigger heart and a huge splanchnic blood volume available for achieving much larger cardiac outputs at lower workload intensities than humans (25). Therefore, it is possible that the challenges presented to the cardiovascular system in terms of cardiac output redistribution and blood pressure regulation at lower exercise intensities are significantly less and therefore, the necessity for functional vasoconstriction would be less than is required in humans.

The reasons for the discrepancy between the conclusions achieved from the findings of the agonist infusion (4, 12, 29, 42) and antagonist blockade studies (5, 7, 26) in humans regarding the degree of maintained alpha-1 vasoconstriction despite higher exercise intensities is not completely clear. Physiological activation of alpha-adrenoreceptors occurs abluminally via endogenous norepinephrine released from the sympathetic boutons which exerts its effects primarily on adrenoreceptors which are proximate to the nerve endings (14). Whether or not intra-luminal administration of exogenous sympathomimetic drugs demonstrates the same receptor affinity, pharmacokinetics, and physiological responses as endogenously released NE has not been identified. Further complicating the issue is the possibility that because intra-luminal agonists most likely need to diffuse through the blood vessel wall and interstitial space to activate the receptors, the interaction may be diffusion limited within the vessel wall. This diffusion limitation may by exacerbated by the intensity dependent increases in blood flow and thus decreases in transit time that are associated with exercise, thereby limiting the time available for diffusion. Previous studies (6, 12, 29, 32, 38) , including some from our group (4, 42), have minimized this concern by flow-adjusting the concentration of infused drug to meet the increased blood flows. By analyzing a wide dose-response range of PE Wray et al (42) and Brothers et al (4) further minimized this concern. In these studies the authors were able to demonstrate a plateau in the FBF and FVC responses to increased doses of PE infusion during exercise, suggesting that the receptors are in fact maximally activated. However, the presence of a plateau in FBF and FVC response may be a result of a balance occurring between the diffusion limitation and the

increased rate of blood flow and not necessarily representative of maximal receptor activation. Collectively, the above mentioned limitations to intra-arterial drug infusions may explain the presence of a maintained increase in $\alpha_1$mediated vasoconstriction during higher intensity exercise observed in the current study using α_1-receptor blockade compared to those studies that use α_1-agonist infusions (4, 12, 29, 42).

Intra-arterial infusion of Tyramine (TYR) is another method that has been utilized to overcome the concerns of intra-luminal agonist infusions (12, 32, 38). TYR exerts its actions by initiating the release of NE from sympathetic nerve endings thus resulting in physiological activation of adrenoreceptors (14). It has been reported that the decreases in FVC induced by TYR infusion (4 $\mu g \cdot dl$ forearm volume$^{-1} \cdot min^{-1}$ and 8 $\mu g \cdot dl$ forearm volume$^{-1} \cdot min^{-1}$) during control rest were attenuated by 15% and 37%, respectively during moderate intensity (10-15% maximal voluntary contraction exercise) forearm exercise (38). The same mode and intensity of exercise resulted in ~65% attenuation of PE (α_1) mediated reductions in FVC (12, 29). Additionally in a dog model exercising at a very high intensity (6mph, 10% grade) the reductions in iliac conductance illicited by a 3 $\mu g \cdot ml^{-1} \cdot min^{-1}$ infusion of TYR were attenuated by ~45% when compared to control rest (32) while in a separate study the same intensity of exercise attenuated the vasoconstrictive actions of PE by ~60% (8). It should also be noted that other investigators have reported a 60 – 70% exercise mediated attenuation of the vasoconstriction elicited by endogenous adrenoreceptor activation. Keller et al (18) demonstrated that one-legged kicking at a rate of 30 kicks per minute and an intensity of

7W and 25W attenuated neck pressure mediated reductions in leg vascular conductance by 60 and 70% respectively. In a similar model with subjects kicking at a rate of 60 kicks per minute and intensity of 50% maximal work rate, Wray et al. (41) reported a 73% attenuation of the vasoconstriction elicited by a 3 minute cold pressor challenge.

While the different modes, intensity, limbs, and species being exercised may explain for the variability in the results it is possible that the findings of the present study can be explained by a "disconnect" between the pharmacological actions of intra-luminal receptor activation vs. the physiological action of endogenous receptor activation. Specifically the diffusion dynamics of the intra-arterially infused agoinst through the blood vessel wall and interstitial space and subsequent receptor activation may be limited by the exercise mediated increases in blood flow and thus decreases in transit time.

α-receptor Mediated Vasoconstriction in the NEL during Exercise

In the present study, blockade of the α_1-adrenergic receptor with Prazosin resulted in significant increases in FBF and FVC in the NEL. However unlike in the EL the increases in the NEL occurred in an intensity dependent manner (Figure 4B and D). Additionally the magnitude of increases in FBF and FVC were significantly greater in the NEL vs. the EL (Figures 3 and 4). These results were expected and can be easily explained by i) the blockade of the basal tone mediated by α_1-receptors (10, 11); and ii) the blockade of the effects on α_1 receptors of exercise induced increases in global sympathetic activity (22, 31). Exercise results in an intensity dependent activation of the

sympathetic nervous system (15, 22, 31), therefore it would be expected that the effects of Prazosin in the NEL would be greater with increasing exercise intensities and subsequent increases in sympathetic outflow. The finding of a greater magnitude in the effect of Prazosin in the NEL compared to the EL was also expected. This was easily explained by the sympatholytic nature of exercise. Because the α_1 induced vasoconstriction was already compromised in the EL (and not the NEL) before Prazosin treatment it was expected that the NEL would respond with a larger degree of vasodilation.

Experimantal Limitations

The use of an oral drug that exerts systemic actions as opposed to local actions is a potential limitation of the present study. The effects of oral Prazosin ingestion during rest (19, 23) and during upright cycling exercise (19) have been previously reported with similar results as observed in the present investigation. In the present study Prazosin resulted in significant increases in HR and plasma NE and significant reductions in MAP during all conditions. There was no effect of the drug on plasma epinephrine or osmolality (Table 1). Therefore it was possible that the observed increases in FBF in the NEL and EL that were observed with Prazosin resulted from the decreases in MAP. While we agree that this would affect FBF, we corrected for the changes in MAP by calculating FVC and identified that the increases in FBF were due to vasodilation resulting from the Prazosin blockade of the α_1-adrenergic receptors. Also prazosin treatment only resulted in partial blockade (i.e. about 75%) of the α_1-receptors (Fig 2).

Therefore, the arterial baroreflex reflex activation of the sympathetic nervous system in response to the decreases in MAP limit the increases in FBF and FVC. By comparing the effect of Prazosin blockade in the EL and NEL across rest and each exercise intensity condition the intensity related reductions in vasodilation as a result of α_1-adrenoreceptor blockade confirm the presence of an intensity related functional sympatholysis.

Perspectives

While the present study evaluated only young healthy subjects, knowledge of the functional role of α_1 adrernoreceptors in this population may be relevant to clinicians through providing a more comprehensive understanding of the effects of adrenoreceptor blockade on the vascular control in metabolically activated organs and skeletal muscle especially in cardiovascular aging and various cardiovascular pathologies. For example, several investigations have reported a reduced responsiveness of α_1-adrenoreceptors associated with healthy aging (13) and enhanced heart rate fluctuations in humans (20). Furthermore, diabetes is associated with increased vascular tone, a consequence that appears to be selectively expressed by α_1 (but not α_2) adrenoreceptors (1), mediated by an increased activity of alpha-1 specific G-proteins (40). Additionally, it has been demonstrated that patients suffering from various forms of heart failure have a reduced hyperemic response in the active skeletal muscle during submaximal and maximal exercise (35). It has been suggested that the increased vascular resistance and reduced hyperemic response observed in these patients is related to a reduced "functional sympatholytic" ability (17, 24, 37). Further studies in specific patient populations are

needed to determine whether the current findings regarding α_1-adrenoreceptor control of vascular tone and peripheral blood flow may be extended to these disease states.

Conclusion

Through comparison of the FBF and FVC responses during rest and three different intensities of dynamic one-legged kicking exercise with and without Prazosin, the present study has identified in humans a significant maintenance of α_1-mediated vasoconstriction during exercise at moderately high intensities.

Acknowledgments

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Figure Legends

Figure 1. Protocol outline for the control (no drug) and experimental (Prazosin) conditions.

Each condition was separated into four different trials; rest, 40%, 60%, and 75% (of each subject's respective workload maximum) one-legged knee extension exercise. The time course of each trial is identified on the top axis in minutes. The control condition was always first and was performed in the morning, this was followed by a 2 hour rest periodat the beginning of which each subject ingested their individual dose of Prazosin (1mg / 20 Kg body weight). At the end of the two hour break the experimental condition was performed. Measurements of FBV and FAD were made at minutes 5, 10, and 15 of each trial and are indicated by the small arrows. A pressor dose of PE was infused intra-venously at time 0 in the morning, immediately prior to the afternoon experimental protocol and then again immediately upon completion of the afternoon protocol to determine the effectiveness of α_1-blockade with Prazosin.

Figure 2. Effectiveness of α_1-blockade with Prazosin.

Prior to beginning the control protocol an intra-venous bolus infusion of PE (1µg / Kg body weight) increased MAP by 14.4±0.4 mmHg. This same dose of PE when given 2 hours post Prazosin treatment (before the start of the experimental protocol) and immediately upon completion of the experimental protocol increased MAP by 3.6±0.2 (i.e. 75±2% blockade) and 3.1±0.1 (i.e.78±1% blockade), respectively ($P<0.05$ when compared to control PE infusion for both time points).

Figure 3. Femoral blood flow and conductance in the exercising leg.

Prazosin resulted in significant increases in absolute FBF (panel A) and FVC (panel B) in the exercising leg during rest and all three exercise trials. The effectiveness of Prazosin, represented as percent change from each respective control condition, to increase relative FBF (panel C) and FVC (panel D) was decreased in an exercising intensity dependent manner. *, $P < 0.05$ compared to respective control baseline condition; †, $P < 0.05$ compared to resting condition; ‡, $P < 0.05$ compared to low intensity exercise condition.

Figure 4. Femoral blood flow and conductance in the non-exercising leg.

Prazosin resulted in significant increases in absolute FBF (panel A) and FVC (panel B) in the non-exercising leg during rest and all three exercise trials. The effectiveness of Prazosin, represented as percent change from each respective control condition, to increase relative FBF (panel C) and FVC (panel D) increased in an exercising intensity dependent manner. *, $P < 0.05$ compared to respective control baseline condition; †, $P < 0.05$ compared to resting condition; ‡, $P < 0.05$ compared to low intensity exercise condition; £, $P < 0.05$ compared to mid exercise intensity condition; €, $P < 0.05$ compared to the effect of Prazosin when compared to the EL within the same exercise intensity.

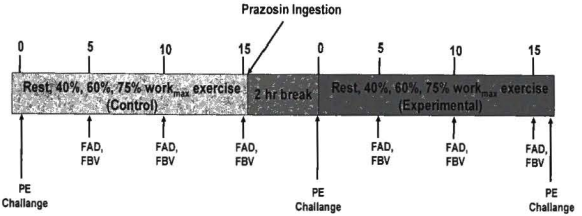
Table 1. Hemodynamic values during rest low (40%), moderate (60%), and high (75%) exercise intensities with and without Prazosin.

Values for HR (heart rate), MAP (mean arterial pressure), circulating plasma norepinephrine, epinephrine, and osmolality during control (no drug) and experimental (Prazosin) rest and three intensities of one-legged dynamic knee extension exercise. *, $P < 0.05$ compared to no drug (within the same exercise intensity); ¥, $P < 0.05$ compared rest within respective exercise intensity.

Table 1: Hemodynamic values during rest low (40%), moderate (60%), and high (75%) exerciseintensities with and without Prazosin.

	Rest		Low		Moderate		High	
	BL	**Drug**	**BL**	**Drug**	**BL**	**Drug**	**BL**	**Drug**
HR (beats · min^{-1})	65±4	73±4*	79±3¥	96±3¥*	90±1¥	115±5¥*	112±2¥	136±5*
MAP (mmHg)	94±3	87±3*	100±4	93±3*	101±3¥	93±4*	109±3¥	101±3¥*
Norepinephrine (pmol/ml Plasma)	2.0±0.1	3.3±0.4*	2.4±0.3	4.7±1.0*	3.0±0.3¥	6.1±1.3¥*	3.7±0.4¥	7.5±1.4¥*
Epinephrine (pmol/ml Plasma)	0.4±0.1	0.6±0.1	0.6±0.1	0.6±0.2	0.8±0.2	0.6±0.1	0.6±0.2	0.6±0.1
Osmolality (mOsm)	288±1.0	289±0.9	290±1.0	288±0.5	291±2.0	290±1.3	292±2.0	293±1.2

Values for HR (heart rate), MAP (mean arterial pressure), circulating plasma norepinephrine, epinephrine, and osmolality during control (no drug) and experimental (Prazosin) rest and three intensities of one-legged dynamic knee extension exercise. *, $P < 0.05$ compared to no drug (within the same exercise intensity); ¥, $P < 0.05$ compared to rest within respective exercise intensity.


Prazosin Ingestion
0
5
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15
0
5
10
15
Rest, 40%, 60%, 75% work$_{max}$ exercise (Control)
2 hr break
Rest, 40%, 60%, 75% work$_{max}$ exercise (Experimental)
FAD, FBV
FAD, FBV
FAD, FBV
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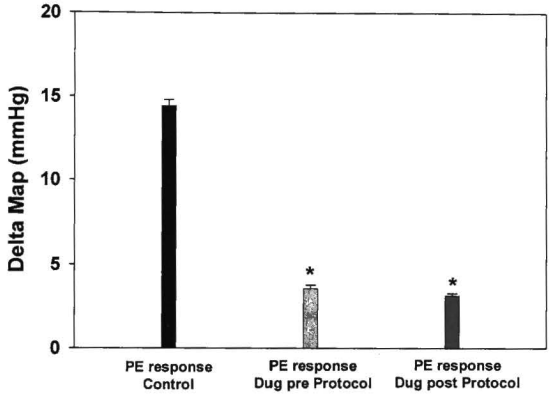
FAD, FBV
PE Challange
PE Challange
PE Challange

Figure 1


20
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PE response
Control
PE response
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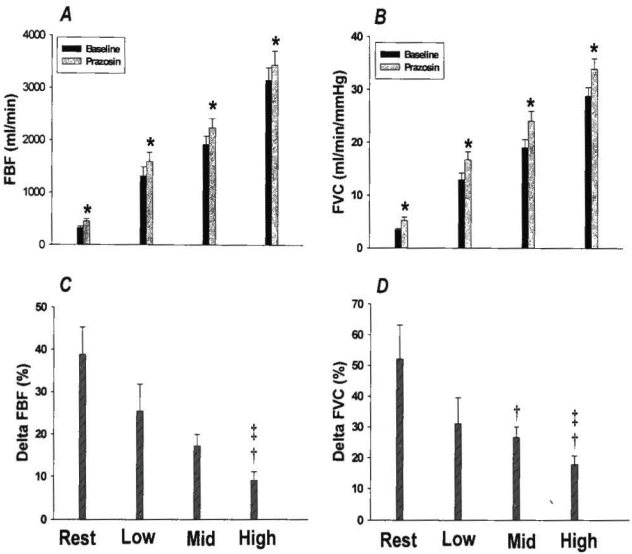
PE response
Dug post Protocol

Figure 2


A
Baseline
Prazosin
FBF (ml/min)
4000
3000
2000
1000
0
B
Baseline
Prazosin
FVC (ml/min/mmHg)
40
30
20
10
0
C
Delta FBF (%)
50
40
30
20
10
0
Rest
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D
Delta FVC (%)
70
60
50
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30
20
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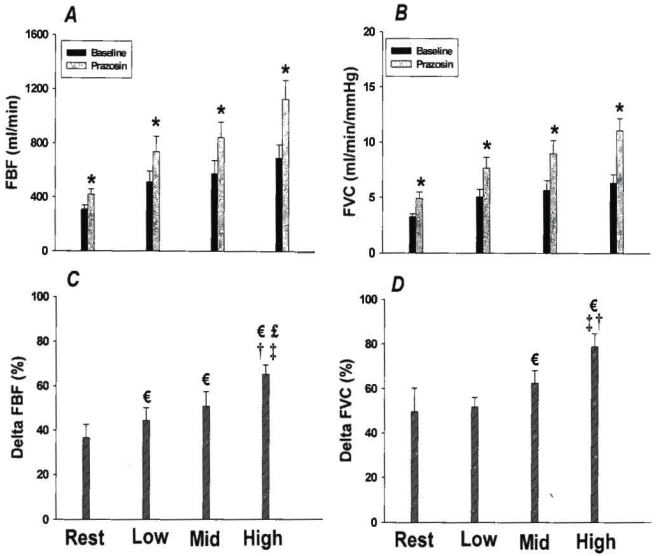
Rest
Low
Mid
High

Figure 3


A
Baseline
Prazosin
FBF (ml/min)
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1200
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B
FVC (ml/min/mmHg)
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Rest
Low
Mid
High

Figure 4

CHAPTER III

Exercise-induced inhibition of angiotensin-II-vasoconstriction in human thigh muscle.

R.Matthew Brothers, Mads L. Haslund, D.Walter Wray,

Peter B. Raven, and Mikael Sander

Journal of Physiology (577.2: 727-737, 2006)

Abstract

Background: It is well established that metabolic inhibition of adrenergic vasoconstriction contributes to the maintenance of adequate perfusion to exercising skeletal muscle. However, little is known regarding non-adrenergic vasoconstriction during exercise. We tested the hypothesis that a non-adrenergic vasoconstrictor, angiotensin-II (AngII), would be less sensitive to metabolic inhibition than an alpha1-agonist, phenylephrine (PE), in the exercising human thigh.

Methods and Results: In 11 healthy men, femoral blood flow (FBF, ultrasound Doppler and thermodilution) and blood pressure were evaluated during wide-ranging intra-arterial (femoral) infusions of PE and AngII at rest and during two workloads of steady-state knee-extensor (KE) exercise (7W and 27W). At rest, the maximal decrease in femoral artery diameter (FAD) during AngII (9.0±0.2 to 8.4±0.4mm) was markedly less than during PE (9.0±0.3 to 5.7±0.5mm), whereas maximal reductions in FBF and femoral vascular conductance (FVC) were similar during AngII (FBF: -65±6 and FVC: -66±6%) and PE (-57±5 and -59±4%). During exercise, FAD was not changed by AngII, but moderately decreased by PE. The maximal reductions in FBF and FVC were blunted compared to rest for both AngII (7W: -28±5 and -40±5%; 27W:
-15±4 and –29±5%) and PE (7W: -30±4 and -37±6%; 27W: -15±2 and -24±6%), with no significant differences between drugs.

Conclusions: The major new findings are 1) an exercise-induced intensity-dependant metabolic attenuation of non-adrenergic vasoconstriction in the human leg; and 2) functional evidence that angiotensinII-vasoconstriction is predominantly distal, whereas

alpha1-vasoconstriction is predominantly proximal within the muscle vascular bed of the human thigh.

Introduction.

Exercise is accompanied by powerful intensity-dependent vasodilation and increases in blood flow to the active muscle (2, 30, 56). This exercise-hyperemia is challenged by the activation of sympatho-adrenergic and non-adrenergic vasoconstrictor systems. During increasing intensity of exercise the increasing activity in sympathetic vasocontrictor efferents is driven by a combination of central nervous co-activation of motor control and sympathetic outflow (ie. "central command") and muscle-derived afferents (ie. "mechano- and metaboreflexes") (49, 53, 54). During higher levels of sympathetic activity, plasma renin acivity increases leading to increased production of the non-adrenergic vasoconstrictor angiotensin-II (AngII) (5, 38, 47). Several studies indicate that both resting and exercise-induced sympathetic activity and AngII-production are elevated in human cardiovascular conditions such as hypertension and heart failure (11, 22, 23, 25, 36). In addition, the non-adrenergic vasoconstrictor endothelin-1 seems to have an increased functional significance in patients with cardiovascular disease compared to controls (26, 58). Thus, the exercise-induced activation and efficacy of vasodilator- and vasoconstrictor-systems within skeletal muscle is dependant upon the type and intensity of exercise as well as pathophysiological state.

The interplay between the vasoactive systems results in a complex integrative control of local blood flow within exercising skeletal muscle. In some models of exercise, there is experimental evidence that sympathoexcitation may cause vasoconstriction in the active skeletal muscle (6, 20). However, it has been acknowledged for decades that the response to adrenergic stimuli may be attenuated in exercising muscle compared to

resting muscle (31), a phenomenon termed functional sympatholysis. More recently, a variety of studies in rodent, canine and human models of exercise have widely agreed, that the vasoconstrictor responses to sympathetic nerve activity or intra-arterial sympathomimetic drugs are at least partially inhibited by metabolic products of muscle contraction (9, 14, 18, 21, 34, 42, 44-46, 56). The significance of this phenomenon has been illustrated in rats with experimental heart failure (46) and in children with Duchenne's muscular dystrophy (34). In both these conditions metabolic inhibition of sympathetic vasoconstriction is diminished, which lead to hypo-perfusion of the exercising muscles during sympathoexcitation. Thus, subnormal metabolic inhibition of sympathetic vasoconstriction could contribute to development of muscle fatigue. Specifically, it has been hypothesized that this mechanism constitutes part of the "peripheral factor" of exercise intolerance in human heart failure (17).

While the exercise-induced attenuation of adrenergic vasoconstriction has been well characterized, little is known to what extent non-adrenergic vasoconstrictor systems are susceptible to the metabolic events of exercise. Thus, the present study focuses on AngII-mediated vasoconstrictor effects in human thigh muscle. Specifically, we hypothesized that in exercising human muscle AngII-vasoconstriction would be significantly less inhibited than alpha-1-vasoconstriction. If proven, this hypothesis would strongly suggest that attenuation of alpha-1-mediated vasoconstriction is related to a specific inhibitory action of metabolic events. If refuted, this would provide evidence in humans that non-adrenergic vasoconstriction is also sensitive to metabolic inhibition. To this end, we measured femoral blood flow directly at rest and during two workloads of

dynamic steady-state knee-extensor exercise with superimposed intra-arterial administration of the endogenous AT-receptor agonist AngII and a selective α_1-agonist phenylephrine (PE).

Methods

Subjects and general procedures

Eleven healthy young men (age: 25±2 years, BMI 25±1 $kg \cdot m^{-2}$) gave written informed consent to participate. The study was approved by the local ethics committee of Copenhagen and Frederiksberg and conformed to the Helsinki declaration. All experiments were performed in a thermoneutral environment, with subjects seated in a semirecumbent position (approximately 45 degree hipflexion). Heart rate (HR) was obtained from an ECG recording (BioAmp, ADInstruments). One-legged knee-extensor exercise was performed at 60 rpm on a modified cycle ergometer as previously described (1, 56). The knee extensor force and rhythm were recorded via a strain gauge (customized signal processor, FBJ Industries).

Catheterization

Under local anesthesia (lidocaine; Danish county pharmaceutical corporation), the arterial (Arrow, 20 gauge) and venous (Cook,18 gauge) catheters were inserted into the femoral artery and vein of the right leg. The catheters were used for intra-arterial drug infusions, direct phasic blood pressure measurements (BP), and femoral blood flow measurements via thermodilution (FBF_{TD}) (56). BP was measured at the level of the heart by adjustment of the position of the pressure transducer (Baxter), which was interfaced with a blood pressure amplifier (BPamp, ADinstruments). The thermodilution technique has been previously described and validated in detail (2, 56), and provides accurate and reproducible measurements of leg blood flow during knee extensor exercise.

Thermodilution was not used during rest because it is not suitable for determination of the low blood flows seen during infusions of vasoconstricting drugs.

Ultrasound imaging and Doppler was used to determine femoral artery diameter (FAD) and blood velocity (FBV) (7.5 MHz mechanical sector transducer, CFM 800, GE Medical). Resting femoral blood flow by ultrasound (FBF_D) was calculated as previously described (56).

Drugs

PE (Danish county pharmaceutical corporation) was used as a specific α_1-adrenergic agonist. AngII (Clinalfa, Switzerland) was used as an AT-receptor agonist. Drugs were diluted with normal saline and intra-arterial infusion rates ranged from 0.2 to 6 $ml \cdot min^{-1}$.

Experimental protocols

The protocol is outlined in Figure 1. In all subjects we measured BP, HR, FBF, and FAD during 1) rest; 2) 7W (low workload); and 3) 27W one-legged knee-extensor exercise (moderate workload). The resting condition was always first, whereas the order of the two exercise-intensities was alternated. Measurements during rest and exercise were obtained before and during continuous intra-arterial infusions of 6-7 incremental doses of PE and AngII. The measurements were made during the last 45 seconds of each dose (total time was 2-3 minutes on each dose). The doses used during rest and exercise were: PE, 0.0125-0.8 and 0.05-1.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$; AngII, 0.25-16 and 1.0-32 $ng \cdot kg^{-1} \cdot min^{-1}$. The

wide range of drug doses allowed for evaluation of saturation kinetics, and *post hoc* comparison of doses normalized to femoral blood flow using linear interpolation between individually obtained values. The order of the two vasoconstrictors was random and balanced; and the drugs were separated by at least 15 minutes, which was accompanied by restoration of pre-drug hemodynamic values. To avoid the initial non-steady state, measurements were not made during the first 10 minutes of each exercise bout. We used recovery periods of at least 45 minutes between exercise conditions.

Data analysis and statistics

All data were sampled at 400 Hz, recorded on a PC, and analyzed offline (PowerLab, ADInstruments). Mean arterial pressure (MAP) was derived from the arterial pressure waveform. Femoral vascular conductance (FVC) was calculated by the formula: FVC = FBF/MAP, and vascular resistance (FVR) by: FVR = MAP/FBF. The variables analyzed did not depart from normal distributions by F-test, or homogeneity of variances by Bartlett's Chi-Square test. Student's t-test for paired data was applied to test for differences between baseline values (e.g. steady state values before drug-intervention). Within group differences were assessed by one- or two-way ANOVA for repeated measures, for drug, dose, and exercise intensity. *Post-hoc* analysis was performed by Dunnett's procedure for one-way ANOVA and by Tukey's procedure for two-way ANOVA. Statistical significance was set at $p<0.05$ and adjusted by the Bonferroni-method as appropriate.

Results

At rest, continuous intra-arterial infusions of PE and AngII caused dose-dependent steady-state decreases in femoral blood flow (Figure 3). The decrease in flow was accomplished by different effects of the two drugs (Figure 2). The lower doses of PE caused minor decreases in FBV, and moderate decreases in FAD, whereas the higher doses of PE caused substantial decreases in FAD (from 9.0±0.3 at baseline to 5.7±0.5mm during PE 0.8μg•kg^{-1}•min^{-1}, p<0.05), and no change in FBV (from 9.7±0.6 at baseline to 10.8±1.5cm•s^{-1} during PE 0.8μg•kg^{-1}•min^{-1}, p=ns). In contrast, AngII had minor effects on FAD (from 9.0±0.2 at baseline to 8.4±0.4mm during AngII 16ng•kg^{-1}•min^{-1}, p<0.05) and marked effects on FBV (from 8.5±0.7 at baseline to 2.9±0.5cm•s^{-1} during AngII 16ng•kg^{-1}•min^{-1}, p<0.05). The resultant effects on FBF and FVC were similar for the two drugs (Figure 3 and Table 1). The maximum decreases in flow and conductance were not significantly different for the two drugs (Table 1), and the two dose-response curves both show saturation at 60-65% decreases for both variables (Figure 3). The maximal decreases were 57±5 and 65±6% for FBF; and 59±4 and 66±6% for FVC during PE and AngII, respectively (p=ns). The nominal difference between the two drugs did not reach significance, and was caused mainly by one subject with a low response to PE (around a 20% decrease). There were only minor changes in MAP and no significant changes in HR at rest (Table 1).

During both exercise intensities, continuous intra-arterial infusions of PE and AngII caused dose-dependent steady-state decreases in femoral blood flow. These decreases were of a similar magnitude for measurements based on thermodilution and

ultrasound (Table 1). The underlying patterns of effects on FAD and FBV seen at rest were also present during both exercise-intensities (Figure 2). Thus, PE caused moderate decreases in FAD (7W: from 9.1±0.2 to 7.3±0.3mm, and 27W: 9.1±0.3 to 8.7±0.3mm during PE 1.6μg•kg^{-1}•min^{-1}, $p<0.05$ for both); but no change in FBV. In contrast, AngII caused no significant changes in FAD, but decreased FBV (7W: from 34±3 to 25±2cm•s^{-1}, and 27W: from 66±5 to 54±3cm•s^{-1} during AngII 32ng•kg^{-1}•min^{-1}, $p<0.05$ for both). Again, the maximal decreases and dose-response curves for changes in FBF and FVC were similar between AngII and PE (Figure 3). The absolute decreases in femoral blood flow caused by both drugs during exercise were nominally (but not significantly) higher during exercise than at rest (Figure 3). Thus, the absolute FBF decrease identified saturation around values of 210ml•min^{-1} at rest; around 350ml•min^{-1} at 7W; and around 450ml•min^{-1} at 27W, for both drugs. However, the relative decreases in femoral blood flow and vascular conductance were markedly and significantly smaller during exercise compared to the resting condition (Figure 3). Thus, the relative FBF decrease had saturation values around 60% at rest; around 30% at 7W; and around 15% at 27W, for both drugs; and the relative FVC decrease had saturation values around 60% at rest; around 40% at 7W; and around 25% at 27W; for both drugs The vasoconstriction obtained were not significantly different between PE and AngII at 7 or 27W exercise.

When flow-adjusting PE and AngII-doses (*post-hoc*), the relative changes in FBF, FVC, and FVR remained significantly smaller during both exercise intensities compared to rest for both drugs (Figure 4). There were no significant differences in the responses to either drug between 7 and 27W exercise.

During exercise, HR increased from 55±3 at rest to 65 ± 3 and 77 ± 3 beats min^{-1} at 7 and 27W, respectively. Both drugs caused larger changes in MAP and HR during both exercise intensities than at rest (Table 1). PE caused increases in MAP of up to 12-14mmHg with concomitant decreases in HR of 13-14beats•min^{-1}. AngII caused increases in MAP of more than 20mmHg, but caused only minor decreases in HR of 4-6beats•min^{-1}. When the data was analyzed in the resting and two exercise conditions at the last drug dosage where there was no significant increase in MAP (rest: PE, 0.2μg•kg^{-1}•min^{-1}, AngII, 16ng•kg^{-1}•min^{-1}, 7W: PE, 0.2μg•kg^{-1}•min^{-1}, AngII, 2.0ng•kg^{-1}•min^{-1}, 27W: PE, 0.4μg•kg^{-1}•min^{-1}, AngII, 4.0ng•kg^{-1}•min^{-1}) there were no significant changes in the trends of the data (Table 2), i.e. no effect of a baroreflex mediated withdrawal of basal sympathetic tone.

Discussion

The major new finding of the present study is a significant exercise-induced inhibition of AngII-induced vasoconstriction in human thigh muscle. This attenuation of AngII-vasoconstriction was evident during both mild and moderate exercise intensity. The AngII- and PE-induced decreases in blood flow were attenuated to similar degrees during both exercise intensities. This finding demonstrates for the first time that metabolic events within exercising human thigh muscle attenuate both adrenergic and non-adrenergic vasoconstriction. Additionally this finding supports results of others investigations where the vasoconstrictive actions of various non-adrenergic hormones were attenuated in an exercise intensity dependent manner. In an anesthetized dog model Burcher et al. (10) demonstrated that equiactive doses of intra-arterial injections of norepinephrine, vasopressin, and angiotensin II were similarly attenuated at the onset of electrically induced muscle contractions. Additionally, in a conscious dog model, Buckwalter et al. (7, 8) demonstrated that the vasoconstrictive actions of an intra-arterially infused NPY Y_1 receptor agonist and a P2X receptor agonist were significantly attenuated in an exercise intensity dependent manner.

AngII- and PE-induced vasoconstriction.

AngII has previously been identified as a potent vasoconstrictor in the resting human forearm (28, 35). Pharmacological studies have provided evidence that AngII-vasoconstriction is mediated by AT-1 receptors, as the AT-1-receptor blocker losartan effectively inhibits AngII-vasoconstriction in the forearm (3, 35)S. Likewise, PE has been

validated extensively as a potent vasoconstrictor operating as an alpha-1-receptor agonist in the human forearm and leg (12, 56). Thus, the AngII and PE dose-ranges used in the present study probe the maximal responses obtainable with AT-1 and alpha-1 receptor stimulation in the resting and exercising human thigh.

In the present study maximal AT-1 and alpha-1 stimulation produced a similar degree of vasoconstriction in the human leg both at rest and during exercise, but did so by affecting different target sites in the vascular bed. Specifically, AngII and PE have dissimilar effects on femoral artery diameter, with intra-arterial PE being more potent than AngII with regards to large artery constriction (figure 2). At rest, PE caused an average decrease in femoral artery diameter exceeding 3 mm (36%), whereas the maximum effect of AngII was around 0.6 mm (7%). During both exercise-intensities, PE still caused significant decreases in the large artery diameter, whereas AngII became ineffective. Conversely, AngII more potently decreased femoral blood velocity, suggesting a higher maximal response on smaller vessel constriction for AngII than for PE-related vasoconstriction. These findings strongly suggest for anatomically dissimilar locations of the functionally important alpha-1 and AT-1 receptors in the human thigh. Proximally located alpha-1 receptors dominate the PE-responses, whereas functional effects of AT-1 stimulation are limited in the proximal arteries and predominately related to distally located AT-1 receptors within the vascular bed of the human thigh. To our knowledge, these data are the first to identify a heterogeneous distribution of alpha-1 and AT-1 receptors by functional responses in human thigh muscle. The findings concur with previous studies in rodents, where functional microvascular studies have indicated that

alpha-1-related effects are minor at the level of precapillary arterioles (15), and angiotensin-II becomes more potent the smaller the resistance vessel (52). Using immunohistochemistry, AT-1 receptors have been detected throughout the vascular tree of rats (29).

Despite these obvious differences, the dose-response curves for AT-1 and alpha1-stimulation vs. flow and vascular conductance are remarkably similar in appearance during both rest and the two exercise intensities (figure 3). Furthermore the maximally obtained effects of the two drugs were also similar under all circumstances. These maximal effects of the drugs during rest were comparable to previously published human studies of PE-infusion in the femoral (56) and AngII-infusion in the brachial artery (28, 35).

The degrees of metabolic inhibition of AT-1- and alpha1-vasoconstrictor effects produced by both very mild (7W) and moderate (27W) exercise intensities were also very similar (figure 4). The maximally obtained relative decreases in flow were reduced by around 50% during 7W and around 75% during 27W for both drugs; and the maximally obtained relative decreases in vascular conductance by 33% during 7W and around 50% during 27W for both drugs. When normalized for blood flow (i.e. the dilution-effect of the higher flows), the vasoconstrictor potential of the two drugs during exercise was still reduced by at least 50% compared to rest when evaluating relative decreases in blood flow, vascular conductance and resistance (except AngII-induced decreases vascular conductance which was attenuated by around 33%). Interestingly, there were no apparent differences in the exercise-induced attenuation of effects for either drug between 7W and

27W when normalized for blood flow. To our knowledge, the present report is the first on the response to intra-arterial AngII in an exercising human limb. The responses to PE in the exercising thigh are similar to our previously published results in this model (56), and to responses seen during very mild human forearm exercise (14).

Mechanisms of metabolic inhibition of vasoconstriction

The similarity in degree of exercise-induced decrease of AT-1- and alpha-1 related vasoconstriction initially suggests that these two vasoconstrictor-systems could be challenged by the same inhibitory metabolic event. Indeed, AT-1 and alpha-1 receptors have very similar G-protein coupling, and both cause activation of phospholipase C and subsequent production of the intracellular messengers inositol triphosphate and diacylglycerol. Ultimately, smooth muscle contraction depends on the phosporylation state of myosin light chain (MLC), which may be increased via activation of the MLC kinase and via inhibition of the MLC phosphatase through the Rho/Rho-kinase pathway. There is evidence that AT-1 and alpha-1 stimulation both cause MLC kinase activation as well as MLC phosphatase inhibition (16, 19, 39). Furthermore, functional convergence of AT-1- and alpha1-signal-transduction has been illustrated *in vivo* in animal studies. For example, chronic administration of norepinephrine induces hyposensitivity in the vasculature to both norepinephrine and AngII (37), which may be related to down-regulated G-protein coupling to both AT-1 and alpha1-receptors.

However, considering the differing sites of functional importance for AT-1-receptors (distal) and alpha1-receptors (proximal) within the vascular bed, the underlying

mechanisms for the metabolic inhibition of these two vasoconstrictor systems may in fact differ. It seems plausible that metabolites released from the contracting myocytes interfere with the distal AngII-effects at the level of the resistance arterioles, while the same metabolites are unlikely to directly influence the proximal PE-effects (and AngII-effects) at the level of the femoral artery. The inhibition at the level of the large artery could conceptually be related to ascending vasodilation mediated via gap-junction communication between endothelial or smooth muscle cells. However, a recent study using intravital microscopy in isolated hamster vessels lends no support to this possibility, with no evidence of exercise-induced inhibition of feed artery constriction (50). Alternatively, the exercise-induced inhibition of large artery constriction may be unrelated to the metabolic events of the skeletal muscle, but instead due to either physical deformation of the large artery leading to vessel wall relaxation, altered perfusion of *vasa vasorum* of the femoral artery, or decreased time for agonist-receptor interaction due to the shortened transit-time of blood in exercising compared to resting muscle (model- and drug-related). Interestingly, in a completely different setup, using rhythmic handgrip exercise and magnetic resonance imaging to measure brachial artery diameter, a marked exercise-induced inhibition of brachial artery constriction was identified during continuous intra-brachial norepinephrine-infusion (18).

The metabolic inhibition of AngII-vasoconstriction may also be compared to the inhibition of alpha2-vasoconstriction. In the resting leg, our group has observed that alpha2-vasoconstriction was as powerful as alpha-1 and AngII vasoconstriction, but characterized by lack of decrease in femoral artery diameter (56). Thus, AngII and alpha-

2 vasoconstriction are both dominated by distal effects. In the exercising leg, alpha2-vasoconstriction is completely inhibited during knee-extensor exercise (56). In contrast, metabolic inhibition of AngII vasoconstriction in this same model was incomplete, underscoring the specificity by which the distal alpha-2 vasoconstriction is sensitive to exercise.

The mechanisms underlying exercise-induced attenuation of alpha2-mediated vasoconstriction in rodents seems related to skeletal muscle production of nitric oxide (NO) and vascular smooth muscle Katp-opening (21, 24, 42, 45). In contrast, exercise-induced attenuation of AngII vasoconstriction has not previously been studied *in vivo*. Recent evidence suggests points of counteraction between the NO and AngII signal transduction pathways within the peripheral vasculature (57). However, a functional significance at rest of such system interference was detectable only in heart failure and not in control rats (41), and it is unknown whether these mechanisms are important during exercise.

Likewise, the involvement of NO and Katp channels in attenuation of alpha-1 vasoconstiction is controversial. Thus, the involvement of NO and Katp channels in attenuation of alpha-1 vasoconstiction is controversial. Rodent studies provide evidence that alpha-1 responsiveness is not sensitive to NO production (24, 45). In contrast, one recent study has provided evidence that NO production is necessary for the metabolic inhibition of alpha-1 (PE) and not alpha-2 (clonidine) vasoconstriction in exercising hindlimb muscle of conscious dogs (9). Human studies have provided evidence that NO is involved in the normal expression of functional sympatholysis (14, 34). However,

exercise-induced attenuation of alpha-1 and alpha-2 vasoconstriction appear to be equally sensitive to combined cyclooxygenase and NO-inhibition during mild handgrip exercise (14). Thus, the mechanisms underlying metabolic inhibition of alpha-1 mediated vasoconstriction are unclear.

Methodological considerations

Dynamic knee-extensor exercise as a model for studying human vascular control has important advantages: *i*) the fraction of the blood flow through the femoral artery reaching the exercising quadriceps muscle is more than 95% even at mild to moderate intensities; *ii*) one-legged knee-extensor exercise (at least up to 30W) can be sustained for hours with steady state hyperemia, and without significant increases in blood pressure or sympathetic nervous system activity; *iii*) direct reproducible measurements of femoral artery diameter and blood flow by both ultrasound and thermodilution can be obtained in the exercising leg without pausing or otherwise interfering with the exercise. It should also be noted that in this exercising model the systemic hemodynamic variables are not reflective of the local intensity dependent increases in blood flow to the isolated working muscle. These features make it possible to test the effects of several different doses of drugs within the same exercise-period. By this approach we are able to determine dose-response relationships and evaluate saturation kinetics for continuous intra-arterial drug infusions.

This combination of advantages is unavailable in other current models of exercise. For example, in animal models, Doppler-determinations of blood flow assume an

unchanged artery diameter, and thereby may underestimate alpha-1 vasoconstriction. In addition, bolus infusions are used, decreasing reproducibility and making comparisons between drugs difficult due to differences in the time-courses of vasoconstrictor-effects. In human handgrip exercise, a relatively large fraction of brachial blood flow reaches non-active muscle or skin, and steady state can only be accomplished during very mild handgrip and only long enough to test 1 or 2 drugs or doses within the same exercise-bout. In addition, the ultrasound-measurements of blood flow in previous studies have not previously described the brachial artery constriction occurring with intra-brachial alpha-1 agonist infusion (18).

During knee-extensor exercise, a large fraction of cardiac output perfuses the quadriceps muscle. Thus, any increase in the vascular resistance within the exercising muscle may lead to a sizeable increase in blood pressure. Indeed, the higher doses of both PE and AngII, which caused significant decreases in blood flow to the exercising muscle, were accompanied by significant increases in blood pressure. It should be noted that the increases in blood pressure could also be attributed to a larger fraction of the drugs reaching the systemic circulation due to a shorter transit time in the exercising compared to the resting leg.

One potential concern with the present investigation is the lack of a control protocol utilizing a pharmacological vasodilator to match the exercise mediated hyperemia to demonstrate that the exercise induced inhibition of vasoconstriction is not merely a result of the higher blood flows achieved during exercise. However several investigations performed in a human forearm (13, 32, 48); and a human quadriceps

muscle (33), as well as in a rat model (43) have clearly indicated that when phenylephrine, clonidine, and tyramine are superimposed on adenosine, nitroprusside, or hydralazine induced hyperemia the result has always been a significantly large decrease in blood flow. These results therefore indicate that the diminished vasoconstrictor response observed during exercise is the result of increased metabolic activity and not due to the elevated blood flow. Additionally, due to the nature of the study it is important to identify that any attenuation of the vasoconstrictor response to the drugs is due to exercise induced increases in metabolic activity and not the result of a baroreflex mediated removal of tonic sympathetic activity. This concern was addresses by comparing percent changes in FVC in all conditions we have factored in any changes in MAP.

Functional significance of angiotensin during exercise.

There is limited knowledge about the significance of non-adrenergic vasoconstrictor hormones, such as AngII, during exercise. Recent studies have provided evidence that AngII production takes place not only in the liver and lung but also within the vascular wall of limb blood vessels (35). Accordingly, during demanding exercise augmented sympathetic traffic leads to increased renin release from the kidneys, and the circulating renin activates angiotensin converting enzyme and could cause increased AngII-production at several sites including muscle (51). Thus, during exercise AT-1 receptors within the skeletal muscle vascular bed could be stimulated by endocrine effects of increased circulating levels of AngII, as well as paracrine effects of locally produced AngII.

Studies in healthy and hypertensive humans (27, 55) and conscious miniature swine (40) have shown that administration of an AT-1 receptor antagonist significantly attenuates the increase in blood pressure associated with aerobically demanding exercise. The smaller increase in blood pressure seems to be related to a decrease in vascular resistance to "less metabolically active tissues" and a redistribution of cardiac output to these tissues during AT-1 receptor blockade. Taken together, the responsiveness to exogenous AngII in resting skeletal muscle and the modulation of exercise blood pressure by AT-1 blockade indicates that endogenous AngII may contribute to vasoconstriction of non-active tissue during exercise. The functional significance of endogenous AngII for blood flow to exercising muscle has not previously been studied. Our finding that the vasoconstrictor effects of exogenously administered AngII are significantly attenuated in the exercising thigh, suggest that metabolic inhibition of endogenous AngII-vasoconstriction can occur in exercising muscle. In an analogy to functional sympatholysis, this phenomenon could be termed "exercise angiotensinolysis".

Congestive heart failure is characterized by higher baseline and larger exercise-induced increases in AngII-levels compared to healthy controls (23). In a recent study, patients with congestive heart failure already treated with and angiotensin converting enzyme inhibitor were exercise tested before and after 6 months additional therapy with an AT-1 receptor blocker or placebo. The submaximal exercise capacity was improved by 26% in the active treatment arm, compared to only 7% in the placebo arm (4). Based on these previous findings and the current data, it is tempting to speculate that attenuated

"exercise angiotensinolysis" may be a potential novel mechanism by which skeletal muscle blood flow is limited during demanding exercise in heart failure patients.

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Figure Legends

Figure 1. Protocol outline for constant intra-arterial (femoral) infusions of 7 angiotensin-II doses (0.25-16 $ng \cdot kg^{-1} \cdot min^{-1}$) and 7 phenylephrine doses (0.0125-0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$) during rest; as well as 6 angiotensin-II doses (0.5-32 $ng \cdot kg^{-1} \cdot min^{-1}$) and 6 phenylephrine doses (0.05-1.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$) during 7 and 27W ipsilateral knee-extensor exercise. The time course is represented on the top axis in minutes. The order of the drugs and of the exercise-levels were random and balanced. There was a resting period of at least 45 minutes between exercise bouts. Measurements of femoral blood flows during baseline and each drug-dose are marked by arrows. Each dose was administered as a constant intra-arterial infusion for at least 2 minutes with measurements obtained during the last 45 seconds. Each shift of dose was a doubling of the previous dose. BL, baseline, PE, phenylephrine, AngII, angiotensin-II.

Figure 2. Femoral artery diameter and blood velocity. Dose-response relationships for intra-arterial angiotensin-II and phenylephrine during rest, 7W, and 27W exercise. AngII, angiotensin-II; PE, phenylephrine; FAD; femoral artery diameter; FBV, femoral blood velocity. *, $P < 0.05$ compared to baseline; § $P < 0.05$ between PE and AngII.

Figure 3. Femoral blood flow and conductance. Dose-response relationships for intra-arterial angiotensin-II and phenylephrine during rest, 7W, and 27W exercise. AngII, angiotensin-II; PE, phenylephrine; ΔFBF, changes in femoral blood flow (absolute in top panels and relative in middle panels); ΔFVC, changes in femoral vascular conductance

(relative in bottom panels). *, $P < 0.05$ compared to baseline; †, $P < 0.05$ between rest and 7W; ‡, $P < 0.05$ between rest and 27W; δ, $P < 0.05$ between rest and 7W.

Figure 4. Femoral blood flow, conductance and resistance. Flow-adjusted dose-response relationships for intra-arterial angiotensin-II and phenylephrine during rest, 7W, and 27W exercise. AngII, angiotensin-II; PE, phenylephrine; ΔFBF, relative changes in femoral blood flow; ΔFVC, relative changes in femoral vascular conductance; ΔFVR, relative changes in femoral vascular resistance. †, $P < 0.05$ between rest and 7W; ‡, $P < 0.05$ between rest and 27W.

Table 1: Values during baseline and maximal drug doses during rest, 7W, and 27W.

		Resting		7W		27W	
		BL	Drug	BL	Drug	BL	Drug
HR (beats•min^{-1})	PE	55±3	52±2	67±3	54±3*	77±3	65±3*
	AngII	55±3	55±2	65±4	59±4*	76±3	73±4*
MAP (mmHg)	PE	88±3	92±3*	89±4	104±6*	93±4	105±7*
	AngII	88±3	90±4	87±3	108±6*	90±3	111±6*
FBF_D (l•min^{-1})	PE	0.37±0.03	0.16±0.02*	1.27±0.05	0.91±0.07*	2.50±0.20	2.16±0.10*
	AngII	0.32±0.03	0.09±0.02*	1.25±0.07	0.90±0.07*	2.68±0.15	2.21±0.15*
FBF_{TD} (l•min^{-1})	PE	-	-	1.06±0.04	0.82±0.04*	2.90±0.14	2.46±0.11*
	AngII	-	-	1.17±0.11	0.81±0.04*	2.87±0.18	2.41±0.15*
FVC_D (ml•mmHg•min^{-1})	PE	4.2±0.3	1.7±0.2*	14.4±1.0	9.3±1.3*	28.5±3.5	21.1±2.1*
	AngII	3.6±0.4	1.0±0.2*	14.5±0.9	8.4±0.6*	30.6±2.7	20.8±2.0*
FVC_{TD} (ml•mmHg•min^{-1})	PE	-	-	13.6±1.0	8.2±0.5*	32.8±2.6	24.6±2.3*
	AngII	-	-	13.6±1.4	7.6±0.4*	32.5±2.7	22.5±2.1*

AngII, angiotensin II; PE, phenylephrine; HR, heart rate; MAP, mean arterial pressure; FBF_D, femoral blood flow by Doppler; FBF_{TD}, femoral blood flow by thermodilution; FVC_D, femoral vascular conductance by Doppler; FVC_{TD}, femoral vascular conductance by thermodilution; *, $P < 0.05$ compared to baseline.

Figure 1

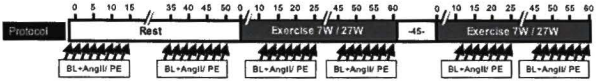

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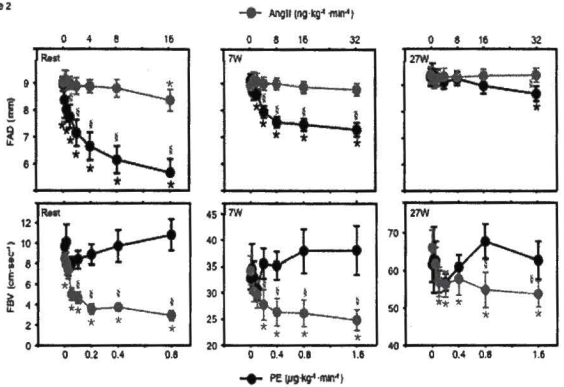
Figure 2



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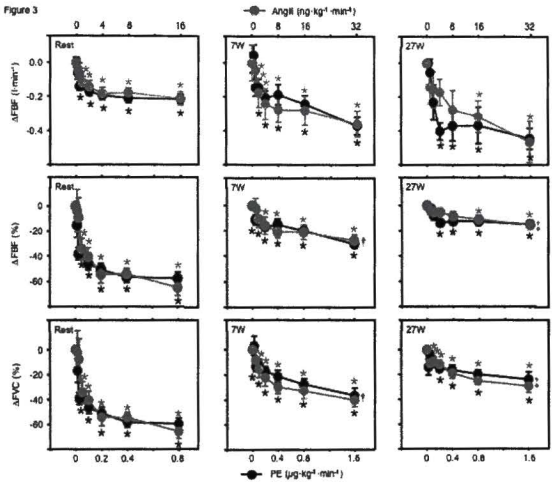

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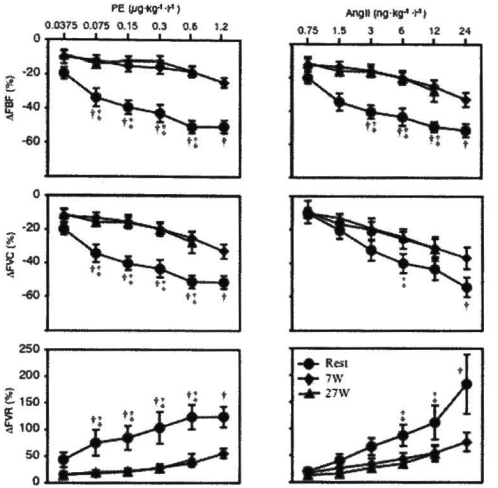
Figure 4



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CHAPTER IV

The role of Angiotensin II in the control of the peripheral vasculature during dynamic exercise.

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Abstract

The effect of increases in sympathetic activity on the control of the peripheral vasculature during exercise is well documented. Additionally this increased sympathetic activity results in increased production of the non-adrenergic vasoconstrictor angiotensinII. However knowledge of the functional role of AngII in the control of the peripheral vasculature is limited. We tested the hypothesis that oral blockade of the AT_1-receptor with Valsartan will identify a significant role of angiotensinII (AngII) in vascular control in a "non-active" muscular bed during exercise. Furthermore, we hypothesize that the effects of Valsartan will increase as exercise intensity increases. In 5 men, and 4 women, femoral blood flow (FBF) using Doppler Ultrasound and blood pressure using radial artery catheterization were measured in the exercising (EL) and non-exercising (NEl) during rest and three varying intensities (~40, 60 & 75% maximum workload, WL_{max}) of dynamic one-legged kicking exercise with and without Valsartan. Valsartan had no effect on FBF and FVC in the exercising leg (EL) during rest and 40, 60, and 75% WL_{max} exercise compared to the control condition. In the NEL Valsartan had no effect on and FBF and FVC during rest and 40% and 60% WL_{max} exercise; however during 75% WL_{max} exercise FBF and FVC were significantly elevated with Valsartan when compared to control (10±3% and 18±3% respectively). These findings indicate that AngII plays a significant role in the control of the peripheral vasculature in a "non-active" muscular bed during exercise, and the effect of AngII increases in an exercise intensity dependent manner.

Introduction

With progressive increases in the intensity and duration of dynamic exercise there is an incremental increase in sympathetic nervous system activity (14). In addition to its well documented role in distributing the cardiac output to "active" tissues and organs (2, 27), the exercise induced sympatho-excitation results in an increased stimulation of renal beta I adrenergic receptors leading to increased plasma renin activity and plasma concentrations of Angiotensin II (AngII) (4, 36, 40). Despite there being abundant evidence identifying the physiologic and pathophysiologic roles of AngII as a constrictor of blood vessels (18, 41, 45) the understanding of its role in the control of the peripheral vasculature during exercise is limited.

A number of investigations have reported an exercise intensity dependent increase in the concentration of circulating AngII with exponential increases occurring at exercise intensities equal to approximately 55% maximal oxygen uptake (VO_{2max}) (4, 36, 40). In the limited number of studies investigating the role of AngII using Angiotensin Type 1 (AT_1) receptor blockade during exercise a significant role of AngII activity in modifying the control of the peripheral vasculature was identified, by decreases in MAP (26, 44), systemic vascular resistance (SVR), and increases in blood flow and vascular conductance in "non-active" tissues and organs (no change was observed in "active" tissues and organs) (37, 39). In an exercising human model Warren et al. (44) demonstrated that selective blockade of the angiotensin type I (AT_1) receptor using Valsartan resulted in intensity dependent reductions in the MAP response to exercise.

This study however, did not investigate blood flow or vascular conductance in either the exercising or non-exercising vasculature.

The α_1-receptor and AT_1 receptor share very similar G-Protein coupling signaling mechanisms. When activated both of these receptor classes result in activation of phospholipase C subsequently resulting in downstream production of the intracellular messengers inositol triphosphate and diacylglycerol (13, 15, 38). Furthermore, several investigators have reported that activation of the α_1 and AT_1 receptor results in both the activation of myosin light chain kinase and the inhibition of myosin light chain phosphatase, two intracellular events that ultimately lead to smooth muscle contraction (13, 15, 38). It has been well documented that the effects of α_1-adrenoreceptor activation are reduced in metabolically active tissue while still being maintained in "non-active tissue" (5, 8, 11, 29, 46). This phenomenon, termed "functional sympatholysis" is the result of metabolic inhibition of post-junctional adrenergic signaling (28). In an attempt to further determine the role of AngII in the vasculature of a "metabolically active" muscle group it was recently reported that the vasoconstriction elicited by a wide dose range of intra-arterial AngII infusions was attenuated during exercise to a similar degree as that elicited by intra-arterial infusions of the α_1-adrenergic receptor agonist Phenylephrine (PE) (5).

With this information as a background the present investigation was designed to test the hypothesis that as exercise intensity increases there is a subsequent increase in circulating concentrations of the non-adrenergic hormone AngII. Furthermore, we hypothesize that

the vasoconstrictive actions of AngII will be attenuated in vasculature of metabolically "active" muscle while it will be maintained in the vasculature of "non-active" muscle. This was accomplished by measuring femoral blood flow (FBF) and femoral vascular conductance (FVC) in both the exercising leg (EL) and non-exercising leg (NEL) during rest and three different intensities of dynamic one-legged kicking exercise with and without oral administration of the AT_1- receptor antagonist Valsartan.

METHODS

Subjects and general information

Five men and four women (age, 26±1 years; height, 175±4 cm; weight, 73±6 kg; mean ± s.e.m.), volunteered to participate in the present investigation. All subjects were healthy, non-smokers, free of known cardiovascular and respiratory disease, and were not using prescription or over-the-counter medications. Prior to the study all subjects provided written informed consent, were familiarized with all the testing protocols, and were asked to abstain from drinking alcohol and caffeine and to not exercise for the 24 hour time period prior to any scheduled experiments. All experimental procedures were approved by the Institutional Review Board at the University of North Texas Health Science Center and were in accordance with the guidelines of the *Declaration of Helsinki*.

All experimental protocols were performed with the subject seated in a semi-recumbent position (~45 degrees) in a modified car seat which allowed for optimal exercise performance while enabling Doppler ultrasound measurements of blood velocity and vessel diameter of the desired femoral artery to be obtained. One-legged kicking exercise was performed on a modified cycle ergometer as previously described (1) in the right leg at a rate of 60 kicks per minute. A standard four-lead electrocardiogram was used for HR monitoring and blood pressure was assessed invasively from a radial artery catheter.

Experimental Protocols

Protocol 1: Effect of Valsartan on the peripheral vasculature during different intensities of exercise.

Experimental day 1. Using a modified Monark cycle ergometer (Ergomedic 874 E, Monark) (31) with resistance against knee extension, each subject performed a graded, dynamic one-legged knee extension maximal exercise test to determine their respective maximal workload intensity (WL_{max}). The subjects began exercising at a 0.1 kg workload for 2 minutes, after this warm-up period an additional 0.1 kg of weight was added in one minute increments until the subject was no longer able to perform the exercise at a rate of 55 - 60 kicks per minute (kpm) despite verbal encourgament. The subject's individual maximum power was used to calculate the individual weight (Kg) required to achieve intensities equal to 40, 60, and 75% of their workload maximum (WL_{max}).

Experimental day 2.

Morning: The protocol for experimental day 2 is outlined in Figure 1. Heart rate (HR), blood pressure (BP), femoral arterial diameter (FAD), and femoral blood velocity (FBV) were measured in all subjects during rest and three different exercise intensities of ~40 (low), ~60 (moderate), and ~75% (high) of each subjects respective WL_{max}. The resting condition was always performed first which was immediately followed by the low, moderate, and high exercise intensities (i.e. there was no rest period between changes in intensities). The HRs and BPs were continuously measured throughout the duration of

the protocol. During the rest condition measurements of FAD and FBV were performed in both the exercising (EL) and non-exercising leg (NEL) following a 5 minute seated rest steady-state period. During each of the exercise conditions FAD and FBV were assessed following a 5 minute exercising period to achieve steady state. Each rest and submaximal exercise workload lasted approximately 15 minutes (i.e. the entire duration of the control protocol approximated one hour). The FAD and FBV were assessed in both the EL and the NEL at minutes 5, 10, and 15 of rest and each submaximal exercise intensity. Additionally, at the completion of each rest and each submaximal intensity (i.e. minutes 15, 30, 45, and 60 of protocol) a 6ml blood sample was drawn from the radial catheter for analysis of osmolality, plasma catecholamines and plasma AngII.

Afternoon: The experimental protocol performed and measurements obtained in the afternoon were exactly the same as in the morning. The only difference between the two protocols was that the afternoon protocol was performed at least 2 hours following oral ingestion of the AT_1-receptor antagonist Valsartan. The 2 hour resting duration was chosen for two reasons; i) to allow for recovery from the exercise performed in the morning; and ii) it has been demonstrated that peak Valsartan activity is achieved approximately 2 hours post-ingestion (16, 25).

Protocol 2. Validation of Valsartan mediated AT_1-receptor blockade.

The validity of these results requires that the dose of Valsartan was capable of significantly inhibiting the pressor effects of AT_1-receptor activation throughout the

duration of the afternoon protocol. Therefore in the morning following catheterization (prior to any data collection) a bolus dose of 1.5 μg of AngII was injected intra-venously (25). This exact same bolus dose of AngII was injected at 2 hours post Valsartan ingestion (immediately prior to the afternoon protocol) as well as immediately following completion of the study (Figure 1). The dose of AngII was chosen because it has been demonstrated to evoke an increase in mean arterial blood pressure of approximately 15 - 20mmHg (25). During all AngII injections arterial blood pressure was closely monitored to determine that the Valsartan dose sufficiently antagonized the pressor response to AngII. Due to drug availability from Clinalfa the challenge protocol was only performed in 5 subjects.

Catheterization

A 4.45 cm, 20 gauge catheter (Arrow) was inserted into the radial artery using sterile techniques under local anesthesia with ~2 ml of lidocaine and aseptic conditions, and was connected to a pressure transducer (Maxxim Medical, Athens, TX, USA) positioned at the level of the heart. The arterial catheter allowed for assessment of beat-to-beat arterial blood pressure (ABP). Additionally a 1.2 mm, 18 gauge venous catheter was inserted into the median antecubital vein and was used for bolus drug injections during the AngII challenge protocols.

Femoral Blood Flow

Measurements of femoral arterial diameter (FAD), and femoral blood velocity (FBV) and subsequent FBF measurements, were obtained using a doppler ultrasound machine (Phillips, model: HDI 5000 Sono CT; Bothell, WA, USA). Ultrasound imaging of femoral artery diameter was measured using a linear array transducer operating at an imaging frequency of 7 - 8 MHz at a site approximately 2 cm distal to the inguinal ligament where the best spatial resolution was achieved. The average of 2 measurements of maximal (systolic) FAD was determined at rest and during one-legged knee extension exercise in the EL and NEL at minutes 5, 10 and 15 of the resting and each exercise trial. Two 20sec segments of FBV profiles were obtained in each the EL and NEL at minutes 5, 10 and 15 of the resting and each exercise trial using the same linear array transducer with a Doppler frequency of 5 MHz and a sample depth of 3.5 – 5 mm. All FBV measurements were taken with the transducer held at an insonation angle as close to 30 - 40° to the femoral artery as possible while optimizing the velocity spectra waveforms and the sample volume centered. The ultrasound and doppler data of FAD and angle-corrected, time-and space-averaged, and intensity-weighted mean FBVs were calculated using commercially available software (Phillips, model: HDI 5000 Sono CT; Bothell, WA, USA). The ultrasound recordings and 20sec segments of blood velocity spectra were saved to the HDI 5000 hard drive for offline analysis.

The reproducibility and validity (when compared to thermodilution) of Doppler ultrasound measurements of FBF during one-legged kicking exercise has been previously

described (27, 46) during exercise intensities up to 70W. The average FBF measurements obtained from all exercise intensities in the current study were in agreement with previously described values.

Drugs

In the afternoon of day 2 each subject ingested an oral dose of the AT_1-receptor antagonist Valsartan (Diovan, Novartis, Basel, Switzerland). The dose of Valsartan ingested was 80mgs. AngII (Clinalfa, Switzerland) was used as a selective AT_1-receptor agonist in the challenge protocols to ensure the efficacy of Valsartan. This same dose of Valsartan has been previously reported to exhibit optimal efficacy 2 hours post-ingestion with significant blockade occurring for up to 24 hours post ingestion (25). Although clinically angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are used in the inhibition of the adverse effects of circulating AngII we decided to use the specific AT_1 receptor antagonist for several reasons: i) ACE inhibitors have been reported to prevent the degradation of the vasodilators bradykinin and substance P (20, 34), and therefore could induce some hemodynamic alterations independent of inhibition of the RAS system; ii) all of the known side effects of AngII receptor activation on the cardiovascular system occur secondarily to AT_1 receptor activation (41, 45); and iii) while other AT_1 receptor antagonists have been reported to have minimal agonist properties, Valsartan only acts as a specific AT_1 receptor antagonist (35). AngII was diluted with physiological saline to yield a bolus dose equal to 1.5 μg.

Blood Sampling

Plasma samples were drawn from the arterial catheter immediately upon completion of each phase of the protocol (i.e. at the completion of rest, and at the completion of 40% WL_{max} intensity exercise, 60% WL_{max} intensity, and 75% WL_{max} intensity exercise for a total of 8 samples).

Plasma Catecholamines: Approximately 2.0 ml of plasma sample was centrifuged and stored at -70°C. Samples were then thawed and plasma concentrations of norepinephrine and epinephrine were separated and analyzed by high-performance liquid chromatography.

Plasma Osmolality: Approximately 1.0 ml of plasma sample was immediately transferred to a heparin treated tube and was centrifuged (4°C) for 10 minutes. The plasma osmolality was then immediately measured by freezing point depression (Advanced Instruments, model 3DII).

Plasma AngII: Approximately 3.0 ml of plasma sample was collected into a pre-chilled EDTA venipuncture tube, which was immediately treated with 100µl of Bestatin solution (ALPCO Diagnostics, Salem, NH, USA) and stored in a 2°C freezer. Samples were then thawed and centrifuged for one minute at 10,000 X g. AngII concentration was then measured by radioimmunoassay as outlined by ALPCO Diagnostics and previously described (21, 22).

Data analysis and statistics

Mean arterial pressure (MAP) was derived from the integration of the arterial pressure waveform. Femoral blood flow (FBF) in the EL and NEL is represented as the average of measurements obtained at min 5, min 10, and min15 of each phase of the protocol and was calculated by the formula: FBF (ml/min) = $FBV_{mean} \cdot \pi \cdot (FAD/2)^2$. Femoral vascular conductance (FVC) is also represented as the average taken at the above mentioned time points and was calculated by the formula: FVC = FBF/MAP. A student's paired T-Test allowed for comparisons of the pressor response to AngII pre-and post-Valsartan ingestion. Two-way analysis of variance and repeated measures (two-way RM-ANOVA) were used for comparisons of physiological variables between rest and the three exercise intensities and with and without Valsartan, as well as for comparisons of the effect of Valsartan between the EL and NEL and the three exercise intensities. In the absence of a significant interaction between drug and exercise intensity and limb and exercise intensity a *Post-hoc* analysis was performed by Tukey's procedure for further analysis when a significant main effect was determined. All data is expressed as means ± SE. Statistical significance was set at $p<0.05$.

RESULTS

Cardiovascular and Hemodynamic Responses to Seated Rest and Three Intensities of Dynamic One-Legged Kicking Exercise

The mean values for cardiovascular and hemodynamic variables obtained at rest and during the three intensities of exercise with and without Valsartan are presented in Table 1. Compared to rest HR was increased during all three exercise intensities. HR was similar with and without Valsartan during rest and three exercise intensities when compared to the control condition. MAP increased significantly above rest during all three intensities of exercise. Although MAP was reduced in all conditions following Valsartan treatment the difference was only statistically significant during 75% WL_{max} intensity exercise. Although Plasma AngII concentration was elevated during all three WL_{max} exercise intensities both with and without Valsartan when compared to resting measurements, the effect of exercise intensity only reached significance during the highest WL_{max} exercise intensity both with and without Valsartan. Additionally AT_1-receptor blockade with Valsartan significantly increased plasma concentration of AngII when compared to control values during rest and all three submaximal exercise conditions. Moderate and high intensity exercise resulted in sympathetic activation as indicated by significant increases in plasma norepinephrine (NE) during moderate and high intensity exercise. Valsartan had no effect on plasma NE.

Effectiveness of Valsartan mediated AT_1-receptor blockade.

The pressor responses to a 1.5 μg bolus intra-venous infusion of AngII are illustrated in Figure 2. During control rest AngII yielded an increase in MAP of 15±0.8 mmHg. Two hours post Valsartan ingestion (i.e. immediately prior to the start of the afternoon experimental protocol) the same dose of AngII yielded an increase in MAP of 3.6±0.4 mmHg (76±4% blockade). The same dose of AngII administered at the completion of the afternoon experimental protocol yielded an increase in MAP of 3.7±0.4 mmHg (77±3% blockade). These data identify the presence of significant blockade of the AT_1-receptor throughout the experimental protocol.

Effect of Valsartan on Femoral Blood Flow and Femoral Vascular Conductance during Seated Rest and three Intensities of Dynamic One-Legged Kicking Exercise

AT_1-receptor blockade with Valsartan had no significant effect on FBF and FVC in the EL during rest and the three exercise intensities (Figure 3). However, in the NEL there was an exercise intensity dependent effect of Valsartan. For example, during rest FBF in the NEL was unchanged with Valsartan when compared to control rest, whereas during the high intensity workload FBF increased by 10±3% (Figure 4A and C). A similar pattern was observed for FVC in the NEL such that during rest FVC was unchanged with Valsartan when compared to control rest, whereas during high intensity exercise FVC increased by 18±3% (Figure 4B and D).

Discussion

In the present study, we found that the AT_1 blockade with Valsartan had no significant effect on hemodynamic variables during low and moderate intensity exercise. As exercise intensity increased, however, Valsartan resulted in significant decreases in MAP and increases in FBF and FVC in the vasculature of the non-exercising leg (NEL). These findings suggest that in humans AngII plays a significant role in the pressor response and redistribution of cardiac output that is associated with exercise. While other studies have examined the effects of AT_1 blockade on the pressor response during exercise in humans, this is, to our knowledge, the first study that has examined the effects of AT_1 blockade on blood flow in an exercising and a non-exercising limb.

Exercise and Angiotensin II Production

While the functional knowledge of the role of AngII in the cardiovascular responses to exercise in humans is limited there is convincing evidence of its intensity related production during exercise (4, 36, 40). It has been reported that dynamic exercise at intensities as low as 30% WL_{max} results in significant activation of the renin-angiotensin system (36, 40) with significant exponential increases in circulating concentrations of AngII being observed at intensities equal to approximately 55% VO_{2max} (36, 40). Furthermore, recent investigations have provided evidence for a local renin-angiotensin system within the vessel wall of smooth muscle which is capable of producing AngII in addition to global AngII production via the liver and lungs (33). Therefore it is plausible that during exercise mediated increases in sympathetic outflow there could be local

production of AngII in several tissues and organs including skeletal muscle as well as a global production of AngII (43).

Hemodynamic response to AT_1 blockade during exercise

Previous investigations have reported a decreased pressor response during exercise following AT_1 blockade in healthy individuals cycling for 10 minutes at 60% and 80% of their respective heart rate reserve (HRR) (44), in miniswine exercising for 20 minutes at 80% of their respective HRR (37), and in healthy rats running on a treadmill for 5 minutes at approximately 50% maximal oxygen uptake (VO_{2max}) (39). Although the study by Warren et al. (44) was not designed to assess regional blood flow, Stebbins et al. (37) and Symons et al. (39) did report a decrease in systemic vascular resistance and an increase in blood flow to "non-active" organs and tissue beds during exercise following AT_1 blockade. The finding in the present investigation of an intensity related decrease in MAP and increase in FBF and FVC in the NEL following AT_1 blockade is in agreement with the findings in these animal studies. Taken together the results of the above mentioned AT_1 blockade studies during exercise strongly indicate that AngII plays a significant role in the modulation of MAP and redistribution of Q to "more metabolically active tissues" that occurs during exercise. Furthermore, it appears that the hemodynamic effects of AngII become more pronounced as the intensity of exercise increases.

It is interesting to note that while AT_1 blockade results in an attenuation of the pressor response to exercise and a redistribution of Q to "non-active" tissue there appears to be

no effect on blood flow to the "metabolically active" tissues as observed in the present study and reported in previous studies (37, 39). In the current study it is possible that the exercise induced demands placed on the cardiovascular system even during the highest intensity of exercise were not "severe" enough to identify the full magnitude of AngII mediated hemodynamic alterations. In this regard it is interesting to note that in the study by Warren et al. (44) where subjects performed upright cycling for 10 minutes at 80% of their HRR, AT_1 blockade with Valsartan significantly reduced MAP, however, it had no effect on Q. While they did not report regional blood flow or vascular conductance in exercising limbs, it has been reported that during near-maximal and maximal exercise approximately 80% - 90% of ones Q is perfusing "active" skeletal muscle (2, 30, 32), thus these vascular beds become the major site of arterial blood pressure regulation during high intensity exercise (2, 30, 32). Therefore it is plausible to speculate that Valsartan mediated decreases in MAP might have altered vascular conductance in these "active" limbs. Likewise the results of Symons et al. (39) identify a non-significant increase in blood flow and vascular conductance in the skeletal muscle vasculature of rats running on a treadmill for 5 minutes at approximately 50% of their VO_{2max}. Similar to the present study, where we observed no effect on "active skeletal" muscle blood flow or conductance, it is possible that they would have observed a greater effect of AT_1 blockade in the active muscle if the intensity and thus production of AngII was increased.

The previous investigations (4, 36, 40) which measured plasma AngII during exercise in humans differ somewhat from the current investigation in that whole body dynamic cycling exercise was performed where as in the current investigation subjects were

performing dynamic one-legged knee extension exercise. While we recognize that near maximal one-legged knee extension exercise does not stress the cardiovascular system to the same degree as near maximal whole body exercise, we still observed significant increases in plasma NE and AngII (Table 1) as well Valsartan mediated alterations in hemodynamic variables (Table 1, Figure 4) therefore, indicatig that there was significant activation of the sympathetic nervous system and subsequent production of AngII.

The lack of effect of AT_1 blockade in the exercising limb could also be a result of an attenuated vasoconstrictor response to AngII via metabolites produced within the exercising limb. It has been extensively documented that the vasoconstrictor effects of alpha-adrenergic activation in humans are significantly attenuated in metabolically active tissues (5, 11, 29, 46). Additionally, previous investigations have reported that there is an exercise intensity dependent attenuation of the vasoconstrictor response to intra-arterial infusions of Neuropeotide Y (NPY) Y_1 and P2X receptor agonists (6, 7) as well as to the non-adrenergic receptor vasconstrictors AngII, and vasopressin (9). More recently it has been reported that the vasoconstrictor responses to intra-arterially infused AngII and phenylephrine (PE) are attenuated to a similar degree during exercise when compared to rest (5). In this regard the AT_1 receptor and the alpha-1 adrenergic receptor activity share a very similar Gq-protein coupling pathway which upon stimulation results in activation of myosin light chain kinase and the inhibition of myosin light chain phosphatase and thus subsequent smooth muscle contraction (13, 15, 38).

Therefore in the present study it is possible that there was significant AT_1 receptor activation within the exercising limb, however much like alpha-receptor activation, the intracellular signaling mechanisms resulting in AngII mediated vasoconstriction were attenuated by the metabolic by-products of skeletal muscle contraction.

Experimantal Limitations

The use of an oral drug that exerts systemic actions as opposed to local actions is a potential limitation of the present study. In fact, it has been reported that circulating AngII is able to access the central nervous system through the area postrema and other circumventricular organs which lack a blood brain barrier (12). In this regard it has been demonstrated that centrally acting AngII can result in a depressed bradycardic response to increases in blood pressure (12, 24), decreases in parasympathetic outflow (12, 24, 42), as well as increased central sympathetic nervous system activity (12, 24). Therefore it is possible that the hemodynamic changes observed in the present investigation following AT_1 receptor blockade could be the result of attenuated actions of AngII on central autonomic activity. However, previous investigations have examined the effects of AT_1 receptor blockade on various hemodynamic variables during rest and during upright cycling exercise (17, 44) and also observed that the augmented MAP response to exercise was attenuated following AT_1 receptor blockade. More importantly there were no changes in indices of autonomic function (44), suggesting that that the AT_1 receptor blockade mediated reductions in MAP were due to peripheral vasodilation and not to changes in central autonomic activity. In the present study Valsartan resulted in

significant increases in FBF and FVC in the NEL during the highest intensity of exercise but had no effect on HR and plasma NE concentration. Therefore it is unlikely that the increases in FBF and FVC with Valsartan were due to changes in central autonomic activity.

Another concern with systemic acting vasodilators is the fact that they often lead to reductions in MAP. Therefore it was possible that the observed increases in FBF in the NEL and that were observed with Valsartan resulted from the decreases in MAP. While we agree that this would affect FBF, we corrected for the changes in MAP by calculating FVC and identified that the increases in FBF were due to vasodilation resulting from Valsartan blockade of the AT_1-receptors. Furthermore, if anything, arterial baroreflex reflex activation of the sympathetic nervous system in response to the decreases in MAP would act to further limit the increases in FBF and FVC.

Perspectives

While the present study evaluated only young healthy subjects, knowledge of the functional role of AT_1-receptor activation in this population may be relevant to clinicians through providing a more comprehensive understanding of the effects of AngII on peripheral vascular control during exercise. Specifically a variety of cardiovascular pathologies such as hypertension and heart failure are associated with increased resting and exercise-induced sympathetic activity and subsequent AngII-production, both of

which have been implicated as major contributors to the "viscious cycle" and rapid decline in cardiovascular hemodynamic function in these disease models (3, 10, 19, 23, 26). In a recent study in patients with congestive heart failure, 6 months of local intra-arterial ACE inhibitor therapy increased exercise capacity by approximately 25% in the treated limb, whereas in the untreated limb exercise capacity was only increased by approximately 10% (3) further indicating the involvement of this hormone during exercise as well as pathological disease states.

Conclusion

Through comparison of the FBF and FVC responses during rest and three different intensities of dynamic one-legged kicking exercise with and without Valsartan, the present study has identified in humans a significant role of AT_1-receptor mediated vasoconstriction in a"non-metabolically active" muscular bed during exercise at moderately high intensities.

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Figure Legends

Figure 1. Protocol outline for the control (no drug) and experimental (Valsartan) conditions.

Each condition was separated into four different trials; rest, 40%, 60%, and 75% (of each subject's respective workload maximum) one-legged knee extension exercise. The time course of each trial is identified on the top axis in minutes. The control condition was always first and was performed in the morning, this was followed by a 2 hour rest period at the beginning of which each subject ingested an 80mg dose of Valsartan. At the end of the two hour break the experimental condition was performed. Measurements of FBV and FAD were made at minutes 5, 10, and 15 of each trial and are indicated by the small arrows. A pressor dose of AngII (1.5μg) was infused intra-venously at time 0 in the morning, immediately prior to the afternoon experimental protocol and then again immediately upon completion of the afternoon protocol to determine the effectiveness of AT_1-blockade with Valsartan.

Figure 2. Effectiveness of AT_1-blockade with Valsartan.

Prior to beginning the control protocol an intra-venous bolus infusion of AngII (1.5μg) increased MAP by 15.1±0.8 mmHg. This same dose of AngII when given 2 hours post Valsartan treatment (before the start of the experimental protocol) and immediately upon completion of the experimental protocol increased MAP by 3.6±0.4 (i.e. 76±4%

blockade) and 3.7±0.4 (i.e.77±3% blockade), respectively (*, $P<0.05$ when compared to control AngII infusion for both time points).

Figure 3. Femoral blood flow and conductance in the exercising leg.

AT_1-blockade with Valsartan had no significant effect on FBF and FVC in the exercising leg during rest and all three exercise trials.

Figure 4. Femoral blood flow and conductance in the non-exercising leg.

AT_1-blockade with Valsartan resulted in significant increases in absolute FBF (panel A) and FVC (panel B) in the non-exercising leg during the highest intensity of exercise (75% WL_{max}). The effectiveness of Valsartan to increase relative FBF (panel C) and FVC (panel D) increased in an exercising intensity dependent manner. *, $P < 0.05$ compared to respective control baseline condition; †, $P < 0.05$ compared to resting condition; ‡, $P < 0.05$ compared to the moderate intensity exercise condition.

Table 1. Hemodynamic values during rest low (40%), moderate (60%), and high (75%) exercise intensities with and without Valsartan.

Values for HR (heart rate), MAP (mean arterial pressure), circulating plasma norepinephrine, epinephrine, angiotensin II and osmolality during control (no drug) and experimental (Valsartan) rest and three intensities of one-legged dynamic knee extension exercise. *, $P < 0.05$ compared to control (within the same exercise intensity); ¥, $P < 0.05$ compared to rest within respective exercise intensity.

Table 1: Hemodynamic values during rest low (40%), moderate (60%), and high (75%) exercise intensities with and without Valsartan.

	Rest		Low		Moderate		High	
	BL	**Drug**	**BL**	**Drug**	**BL**	**Drug**	**BL**	**Drug**
HR (beats · min^{-1})	64±3	69±3	82±3¥	89±5¥	96±3¥	98±4¥	115±2¥	119±3¥
MAP (mmHg)	90±2	85±5	100±4¥	97±4¥	100±5¥	97±4¥	110±6¥	103±6*¥
Norepinephrine (pmol/ml Plasma)	1.9±0.1	2.2±0.6	2.1±0.2	2.8±0.3	3.6±0.3¥	3.3±0.3¥	4.8±0.3¥	4.9±0.3¥
Epinephrine (pmol/ml Plasma)	0.4±0.07	0.4±0.05	0.4±0.06	0.4±0.04	0.4±0.07	0.5±0.07	0.5±0.07	0.6±0.05
Osmolality (mOsm)	287±3.0	287±1.6	285±0.5	288±1.7	287±1.2	288±1.1	289±1.5	288±1.4
Angiotensin II (pg/ml Plasma)	9.7±2.0	22.0±6.1	10.2±1.0	23.9±8.0	10.8±1.7	24.1±7.9	17.5±1.4¥	35.4±11.3*¥

Values for HR (heart rate), MAP (mean arterial pressure), circulating plasma norepinephrine, epinephrine, and osmolality during control (no drug) and experimental (Valsartan) rest and three intensities of one-legged dynamic knee extension exercise. *, $P < 0.05$ compared to no drug (within the same exercise intensity); ¥, $P < 0.05$ compared to rest within respective drug condition.

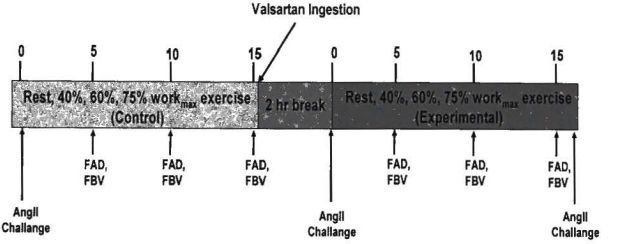
Valsartan Ingestion
0
5
10
15
0
5
10
15
Rest, 40%, 60%, 75% work$_{max}$ exercise (Control)
2 hr break
Rest, 40%, 60%, 75% work$_{max}$ exercise (Experimental)
FAD, FBV
FAD, FBV
FAD, FBV
FAD, FBV
FAD, FBV
FAD, FBV
FAD, FBV
AngII Challange
AngII Challange
AngII Challange

Figure 1

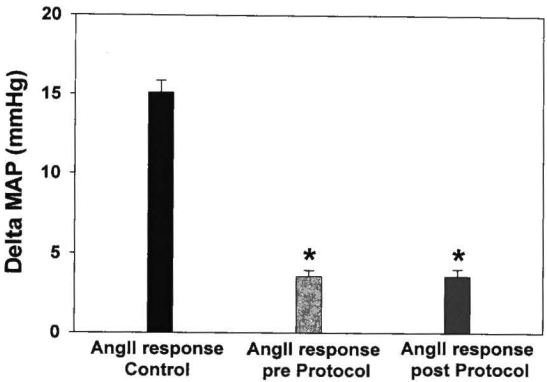
Delta MAP (mmHg)
20
15
10
5
0
*
*
AngII response Control
AngII response pre Protocol
AngII response post Protocol

Figure 2

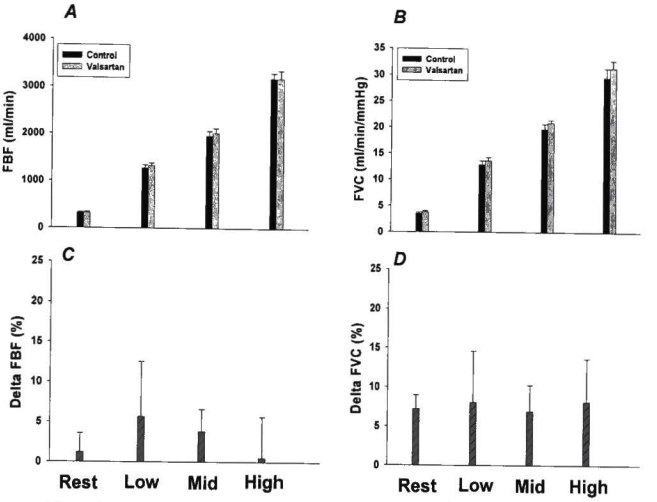
A
B
C
D
Control
Valsartan
FBF (ml/min)
FVC (ml/min/mmHg)
Delta FBF (%)
Delta FVC (%)
Rest
Low
Mid
High

Figure 3

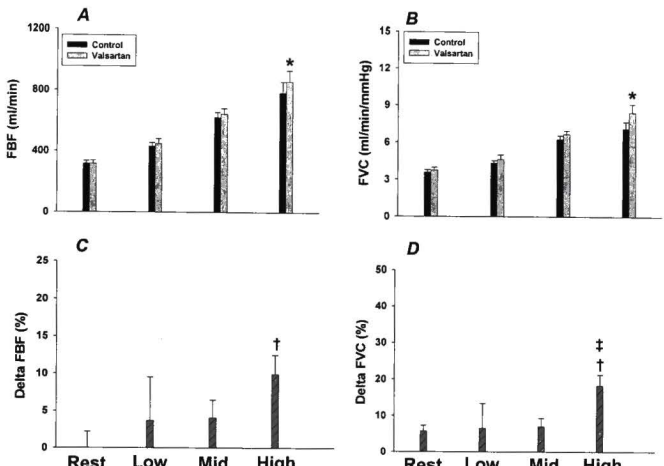
A
Control
Valsartan
FBF (ml/min)
1200
800
400
0
*
B
Control
Valsartan
FVC (ml/min/mmHg)
15
12
9
6
3
0
*
C
Delta FBF (%)
25
20
15
10
5
0
†
Rest
Low
Mid
High
D
Delta FVC (%)
50
40
30
20
10
0
‡
†
Rest
Low
Mid
High

Figure 4

CHAPTER V

CONCLUSIONS

The findings from the three investigations described in this dissertation demonstrate that there is a greater maintenance of alpha-1-adrenoreceptor mediated vasoconstriction during moderately heavy exercise in humans, suggesting that probing the effects of this receptor using intra-arterial infusion of agonists overestimates both the degree and onset of exercise mediated sympatholysis. Additionally they demonstrate that there is an exercise mediated increase in the circulating concentrations of the non-adrenergic vasoconstrictor angiotensin II and that this hormone plays an important role in the pressor response and redistribution of cardiac output that is associated with exercise.

In the first investigation, we demonstrated that pharmacological blockade of the alpha-1 receptor with Prazosin results in significant decreases in MAP both during exercise and during low, moderate, and heavy exercise intensities in humans. Additionally we demonstrated that alpha-1-receptor blockade results in significant activation of the sympathetic nervous system as identified by increases in HR and in circulating concentrations of plasma norepinephrine. Furthermore, we demonstrated that the effects of alpha-1-blockade in the exercising limb decreases in an exercise intensity dependent manner. This finding was expected and further confirms that the mechanisms of "functional sympatholysis" are operating and become more prominent as metabolic

femoral blood flow and femoral vascular conductance in the non exercising leg, however, unlike in the exercising leg the effects of Prazosin increased with increasing intensities of exercise. Again this finding was expected and confirms that the mechanisms leading to "functional sympatholysis" are isolated to metabolically active tissues and muscular beds.

The second investigation examined the effects of exercise on the metabolic attenuation of vasoconstriction elicited by activation of the non-adrenergic AT_1-receptor. In this investigation we demonstrated that the vasoconstrictive effects of a wide-dose range on intra-arterial angiotensin II infusion were attenuated during exercise to a similar degree as the vasoconstrictive effects of intra-arterial alpha-1-receptor activation via a wide-dose range of phenylephrine infusions. These findings suggest that the mechanisms resulting in exercise mediated "functional sympatholysis" are not limited to the adrenergic receptors but also effect non-adrenergic receptors as well.

The third investigation examined role of AT_1-receptor via pharmacological blockade during rest and three different intensities of one-legged kicking exercise. In this investigation we demonstrated that Ang II plays a significant role in the pressor response that is associated with exercise. Furthermore we identified that this effect is intensity related such that MAP was only decreased during "moderately heavy" exercise. More importantly we identified that Valsartan had no effect on FBF or FVC in the exercising leg but significantly increased FBF and FVC in the non-exercising leg. Again this effect of Valsartan in the non-exercising leg was intensity dependent such that it was only observed during "moderately high" intensity exercise. These findings are in agreement with the results observed in the second investigation of this investigation. Specifically,

because there were no observed effects in the exercising leg they further demonstrate that the vasoconstrictive properties of AT_1-receptor activation are attenuated by the metabolic byproducts of skeletal muscle contraction. Additionally the findings provide further support for a major role of angiotensin II in the control of blood pressure and the redistribution of cardiac output that occurs during exercise.

CHAPTER VI

SUGGESTIONS FOR FUTURE RESEARCH

Many important questions have remain unanswered regarding the control of the peripheral vasculature, especially during dynamic exercise. Listed below are a few suggestions for future research which would provide some beneficial insight into the different mechanisms involved in the control of the peripheral vasculature during exercise, especially as they pertain to adrenergic and non-adrenergic vasoconstrictors.

I. To expand our understanding of the non-adrenergic control of the peripheral vasculature, an experimental model that incorporates higher intensities of exercise as well as involves a larger percentage of ones muscle mass would be beneficial. This would become of particular interest during higher intensities of exercise when presumably alpha-receptor mediated vasoconstriction is close to or completely absent. Therefore, when you consider that during this magnitude of exercise approximately 85% - 90% of ones cardiac output is directed towards active skeletal muscle, the lack of a vasomodulatory control of the vasculature in exercising muscle may have significant consequences on blood pressure regulation. While the one-legged

kicking exercise technique is a sound exercise paradigm in these types of studies it is likely that a different mode of exercise, such as upright cycling might be required to reach the necessary intensity of exercise to fully accomplish these goals.

II. Another line of future research that would provide some beneficial insight into the control of the peripheral vasculature during exercise and the role of non-adrenergic receptors would be to utilize the well established neck suction technique. It is commonly accepted that unloading of the carotid baroreceptors using neck suction results in withdrawal of sympathetic activity. Therefore, if the activity of the AT_1-receptors is functionally blocked with Valsartan during high intensity exercise (when the role of the alpha-receptors is already significantly attenuated) and if sympathetically mediated vasoconstriction is, functionally removed with NS stimuli, it would be possible to further identify the role of the AT_1-receptor in the control of the peripheral vasculature during exercise. Again a mode of exercise that allows for near-maximal intensities to be achieved would be preferable to investigate this proposed project as the effect of angiotensin II appears to be exercise intensity related.

III. Lastly it would be very beneficial to investigate the role alpha-receptor and AT_1-receptor activation during exercise in normal physiological aging as well as in a variety of cardiovascular conditions, including hypertension, diabetes, and heart failure. All of these above mentioned conditions are associated with increased sympathoexcitation and thus subsequent increases in circulating concentrations of vasoactive hormones including angiotensin II, conditions that contribute to the exercise intolerance, increased incidence of cardiovascular morbidity, and "viscous cycle of heart failure" that is commonly present in these patients. Furthermore, exercise is commonly used as a preventative tool as well as a treatment for many patients suffering from cardiovascular pathologies. Additionally many of these patients are on various medications including alpha-receptor blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), however, their effect on the control of the peripheral vasculature during exercise in incompletely understood.