Adrenergic Control of Vascular Resistance Varies in Muscles Composed of Different Fiber Types: Influence of the Vascular Endothelium

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Abstract

The influence of the sympathetic nervous system (SNS) upon vascular resistance is more profound in muscles comprised predominately of low-oxidative type IIB versus high oxidative type I fiber types. However, within muscles containing high-oxidative type IIA and IIX fibers, the role of the SNS on vasomotor tone is not well established. The purpose of this study was to examine the influence of sympathetic neural vasoconstrictor tone in muscles composed of different fiber types. In adult male rats, blood flow to the red and white portions of the gastrocnemius (GastRed and GastWhite, respectively) and the soleus muscle was measured pre- and post-denervation. Resistance arterioles from these muscles were removed and dose responses to alpha1 (\(\alpha_1\))(phenylephrine) or alpha2 (\(\alpha_2\))(clonidine) adrenoreceptor agonists were determined with and without the vascular endothelium. Denervation resulted in a 2.7 fold increase in blood flow to the soleus and GastRed and an 8.7 fold increase in flow to the GastWhite. In isolated arterioles \(\alpha_2\)-mediated vasoconstriction was greatest in GastWhite (~50%), and less in GastRed (~31%) and soleus (~17%), differences among arterioles were abolished with the removal of the endothelium. There was greater sensitivity to \(\alpha_1\)-mediated vasoconstriction in the GastWhite and GastRed versus the soleus, which was independent of whether the endothelium was present. These data indicate that 1) control of vascular resistance by the SNS in high-oxidative fast-twitch muscle is intermediate to that of low-oxidative fast-twitch and high-oxidative slow-twitch muscles, and 2) the ability of the SNS to control blood flow to low-oxidative type IIB muscle appears to be mediated through post-synaptic \(\alpha_1\)- and \(\alpha_2\)-adrenoreceptors on the vascular smooth muscle.

Key Words: adrenergic receptors, clonidine, phenylephrine, blood flow, muscle fiber type
Introduction

The ability to match blood flow to oxygen uptake (VO₂) in skeletal muscle varies according to the predominant myosin heavy chain isoform and fiber phenotype present (i.e., type I; type IIA, B, and D/X) (6, 7, 23). Differences in VO₂ are likely due to a combination of tissue oxidative enzymatic activity and the fiber-type dependent regulation of mitochondrial O₂ consumption (8, 34). However, the regulation of vascular resistance, and thus O₂ delivery, in muscles composed of different fiber types is less clear.

Within skeletal muscle there are a myriad of factors which influence vascular resistance at rest and during exercise, including the local muscle fiber metabolism and the consequent release of vasoactive metabolites, circulating vasoactive substances, local myogenic tone, and sympathetic postganglionic neural activity (21, 27, 35). The sympathetic nervous system (SNS) appears to have a fiber-type dependent influence on vasoreactivity in skeletal muscle (23), since there is a greater vasoconstriction in muscle comprised of low-oxidative type IIB fibers during sympathetic neuron stimulation (16, 19). Similarly, Gray (17) demonstrated that resistance vessels from muscle with low-oxidative type IIB fibers were more responsive to topically applied norepinephrine than those from highly-oxidative type I muscle. In vivo, pharmacological blockade of alpha-receptors increases blood flow in muscle composed predominantly of type IIB fibers whereas flow remains unchanged in muscle comprised primarily of type I fibers (24).

Although these studies demonstrate a differential autonomic control of resistance vessel tone in muscles composed of low-oxidative fast-twitch and high-oxidative slow-
twitch fibers, vasomotor control of resistance vessels (i.e., arterioles) from highly-oxidative fast-twitch muscle is relatively unknown. Understanding vascular control mechanisms in this muscle type is important because type IIA and IIX fibers account for approximately 23% of skeletal muscle mass (10) and this fiber type receives the highest blood flow during exercise of the locomotory muscles (e.g., red portion of the gastrocnemius muscle; (Gast_{Red})) (3, 5, 33). Given the heterogeneous composition of the majority of skeletal muscle it is difficult to discriminate vascular adrenergic regulation in muscles of specific fiber type. Therefore, the purpose of this study was to investigate the differential autonomic control of blood flow and resistance vessel tone in skeletal muscle composed primarily of different fiber types, i.e., the soleus (84% type I), the high-oxidative Gast_{Red} (48% type IIA and 13% IIX) and the low-oxidative white portion of the gastrocnemius (Gast_{White}; 92% type IIB) (10). Blood flow was measured in the soleus, Gast_{Red} and Gast_{White} during sequential deprivation of metabolic, sympathetic neural, and sympathetic humoral influences. Within the resistance vasculature of skeletal muscle there are $\alpha_2$-adrenergic receptors located on both the smooth muscle and vascular endothelium (Rev see (38)) which can result in vasoconstrictor or vasodilator influences, respectively, with activation. Therefore, to further delineate the effects of the vascular endothelium on fiber-type associated adrenergic vasomotor control, arterioles from each of these muscles were isolated and alpha$_1$-($\alpha_1$) and alpha$_2$-($\alpha_2$) adrenoreceptor function was studied in vitro.
Methods

Six month old male Sprague-Dawley Rats (482 ± 9 g) were used in this study. All procedures were approved by the Institutional Animal Care and Use Committees at the University of Georgia (where blood flow experiments were performed; M.D.D. and R.B.A) and Texas A&M University (where isolated vessel experiments were performed B.J.B, R.B.A, and M.D.D). Rats were housed 2 per cage at 23°C and maintained on a 12:12 h light-dark cycle. All rats were fed Purina rat chow and water ad libitum.

Blood Flow Measurements

Blood flow to the entire hindlimb, as well as individual hindlimb muscles, was determined using the radionuclide-tagged microsphere technique (25). Initially, rats were anesthetized with isoflurane (2.5%/O₂ balance) and Silastic catheters (ID 0.6 mm, OD 1.0 mm) were implanted in the right carotid and caudal (tail) arteries. The carotid artery catheter was advanced 2-3 mm rostral to the aortic valve and secured. The tail artery catheter was advanced toward the bifurcation of the descending aorta and secured. The tail artery catheter was connected to a 5 ml glass syringe, which was attached to a Harvard withdrawal pump (model 907, Cambridge, MA). The carotid artery catheter was connected to a pressure transducer (BP100, ADInstruments) to monitor mean arterial pressure. A midline abdominal incision was then made to expose the descending aorta and bifurcation of the iliac arteries. An ultrasonic flow probe (Transonic Systems, Inc.; Ithaca, NY) was placed around the descending aorta just proximal to the bifurcation of the common iliac arteries for measuring absolute volume flow rates.
Surgical procedures for denervation: Surgical denervation was performed according to the methods described by Delp and Armstrong (9). Briefly, a 1 cm incision was made through the skin and connective tissue at the junction of the semitendinosus, biceps femoris and gastrocnemius muscles on the posterior aspect of the right hindlimb. The sciatic nerve was isolated using glass probes, and a silk suture (4-0) was loosely looped and tied around the nerve. The incision was then stapled closed for rats used in protocol 1 (see below). All anesthetized animals were placed on a heating pad and body temperature (measured via a rectal thermometer) was maintained at 37° C.

Radiolabeled (\(^{46}\)Sc, \(^{113}\)Sn and \(^{85}\)Sr) microspheres (15 µm diameter; DuPont/NEN; Boston, MA) were used for blood flow measurements as previously described (5, 11). Prior to infusion, the microspheres were agitated by sonication and thirty seconds prior to the microsphere infusion blood withdrawal from the caudal artery was initiated at 0.25 ml/min. The right carotid artery catheter was disconnected from the pressure transducer and ~2.5 \(X\) \(10^5\) microspheres of a specified radiolabel were infused into the ascending aorta and flushed with warmed saline to assure clearance of the beads. Blood withdrawal from the caudal artery continued for 45 seconds after the microsphere infusion.

Following the microsphere infusion, the rats were killed with an overdose of sodium pentobarbital (>80 mg/kg) via the right carotid artery catheter. After verifying correct placement of the carotid catheter, the left and right soleus, gastrocnemius, plantaris, tibialis posterior, flexor digitorum longus, flexor hallucis longus, tibialis anterior and extensor digitorum longus muscles and kidneys were dissected free. The gastrocnemius and tibialis anterior muscles were then sectioned into red (Gast\textsubscript{Red}, TA\textsubscript{Red}), mixed and white (Gast\textsubscript{White}, TA\textsubscript{White}) portions of the muscle, which correspond to the
predominately high-oxidative type IIA and IIX fibers (GastRed, TA\textsubscript{Red}), and the
predominately low-oxidative type IIB fibers (GastWhite, TA\textsubscript{White}) (24). The radioactivity
level of the tissues was determined by a three-channel gamma scintillation counter
(Packard Auto Gamma Spectrometer, model 5230) set to record the peak energy activity
of each isotope for 5 minutes. Total blood flow to each tissue was calculated by the
reference sample method (20, 22) and expressed in milliliters/minute/100 g of tissue.
Vascular resistance was calculated by dividing mean arterial pressure by blood flow and
expressed in mmHg/milliliter/minute/100 g of tissue. Adequate mixing of the
microspheres was verified by demonstrating a <15\% difference in blood flows between
the right and left kidneys.

**Blood Flow Protocol I**

The first protocol was designed to examine blood flow to muscles composed of
different fiber types after a reduction in metabolic activity from conscious standing to an
anesthetized and paralyzed condition (n=8). Animals were instrumented for blood flow
measurements, and allowed 4 h to recover as Flaim et al. (15) demonstrated that cardiac
or circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status are
stable in the awake unrestrained rat 1-6 h after gas anesthesia. Microspheres were
injected during conscious standing. After microsphere infusion, animals were
anesthetized (isoflurane 2.5\%/O\textsubscript{2} balance) and a tracheotomy was performed for artificial
ventilation. Briefly, the trachea was isolated and an 8 cm polyethylene tube (ID 1.4 mm,
OD 1.9 mm) was secured 1 cm into the trachea. Under isoflurane anesthesia (2.5\%/O\textsubscript{2}
balance), neuromuscular blockade was induced with an intra-arterial infusion of 10
in mg/kg gallamine triethiodide. In a separate group of animals (n=7) this dose of gallamine triethiodide was found to block 97.9% of the maximal twitch tension of the soleus, plantaris and gastrocnemius muscles without compromising mean arterial pressure (data not shown). After 50-75% of the dose of gallamine triethiodide was infused, the rats were connected to a rodent respirator (Harvard model 683) and ventilated (88 breaths/min, 2.25-2.5 ml tidal volume) to achieve an arterial pressure of O$_2$ (PaO$_2$) ~ 90 mmHg. Blood flow was then measured in the anesthetized and paralyzed condition.

**Blood Flow Protocol II**

The second protocol was designed to examine blood flow to muscles composed of different fiber types after removal of sympathetic neural influences in the absence of alpha-motoneuronal activation. All experiments were performed under isoflurane anesthesia (2.5%/O$_2$ balance) with the animals connected to the rodent respirator as described above. The group of animals (n=8) was used to follow temporal changes in abdominal aortic blood flow after denervation of one hindlimb to identify the time of the peak blood flow response. Neuromuscular blockade was induced with the infusion of 10 mg/kg gallamine triethiodide. Aortic blood flow, heart rate, and mean arterial pressure were measured for 5 min after neuromuscular blockade. Using the silk loop, the sciatic nerve was quickly isolated and severed unilaterally with microscissors. Temporal changes in aortic blood flow, heart rate and mean arterial pressure were continuously monitored for 60 minutes.

Once the peak blood flow response was identified (20-30 s post-denervation) in the first group, a second group of animals (n=9) was used to measure the distribution of
flow with radiolabeled microspheres to the left and right leg muscles during the pre-
denervation, 30 s post-denervation (peak blood flow response), and 5 min post-
denervation (relative steady-state). The anaesthetized rats were infused with gallamine
triethiodide (10 mg/kg) and 5 min later blood flow distribution, aortic flow, and mean
arterial pressure were measured. Next, the sciatic nerve was severed as described above,
and blood flow distribution, aortic flow, and mean arterial pressure were measured 30 s
and 5 min post-denervation.

**Blood Flow Protocol III**

This protocol was designed to examine blood flow to muscles composed of
different fiber types during stimulation (Group 1; n=8) and/or inhibition (Group 2; n=8)
of adrenergic receptors. Instrumentation for measuring blood flow and denervation was
performed as described above. In the group from Protocol II above (i.e., instrumented for
aortic flow determination), after the 5 minutes post-denervation period various
pharmacological interventions were performed and the temporal responses were
measured to align microsphere infusion with a blood pressure and abdominal aortic flow
steady-state with the interventions. Subsequently, in the group for this protocol blood
flow was determined under the following conditions: A) 1 minute after infusion (via
carotid artery catheter) of the α₁-adrenoreceptor agonist phenylephrine (0.3
mg/kg)(Group 1), B) 3 min after prazosin α₁-antagonist) infusion (3.0 mg/kg)(Group2),
and C) 1 minute after phenylephrine infusion (0.3 mg/kg) in the presence of prazosin
inhibition (Group 2). At least ten minutes of baseline aortic flow, heart rate and mean
arterial pressure were required before any compound(s) was administered.
**Isolated Microvessel Preparation**

In a separate set of animals (n=24) adrenergic vasoconstrictor responses of isolated resistance arterioles from the Gast\textsubscript{Red}, Gast\textsubscript{White} and soleus muscles were investigated.

Animals were anesthetized with sodium pentobarbital (85 mg/kg, i.p.) and killed by exsanguination. The gastrocnemius–plantaris–soleus muscle complex was carefully excised from each leg and placed in cold (4°C) physiological saline solution (PSS) containing (mM): 145.0 NaCl, 4.7 KCl, 2.0 CaCl\textsubscript{2}, 1.17 MgSO\textsubscript{4}, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, 3.0 MOPS buffer and 1 g /100 ml BSA at pH 7.4. Soleus and gastrocnemius muscle first-order (1A) arterioles were then isolated with the aid of a dissecting microscope (Olympus SVH10) as previously described (29, 32). In soleus muscles, 1A arterioles were defined as the first branch off the feed artery perforating the muscle. In gastrocnemius muscles, 1A arterioles were defined as the first branch off the feed artery that runs over the superficial (Gast\textsubscript{White}) or into the deep (Gast\textsubscript{Red}) portions of the muscle. The arterioles (length, 0.5–1.0 mm) were cleared of surrounding muscle fibers, removed from the muscle and placed in Lucite chambers containing MOPS-buffered PSS equilibrated to room air. The arterioles were cannulated on both ends to glass micropipettes, and secured with ophthalmic nylon suture (Alcon 11-0). After cannulation, the chambers were transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (Microcirculation Research Institute, Texas A&M), and data acquisition system (MacLab) for recording of luminal diameter. Intraluminal pressure was set at 75 cm H\textsubscript{2}O...
to coincide with pressures used in previous *in vitro* studies of skeletal muscle arterioles (1, 12). Leaks were detected by pressurizing the vessel and determining whether vessel diameter was maintained. Arterioles that exhibited leaks were discarded. Arterioles free of leaks were warmed to 37°C and allowed to develop spontaneous tone during a 30–60 min equilibration period, upon which arterioles were discarded unless at least 20% baseline tone was achieved prior to addition of vasoactive agents. Sensitivity of the arterioles to agonists was assessed by calculating the dose eliciting 50% of the maximal vasoconstriction (EC$_{50}$).

To determine whether there are fiber-type differences in adrenergic vasoconstrictor function of the skeletal muscle arterioles, responses of 1A arterioles were determined to the cumulative addition of either the $\alpha_2$-adrenoreceptor agonist clonidine ($10^{-9}$ to $10^{-5}$ M) or the $\alpha_1$-adrenoreceptor agonist phenylephrine ($10^{-9}$ to $10^{-4}$ M). A second paired series of studies was performed to determine whether differences in $\alpha_1$ or $\alpha_2$ vasoconstriction were mediated through the vascular endothelium. For these studies, the endothelium was removed from arterioles by passing 3–5 ml of air through the lumen of the vessel as described previously (12). To ensure complete denudation of the endothelium, arterioles were exposed to the endothelium-dependent vasodilator acetylcholine ($3 \times 10^{-5}$ M), and any vessel that exhibited vasodilatation $>5\%$ was excluded. Following exposure to acetylcholine, the vessels were washed several times with PSS and allowed to establish spontaneous tone. Dose-response relations to either the cumulative addition of clonidine ($10^{-9}$ to $10^{-5}$ M) or phenylephrine ($10^{-9}$ to $10^{-4}$ M) were performed in the absence of the endothelium.
**Blood Gas Analysis**

Arterial pH and the partial pressure of oxygen (PaO₂) were measured with a pH/blood gas analyzer (Corning model 170). Body temperature and hemoglobin concentration measurements were used to correct pH and PaO₂ values.

**Data Analysis**

Responses were recorded as actual diameters and expressed as a percentage of possible vasoconstriction according to the following formula:

\[
\text{Vasoconstriction (\%maximal response)} = \frac{(D_b - D_s)}{D_b} \times 100
\]

where \(D_s\) is the steady-state inner diameter recorded after addition of agonist, \(D_b\) is the initial baseline inner diameter before the first addition of a pharmacological agonist, and \(D_m\) is the maximal intraluminal diameter obtained in Ca²⁺-free PSS. Comparison of data as a percentage of the maximal vasoconstriction normalizes for potential differences in maximal diameter or spontaneous tone among vessels. Spontaneous tone is expressed at a percentage of maximal intraluminal diameter (\(D_m\)) according to the formula:

\[
\text{Spontaneous tone (\%)} = \left(\frac{D_m - D_b}{D_m}\right) \times 100
\]

**Statistical Analysis**

Repeated measures analysis of variance (ANOVA) was used to determine differences among blood flow for pre-, 30 s and 5 min post-denervation. ANOVA was also used to determine differences for dose–response-diameters in isolated vessels, in order to detect differences within (dose) and between (muscle fiber type) factors. Post
hoch analysis was performed by Duncan’s multiple range test to determine the significance of differences among means. A one-way ANOVA was used to determine the significance of differences among vessel characteristics and blood flow from conscious standing (Protocol I, Group 1) to anesthetized (Protocol I, Group 2). All data are presented as mean ± S.E.M. Significance was set at $P \leq 0.05$.

**Results**

*Hemodynamics and Neuromuscular Blockade*

With administration of 10 mg/kg gallamine triethiodide there was no change in heart rate, mean arterial pressure, or abdominal aortic blood flow. Arterial pH was 7.46 ± 0.01 and arterial PO$_2$ was 88.5 ± 3.9 mmHg and both variables remained unaltered throughout the entire pre- and post-denervation period (data not shown).

*Protocol I: Blood flow from Conscious Standing to the Anesthetized Condition*

Blood flow decreased and resistance increased in all muscles studied from conscious standing (CS) to an anesthetized and paralyzed condition (AN) (Table 1). The most dramatic decreases in blood flow occurred in the most oxidative muscles. Specifically, there was a 94% and 86% reduction in blood flow to the soleus and Gast$_{Red}$, respectively. In Gast$_{White}$, which is composed predominately of type IIB fibers (Delp & Duan, 1996), blood flow decreased by ~ 53%.
Protocol II: Blood flow and Denervation

After denervation, aortic flow increased from 17 ± 2 ml/min (pre-denervation) to a peak of 26 ± 3 ml/min (P<0.05) at 30 s. At 1 min post-denervation, aortic flow remained ~ 15 % above baseline at 24 ± 3 ml/min (P<0.05; Figure 1A) and remained unchanged through 60 min post-denervation. Mean arterial pressure and heart rate did not change at any time point from pre- to 60 min post-denervation (range 104 ± 7 to 108 ± 4 mmHg).

The increase in abdominal aortic flow was equivalent to the summed increase in flow measured with microspheres to all denervated muscles (Figure 1A). Blood flow increased in all individual denervated leg muscles from pre- to 30 s post-denervation (Table 2). Of the GastWhite, GastRed, and soleus muscles, the greatest increases in blood flow occurred in GastWhite (8.5 fold increase), with an 2.7 fold increase in the GastRed and soleus muscle (Figure 1B).

At 5 min post-denervation, abdominal aortic blood flow decreased by 3 ml/min versus the 30 s post-denervation, but remained elevated compared to pre-denervation (Table 2; Figure 1A). The total summed flow to the denervated leg muscles had a similar decrease in flow at 5 minutes post-denervation, whereas in the contralateral normal leg flow remained unchanged. In the contralateral muscles, blood flow remained unchanged with denervation of the other leg muscles (Figure 1A).

Protocol III: Muscle Vascular Resistance during Adrenergic Receptor Stimulation/Inhibition
In all denervated muscles, there was an increase in vascular resistance with stimulation of \( \alpha_1 \)-adrenoreceptors via systemic phenylephrine infusion. Relative to baseline control blood flow (measured at 5 min post-denervation in protocol II) the greatest increase in resistance occurred in \text{GastWhite} (~19 fold), with a more moderate increase in \text{GastRed} (~6 fold) and soleus (~4.5 fold) muscles (Figure 2). Changes in resistance to normally innervated muscles with phenylephrine paralleled the fold differences found in the denervated muscles (data not shown). When comparing the relationships between vascular resistance changes with phenylephrine infusion and the fiber type composition or oxidative capacity of the individual hindlimb muscles listed in Table 2, there was 1) an indirect relationship with the % of type I and IIA fiber mass (Figure 3A) and oxidative capacity (Figure 3B), and 2) a direct relationship with the % of type IIB fiber mass (Figure 3C) of the individual muscles.

There was no significant change in vascular resistance in any muscle with the administration of the \( \alpha_1 \)-adrenoreceptor antagonist prazosin (Figure 2); prazosin treatment was sufficient to block the vasoconstrictor effects of phenylephrine in all muscle analyzed (Figure 2). Mean arterial pressure increased from control (103 ± 8 mmHg) to phenylephrine treatment (196 ± 4 mmHg; \( P < 0.05 \)); prazosin treatment resulted in a decreased pressure (63 ± 5 mmHg; \( P < 0.05 \)).

\textit{In Vitro Studies}

The maximal intraluminal diameter of arterioles from the soleus muscle (131 ± 9 \( \mu m \)) was smaller than that of the \text{GastRed} (157 ± 7 \( \mu m \)) and \text{GastWhite} (158 ± 11 \( \mu m \))
µm)(P<0.05). There was no difference in spontaneous tone between muscles with
(soleus, 33 ± 2%; GastRed, 32 ± 3%; GastWhite, 30 ± 3%) or without (soleus, 34 ± 3%;
GastRed, 36 ± 3%; GastWhite, 32 ± 3%) the endothelium.

In response to the α2-adrenoreceptor agonist clonidine, there were fiber type-
associated differences in vasoconstriction; the GastWhite demonstrated the greatest
maximal vasoconstriction of the three muscle types and a greater sensitivity (EC50; Table
3) to clonidine versus the soleus muscle arteriole (Figure 4A). In addition, there was
greater maximal vasoconstriction of the GastRed arteriole compared to that of the soleus
(Figure 4A); there were no differences in sensitivity to clonidine between these two
muscle types. All differences in the percent vasoconstriction and sensitivity to clonidine
among muscle types were abolished with the removal of the endothelium (Figure 4B).
Endothelium removal did not change the maximal response or sensitivity to clonidine in
GastWhite arterioles, whereas there was an enhanced vasoconstriction and sensitivity in
GastRed and soleus arterioles with denudation.

Maximal vasoconstrictor responses and sensitivity (EC50; Table 3) to the to the
α1-adrenoreceptor agonist phenylephrine were greater in arterioles from GastWhite and
GastRed than that of the soleus muscle (Figure 5A). Removal of the endothelium did not
alter these differences (Figure 5B).
Discussion

The purpose of this study was to investigate the potential for differential vasomotor control of resistance vessels in skeletal muscle composed of different fiber types. The main findings are: 1) there exists a large metabolic control of blood flow in muscles composed of type I, IIA and IIX versus IIB fibers; 2) removal of sympathetic nerve influence via denervation induces a greater increase in blood flow to muscle of type IIB fiber composition than either type I or IIA and IIX; 3) in vivo, with selective alpha-1 adrenergic receptor stimulation there is a direct relationship between changes in vascular resistance and the % type IIB fiber composition of the individual muscles measured herein, 4) endothelial modulation of vascular tone through alpha-2-adrenergic receptor stimulation is greatest in muscle composed of type I fibers and least in muscle composed of type IIB fibers; and 5) soleus muscle arterioles have a lower alpha-1-adrenergic receptor mediated vasoconstriction versus arterioles from the GastRed and GastWhite. Therefore, vasoconstriction of arterioles from IIA and IIX muscle to alpha-1-adrenoreceptor stimulation is most similar to that of arterioles from muscle composed of type IIB fibers. Conversely, arterioles from muscle composed of high-oxidative type IIA and IIX fibers demonstrate vasoconstrictor responses to alpha-2-adrenoreceptor stimulation that are more similar to that of arterioles from muscle composed of high-oxidative type I fibers than muscle containing low-oxidative glycolytic type IIB fibers. These data indicate that the vasoconstrictor responsiveness of the resistance vasculature in type IIA and IIX muscle is intermediate to that of the low-oxidative and fast-glycolytic muscles. Similarly, the magnitude of control exerted through the sympathetic nerves and adrenergic stimulation over the range of
blood flows occurring in muscle is relatively small in muscles composed of the high-
oxidative type I, IIA and IIX fibers, whereas the magnitude of adrenergic control over the
range of flows occurring in the low-oxidative type IIB muscle is quite large (Figure 6).

*Changes in blood flow from conscious standing to anesthetized*

There is evidence to suggest that local metabolite release is different among
muscle composed of different fiber types (18, 28). For example, muscles composed of a
high percentage of type I fibers generate greater muscle hyperemia to the vasodilator
substance adenosine than muscles with predominately type IIB fibers (26). This greater
hyperemia to adenosine could reflect greater production of adenosine by the type I fibers,
greater sensitivity of the resistance vasculature to adenosine in type I muscle, or a
combination of the two. *In vivo* differences in the quantity and types of vasoactive
metabolites among muscles could result in variations in the level of vascular tone and the
mechanism(s) through which vascular tone is achieved in muscle. In the present study, a
reduction in muscular activity from the conscious standing to the anesthetized condition
resulted in a 2100%, 310%, and 209% increase in vascular resistance in the soleus,
GastRed and GastWhite muscle, respectively (Table 1). It is probable that the difference in
the change in resistance among the muscle types simply reflects the relative changes in
motor unit activity from the conscious to anesthetized state. The soleus muscle, for
example, which is near maximally active during postural maintenance (25, 36, 39), would
presumably have had the greatest decrease in muscle fiber activity from the standing to
the anesthetized paralyzed condition. In contrast, the motor units in the GastWhite are
relatively inactive during conscious standing (12, 18, 28), which is supported by the
relative smaller change in blood flow from conscious standing to the anesthetized condition.

Removal of sympathetic neural influence

The differential influence of the sympathetic nervous system in controlling blood flow to type I and IIB muscle has long been noted (16, 25, 35). Peripheral nerve section was used in the present study to estimate neurally mediated sympathetic tone in the absence of evoked muscular activity. Denervation resulted in a rapid increase in blood flow through the abdominal aorta with a peak response at approximately 20-30 s post-denervation, followed by a small decrease at 1 minute (Figure 1) that remained stable for 60 min post-denervation. The change in the total sum of blood flow to all denervated leg muscle measured with microspheres from pre- to 30 s and 5 min post-denervation could quantitatively account for the change in abdominal aortic flow measured with an ultrasonic flow probe (Figure 1A). The GastWhite, which has the largest proportion of type IIB fibers of the muscles studied, demonstrated the greatest hyperemic response 30 s post-denervation. When comparing denervated to maximal exercising blood flow, the hyperemic response in the denervated GastWhite increased to ~75% of the highest blood flow reported to this muscle during maximal exercise (4, 33) or with tetanic contractions (28). In the GastRed and soleus muscle, the blood flow with denervation reached only 4 and 7%, respectively, of the reported maximal exercising blood flows for these muscles (Figure 6). This suggests that a) sympathetic vasoconstrictor tone is greater in muscles composed of type IIB than in type I, IIA and IIX fibers, and b) the sympathetic nervous system has to potential to modulate virtually the entire adaptive range of blood flow.
measured in conscious exercising animals in muscles comprised of mainly type IIB fibers. Indeed, Thomas and colleagues (37) have demonstrated that sympathetic vasoconstrictor tone is abolished in maximally contracting muscles composed of highly glycolytic type IIB fibers. These same authors have found that sympathetically mediated vasoconstrictor tone is maintained in the more oxidative type I fibers due, in part, to the production of nitric oxide (6, 33).

Alpha adrenergic receptor function in isolated vessels

The observations that resistance vessels from the GastWhite (type IIB muscle) demonstrate greater maximal vasoconstriction and sensitivity with $\alpha_2$-receptor agonism (versus soleus and GastRed; Figure 4) may be explained by a greater postsynaptic $\alpha_2$-adrenergic receptor density (13, 14, 25) or location. In intact muscle, there are no apparent differences in adrenergic innervation density to resistance vessels in type IIB and type I muscle (2). However, within the resistance vasculature of skeletal muscle there are $\alpha_2$-adrenergic receptors located on both the smooth muscle and vascular endothelium (Rev see (38)) which result in vasoconstrictor or vasodilator influences, respectively, with activation. Therefore, the vascular tone of a given resistance vessel in response to a selective $\alpha_2$-adrenergic receptor agonist is determined by the net contribution of these opposing vasoconstrictor and vasodilator influences. Recognizing this, we chose not to infuse a selective $\alpha_2$-adrenoreceptor agonist into the intact animal as we could not discriminate the relative contributions from endothelial versus smooth muscle $\alpha_2$-adrenergic receptor stimulation on the net blood flow response. However, we were able to investigate the modulatory role of the vascular endothelium with selective
the $\alpha_2$-adrenergic receptor stimulation *in vitro*. In arterioles from muscle composed of type I and type IIA and IIX fiber types, the $\alpha_2$-adrenergic receptors on the endothelium appear to have a greater vasodilator influence to oppose the vasoconstriction (Figure 4A), since removal of the vascular endothelium resulted in no differences in maximal vasoconstriction among the three fiber type muscles (Figure 4B). The fact that removing the endothelium did not alter the sensitivity or maximal vasoconstriction to clonidine in GastWhite (Figure 4A & B) supports the notion that the endothelial $\alpha_2$-receptors play a minimal role in setting vascular tone in this muscle type. It should be noted that Aaker and Laughlin (30) found no difference in vasoconstriction to norepinephrine in arterioles from the GastRed and GastWhite. It is possible, however, that since norepinephrine stimulates both $\alpha_1$- and $\alpha_2$-adrenoreceptors, the contribution of $\alpha_2$-adrenoreceptors located on the endothelium was masked by a dominant vasoconstriction elicited by $\alpha_1$- and $\alpha_2$-adrenoreceptors located on the smooth muscle.

With respect to $\alpha_1$-adrenoreceptor function in the intact muscle, the infusion of phenylephrine increased vascular resistance to the greatest extent in the GastWhite (Figure 2). However, there were no differences in the sensitivity or maximal vasoconstriction of isolated arterioles between the GastWhite and GastRed, and arterioles from both these muscle types demonstrated greater responsiveness to phenylephrine than soleus muscle arterioles (Figure 5A & B). In the current study we investigated vasomotor control *in situ* from a major resistance artery (i.e., 1A arterioles); however, the change in vascular resistance with phenylephrine infusion *in vivo* reflects the net effect of all the resistance vasculature. Therefore, given the regional heterogeneity of $\alpha$-adrenoreceptor subtypes in skeletal muscle arterial networks (31), it is possible that the downstream resistance
vessels in the \text{GastWhite} may be more sensitive to phenylephrine compared to those of the \text{GastRed}.

\textit{Conclusion}

The present investigation demonstrates that sympathetic vasoconstrictor tone is greater in muscles composed of type IIB than type I and type IIA and IIX fibers. Furthermore, the \text{GastRed} appears to be more similar to the soleus with $\alpha_2$-adrenoreceptor stimulation and to the \text{GastWhite} with $\alpha_1$-adrenergic stimulation. With respect to the modulating effect of the SNS on blood flow, the \text{GastRed} and soleus are very similar in that denervation resulted in blood flow increasing to less than 7\% of maximal reported values. The ability of the sympathetic nervous system to maintain tighter control of blood flow in muscle with predominately type IIB fibers appears to be primarily mediated though $\alpha_1$- and $\alpha_2$-adrenergic receptors located on the vascular smooth muscle with little effect of endothelial $\alpha_2$-adrenoreceptors. In addition, modulation of sympathetic nerve activity to the muscle composed of type IIB fibers appears to have the potential to control virtually the entire adaptive range of blood flow to this muscle type.

\textbf{Acknowledgements}

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\textbf{Reference}


**Figure Legends**

Figure 1. A) Mean absolute (ml/min) abdominal aortic, denervated, and intact leg muscle blood flow during pre-denervation and 30 s and 5 minutes post-denervation. B) Mean relative (ml/min/100 g) blood flow in the soleus and red (GastRed) and white (GastWhite) portions of the gastrocnemius muscle after denervation. Blood flow to the contralateral (i.e., intact) muscles did not change during over the 5 min period. * P<0.05 versus pre-denervation blood flow (time zero). † P<0.05 versus blood flow at 5 min post-denervation.

Figure 2. Mean vascular resistance in denervated muscles during control (i.e., 5 min post-denervation) and with administration of saline, phenylephrine, prazosin, or prazosin + phenylephrine. * P<0.05 versus control and saline. † P<0.05 versus phenylephrine.

Figure 3. Relationships between the A) percent sum of type I and IIA fibers, B) citrate synthase activity, and C) percent sum of type IIB fibers of the individual muscles listed in table 1 of the rat hindlimb and the change (Δ) in vascular resistance after infusion of
phenylephrine. Based on fiber type composition and citrate synthase activity reported by Delp and Duan (10).

Figure 4. Dose response relations to cumulative additions of the $\alpha_2$-receptor agonist clonidine in arterioles from the soleus, Gast$\text{Red}$ and Gast$\text{White}$ muscle. A) Percent vasoconstriction to cumulative doses of clonidine with the endothelium intact. B) Percent vasoconstriction to cumulative doses of clonidine with the endothelium removed. *P<0.05 versus Gast$\text{White}$. †P<0.05 versus Soleus.

Figure 5. Dose response relations to cumulative additions of the alpha$1$-receptor agonist phenylephrine in arterioles from the soleus, Gast$\text{Red}$ and Gast$\text{White}$ muscle. A) Percent vasoconstriction to cumulative doses of phenylephrine with the endothelium intact. B) Percent vasoconstriction to cumulative doses of phenylephrine with the endothelium removed. *P<0.05 versus vessel responses from Gast$\text{Red}$ and Gast$\text{White}$.

Figure 6. Comparisons of the blood flow response measured in the current study after denervation to that measured during maximal exercise (Armstrong & Laughlin, 1983; Musch et al., 2001) or with tetanic contractions (Mackie & Terjung, 1983). With removal of sympathetic neural tone, blood flow in the Gast$\text{White}$ increased to near maximal reported values.
Figure 3

A. Type I + IIA Fiber Mass (%)

B. Citrate Synthase Activity (μmol/min/100 g)

C. Type IIB Fiber Mass (%)

$r = 0.61, P \leq 0.05$

$r = 0.60, P \leq 0.05$

$r = 0.61, P \leq 0.05$
Figure 4

A

Vasoconstriction (%) vs. Clonidine (log M)

- White Gastrocnemius
- Red Gastrocnemius
- Soleus

B

Vasoconstriction (%) vs. Clonidine (log M)

- White Gastrocnemius-Endo
- Red Gastrocnemius-Endo
- Soleus-Endo

Figure 4
Figure 5
Figure 6

Blood Flow (ml/min/100 g)

- Maximal Treadmill Exercise
- Tetanic Contractions
- 30 s Post-Denervation
- Anesthetized

Soleus

Red Gastroc.

White Gastroc.
Table 1. Hemodynamic data during conscious standing (CS) and anesthesia plus neuromuscular blockade (AN).

<table>
<thead>
<tr>
<th></th>
<th>CS (n=8)</th>
<th>AN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>373 ± 8</td>
<td>320 ± 11*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>114 ± 2</td>
<td>104 ± 5</td>
</tr>
<tr>
<td><strong>Blood Flow (ml/min/100 g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>148 ± 12</td>
<td>8 ± 2 *</td>
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<tr>
<td>Red Gastrocnemius</td>
<td>69 ± 15</td>
<td>10 ± 2 *</td>
</tr>
<tr>
<td>Mixed Gastrocnemius</td>
<td>24 ± 3</td>
<td>9 ± 2 *</td>
</tr>
<tr>
<td>White Gastrocnemius</td>
<td>15 ± 2</td>
<td>7 ± 2 *</td>
</tr>
<tr>
<td>Plantaris</td>
<td>26 ± 7</td>
<td>11 ± 2 *</td>
</tr>
<tr>
<td>Tibialis Posterior</td>
<td>36 ± 5</td>
<td>9 ± 3 *</td>
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<tr>
<td>Flexor digitorum longus</td>
<td>29 ± 3</td>
<td>8 ± 2 *</td>
</tr>
<tr>
<td>Flexor hallicus longus</td>
<td>25 ± 4</td>
<td>9 ± 3 *</td>
</tr>
<tr>
<td>Red tibialis anterior</td>
<td>66 ± 6</td>
<td>10 ± 2 *</td>
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<tr>
<td>White tibialis anterior</td>
<td>21 ± 4</td>
<td>9 ± 2 *</td>
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<tr>
<td>Extensor digitorum longus</td>
<td>32 ± 4</td>
<td>10 ± 1 *</td>
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Values are means ± SEM. * P<0.05 versus mean during conscious standing (CS).
Table 2. Hemodynamic data during anesthesia and neuromuscular blockade (AN) and at 30 s and 5 min post-denervation.

<table>
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<th>30 s</th>
<th>5 min</th>
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<tr>
<td>Heart Rate (bpm)</td>
<td>341 ± 8</td>
<td>351 ± 13</td>
<td>349 ± 12</td>
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<tr>
<td>MAP (mmHg)</td>
<td>108 ± 7</td>
<td>110 ± 6</td>
<td>111 ± 5</td>
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<tr>
<td>Abdominal aorta blood flow (ml/min)</td>
<td>17 ± 2</td>
<td>26 ± 3 *</td>
<td>23 ± 3 * †</td>
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<tr>
<td>Blood Flow (ml/min/100 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>9 ± 1</td>
<td>26 ± 3 *</td>
<td>25 ± 3 *</td>
</tr>
<tr>
<td>Red Gastrocnemius</td>
<td>11 ± 2</td>
<td>30 ± 3 *</td>
<td>30 ± 3 *</td>
</tr>
<tr>
<td>Mixed Gastrocnemius</td>
<td>11 ± 2</td>
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<tr>
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<td>7 ± 1</td>
<td>56 ± 5 *</td>
<td>45 ± 6 * †</td>
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<tr>
<td>Plantaris</td>
<td>11 ± 3</td>
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<tr>
<td>Tibialis posterior</td>
<td>8 ± 1</td>
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<tr>
<td>Flexor digitorum longus</td>
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<td>75 ± 14 *</td>
<td>66 ± 15 *</td>
</tr>
<tr>
<td>Flexor hallicus longus</td>
<td>8 ± 2</td>
<td>86 ± 19 *</td>
<td>63 ± 18 *</td>
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<tr>
<td>Red tibialis anterior</td>
<td>9 ± 1</td>
<td>40 ± 9 *</td>
<td>33 ± 4 *</td>
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<tr>
<td>White tibialis anterior</td>
<td>10 ± 2</td>
<td>81 ± 15 *</td>
<td>45 ± 9 * †</td>
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<td>Extensor digitorum longus</td>
<td>8 ± 1</td>
<td>98 ± 21 *</td>
<td>61 ± 11 * †</td>
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</tbody>
</table>

Values are means ± SEM. *P<0.05 versus anesthesia and neuromuscular blockade (AN).

† P<0.05 versus 30 s post denervation value.
Table 3. Arteriolar sensitivity (EC$_{50}$) to clonidine and phenylephrine before and after removal of the endothelium.

<table>
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<th>Clonidine</th>
<th>Clonidine-Endo</th>
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<td></td>
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<td>$3.1 \times 10^{-8}$ $\pm$ $7.1 \times 10^{-9}$†</td>
</tr>
<tr>
<td>Soleus</td>
<td>$1.1 \times 10^{-7}$ $\pm$ $3.2 \times 10^{-7}$</td>
<td>$3.5 \times 10^{-8}$ $\pm$ $1.9 \times 10^{-8}$†</td>
</tr>
<tr>
<td>Red Gastrocnemius</td>
<td>$4.5 \times 10^{-8}$ $\pm$ $3.1 \times 10^{-8}$*</td>
<td>$2.8 \times 10^{-8}$ $\pm$ $1.1 \times 10^{-8}$</td>
</tr>
<tr>
<td>White Gastrocnemius</td>
<td>$4.2 \times 10^{-6}$ $\pm$ $2.3 \times 10^{-6}$</td>
<td>$2.7 \times 10^{-6}$ $\pm$ $2.1 \times 10^{-6}$</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>$8.9 \times 10^{-7}$ $\pm$ $4.8 \times 10^{-7}$*</td>
<td>$7.5 \times 10^{-7}$ $\pm$ $3.9 \times 10^{-7}$*</td>
</tr>
<tr>
<td>Phenylephrine-Endo</td>
<td>$4.4 \times 10^{-7}$ $\pm$ $3.2 \times 10^{-7}$*</td>
<td>$4.1 \times 10^{-7}$ $\pm$ $2.2 \times 10^{-7}$*</td>
</tr>
</tbody>
</table>

All values are in molar concentration and represented as mean $\pm$ SE. *P<0.05 versus soleus. †P<0.05 versus endothelium intact for same muscle type.