Differential Sensitivity of Arteriolar $\alpha_1$- and $\alpha_2$-Adrenoceptor Constriction to Metabolic Inhibition During Rat Skeletal Muscle Contraction

Karen M. Anderson and James E. Faber

Our previous studies in rat skeletal muscle have determined that neural constriction of large arterioles, which regulate blood flow and peripheral resistance, is mediated by $\alpha_1$-adrenoceptors, whereas small arterioles, which determine effective capillary density, depend on $\alpha_2$-receptors. During physical exercise, metabolic vasodilators from contracting skeletal muscle oppose neural vasoconstriction. By mechanisms that are not understood, adrenergic constriction of small arterioles is particularly sensitive to metabolic inhibition during imbalances in oxygen supply versus demand. This sensitivity may result from the reliance of small arterioles on $\alpha_2$-receptors and a greater sensitivity of $\alpha_2$ constriction to metabolic dilators. We previously demonstrated selective attenuation of arteriolar $\alpha_2$ constriction during a reduction in the oxygen supply/demand ratio subsequent to decreased skeletal muscle perfusion. In the present study, intravital microscopy of rat cremaster skeletal muscle was used to examine the effect of increased oxygen demand on adrenergic constriction of arterioles. The effect of multiple frequencies of skeletal muscle contraction (via genitofemoral nerve stimulation) on $\alpha_1$ (norepinephrine+rauwolscine) and $\alpha_2$ (norepinephrine+prazosin) constriction was used to evaluate neural–metabolic interactions over a wide range of metabolic conditions. Low-frequency ($\leq$2 Hz) skeletal muscle contraction attenuated only $\alpha_2$ constriction; contractions $\geq$4 Hz attenuated $\alpha_1$ constriction and further reduced $\alpha_2$ constriction. Comparison of the frequency of constriction necessary to produce inhibition of 20% of maximal dilation indicated that $\alpha_2$ constriction was approximately 10-fold more sensitive than $\alpha_1$ constriction to “metabolic” inhibition. High-frequency, but not low-frequency, contraction also inhibited intrinsic tone. These data suggest that release of dilator substances during moderate exercise may preferentially attenuate $\alpha_2$ constriction to produce small arteriolar dilation and increased capillary density. In contrast, metabolic signals associated with higher frequency muscle contraction may inhibit both intrinsic tone and large arteriolar $\alpha_1$ tone so that blood flow and oxygen delivery increase to match the elevated oxygen demand of more heavily exercising muscle. (Circulation Research 1991;69:174–184)

The rate of oxygen delivery (blood flow) and capacity for blood–tissue oxygen exchange (functional capillary density) are two major determinants of tissue oxygenation. During physical exercise, effective capillary density and blood flow increase as vasodilators from contracting skeletal muscle oppose adrenergic vasoconstriction associated with sympathetic excitation. Network models of working skeletal muscle predict that extensive capillary recruitment through dilation of terminal arterioles (“precapillary sphincters”) and increased oxygen extraction can maintain muscle oxygenation during light work but that tissue blood flow must increase to provide adequate oxygenation during exercise of increasing intensity.1 Measurements of capillary filtration and blood flow in exercising skeletal muscle

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have verified that an increase in functional capillary density precedes overall increases in tissue blood flow.\textsuperscript{2–4} Generally, skeletal muscle capillary flow is regulated by terminal arterioles, and overall bed resistance and blood flow are controlled by large resistance arterioles and small arteries. Thus, both the network model and the observed preferential increase in capillary density suggest that adrenergic constriction of small arterioles may be particularly sensitive to metabolic inhibition during exercise and that the small and large arterioles can be independently regulated. Other studies have also demonstrated that terminal arteriolar adrenergic constriction is particularly sensitive to inhibition during reduced muscle perfusion (i.e., reduced oxygen supply\textsuperscript{3–6}). The mechanisms by which the small and large arterioles may differentially integrate neural and metabolic signals for independent regulation of capillary density and blood flow are not well understood.

Previous studies from this laboratory\textsuperscript{7,8} have demonstrated that adrenergic constriction of skeletal muscle microvessels follows the stimulation of postjunctional \(\alpha_1\) and \(\alpha_2\)-adrenoceptors. Large resistance arterioles possess both \(\alpha_1\)- and \(\alpha_2\)-receptors, whereas small terminal arterioles have predominantly \(\alpha_2\)-receptors.\textsuperscript{7,8} Although the large arterioles have both receptor types, their response to neural stimulation is mediated only by \(\alpha_1\)-receptors; predictably, neural constriction of terminal arterioles is mediated by \(\alpha_2\)-receptors.\textsuperscript{9} Additional studies from our laboratory\textsuperscript{10} have demonstrated that \(\alpha_2\)-mediated constriction of skeletal muscle arterioles is selectively attenuated during moderate reductions in tissue perfusion. Thus, adrenergic constriction of small arterioles may be preferentially inhibited during modest imbalances of oxygen supply and demand because of their reliance on \(\alpha_2\)-receptors for adrenergic constriction and a particular sensitivity of \(\alpha_2\)-receptor contractile coupling to metabolic vasodilators.

It is well known that multiple tissue metabolites may function as vasodilators in skeletal muscle.\textsuperscript{11,12} Recent evidence indicates that different substances may be responsible for arteriolar dilation during muscle ischemia and muscle contraction\textsuperscript{13} and that different dilator metabolites predominate during successive stages and intensities of exercise.\textsuperscript{11,12} We have demonstrated that acidosis within the physiological range for light-to-moderate skeletal muscle exercise attenuates \(\alpha_2\), but not \(\alpha_1\), constriction of cremaster muscle arterioles.\textsuperscript{14} The purpose of this study was to examine the effect of multiple skeletal muscle contraction frequencies on adrenergic constriction of large and small arterioles to evaluate neural–metabolic interactions over a wide range of metabolic conditions.

**Materials and Methods**

**Surgical Procedures**

Experiments were performed on 6–7-week-old male Sprague-Dawley rats (body weight, 166±5 [mean±SEM] g; \(n=61\)) that were anesthetized with urethane and chloralose (425 and 100 mg/kg i.m., respectively) as described previously.\textsuperscript{7,14} Rectal temperature was maintained at 37°C, and the rats breathed room air spontaneously via tracheostomy. The right cremaster muscle was prepared for in situ microvascular observation via intravital microscopy and an image analysis system as described in detail elsewhere.\textsuperscript{7,9,14}

Cremaster muscle contraction was produced by electrical stimulation of the genitofemoral nerve, which was freed from the underlying psoas muscle, decentralized, and placed on a platinum stimulating electrode for delivery of square-wave stimuli (2–4 V, 0.1 msec). The nerve was covered with mineral oil to prevent drying, and the electrode was covered with stopcock grease to prevent leakage of the current to surrounding tissue. For a given experiment, the voltage used to produce cremaster contraction was set 0.2 V greater than threshold and was not changed over the duration of the experiment. To remove any remaining autonomic innervation of the cremaster muscle, the right lateral cutaneous, iliohypogastric, and ilioinguinal nerves were transected.\textsuperscript{10,14}

**Experimental Protocol**

Microvascular measurements were made on either first-order (diameter, 136±4 [mean±SEM] \(\mu m\); \(n=35\)), second-order (diameter, 89±4 \(\mu m\); \(n=7\)), or third-order (diameter, 18±2 \(\mu m\); \(n=29\)) arterioles as defined previously\textsuperscript{14} and were chosen on the basis of anatomic position and image clarity. A single arteriole was studied per experiment. The adrenergic sensitivity of first- and second-order arterioles does not differ significantly.\textsuperscript{7,14} Thus, the results for first- and second-order arterioles have been combined and reported as large arterioles. The cremaster bath Krebs solution contained, at all times, propranolol (10\textsuperscript{–6} M) for blockade of \(\beta\)-receptors and desipramine (10\textsuperscript{–8} M) and normetanephrine (10\textsuperscript{–5} M) for blockade of neuronal and nonneuronal catecholamine uptake mechanisms, respectively.\textsuperscript{7,8} Drug concentrations expressed here and elsewhere represent final bath concentrations.

Figure 1 shows results from a representative experiment that examined the effect of cremaster contraction on \(\alpha\)-adrenergic constriction of arterioles. After a 30-minute stabilization period, vessel diameter was measured for a 5-minute control period. Norepinephrine (NE) was added to the bath to produce an intermediate level of adrenergic constriction. Previous studies\textsuperscript{7,8,14} have established that a steady-state response to intermediate concentrations of NE is achieved within 4–8 minutes. Thus, the NE-induced constriction in these experiments was determined by measuring arteriolar diameter during the final 3 minutes of a 10-minute exposure period (“constricted diameter”). For large arterioles, intermediate \(\alpha_2\)-adrenergic tone was produced by addition to the bath of NE (3×10\textsuperscript{–7} M) in the presence of the \(\alpha_2\)-antagonist rauwolfscine (1×10\textsuperscript{–6} M, \(n=7\)); intermediate \(\alpha_2\) constriction was induced by NE (3×10\textsuperscript{–7} M).
to $3 \times 10^{-5}$ M) in the presence of the $\alpha_1$-antagonist prazosin ($1 \times 10^{-7}$ M, $n=7$). Only $\alpha_1$ or $\alpha_2$ constriction was evaluated in a given experiment. For small third-order ("terminal") arterioles, intermediate $\alpha_2$ constriction was produced by NE ($3 \times 10^{-8}$ M) in the presence of prazosin. A lower concentration of NE was used to induce $\alpha_1$ tone in these terminal arterioles because of their greater sensitivity to NE. During this intermediate NE constriction, the cremaster muscle was stimulated to contract at a given frequency for 5 minutes. A 5-minute poststimulation period was observed to allow recovery of adrenergic constriction before the sequence was repeated for a different contraction frequency. Each vessel was studied at five frequencies so that vascular behavior could be evaluated over a range of metabolic conditions. Based on preliminary experiments, $\alpha_2$-induced tone was subjected to contraction frequencies of 2, 4, 6, 8, and 12 Hz; for $\alpha_1$ constriction, frequencies of 0.5, 1, 2, 4, and 8 Hz were used. The order of presentation of the different frequencies was randomized among experiments. Stimulation of the muscle produced no change in heart rate or mean arterial pressure (left carotid artery). Contractions $\leq12$ Hz were nontetanic and did not produce skeletal muscle or vascular damage. Control experiments were done during neuromuscular junction blockade (0.5 mM succinylcholine plus 0.5 mM gallamine triethiodide added directly to the cremaster bath) or during combined neuromuscular junction and $\alpha$-receptor blockade ($5 \times 10^{-6}$ M phentolamine) to verify that the stimulation parameters that were used produced selective stimulation of somatomotor fibers. In addition to the imposed skeletal muscle contractions, tissue bath PO$_2$ was reduced 50% from control (from $27 \pm 1$ to $13 \pm 1$ mm Hg, by increasing N$_2$ aeration) to ensure that the bath did not act as a significant source of O$_2$ to the tissue.

At midprotocol, the cremaster bath was washed and refilled with fresh Krebs stock solution adjusted to the reduced bath PO$_2$. This was done to minimize accumulation of by-products of tissue metabolism in the fixed-volume tissue bath after repeated series of muscle stimulations and, therefore, to allow more accurate assessment of the effects of the muscle stimulations imposed during the second half of the experiment. Drugs were reintroduced into the bath at their original concentrations, and 10 minutes passed to permit recovery to constricted diameter. Data were discarded if the arteriole did not recover to within 10% of the original constricted diameter after this wash or after any skeletal muscle contraction period. Previous data indicate that in the presence of an intermediate amount of NE constriction, changing of the bath, per se, had no effect on arteriolar diameter. After completion of the final skeletal muscle stimulation period, nitroprusside ($3 \times 10^{-5}$ M) was added to the bath for 5 minutes to produce complete smooth muscle relaxation for determination of maximal diameter.

Large arterioles and especially small arterioles in this denervated preparation possess significant resting tone (dilation with nitroprusside to $\sim15$–$30\%$ greater than control for large arterioles and 200–300% greater than control for terminal arterioles). It has been shown that this "intrinsic tone" is unaffected by adrenergic antagonists. Experiments were done to examine whether skeletal muscle contraction had an effect on the substantial small arteriolar intrinsic tone. The protocol was the same as that described above, with the exception that no NE was added to the bath (i.e., there was no adrenergic tone).

A weakness of this study is that the protocol did not include methods to obtain direct measurements of tissue metabolic status during the imposed skeletal muscle contractions. Obtaining such measurements would not be trivial, and therefore, this apparent omission was due to technical considerations. For example, collection of blood samples from this microvascular preparation during the in situ conditions of our experiments would have required placement of micropipettes into the microvasculature. This would itself produce changes in blood flow and metabolic disturbances in the tissue. The small samples that could be obtained would be susceptible to significant contamination by atmospheric gases. In
addition, the sophisticated procedures and equipment required to obtain accurate measures of tissue metabolism were not available in our laboratory. Thus, although we recognize that measurements of metabolic indexes would have strengthened our study, we relied on previous work of others (see “Discussion”) to infer the metabolic changes that occur during muscle stimulation.

Data Analysis

During genitofemoral nerve stimulation and recovery periods, vessel diameter was measured at 30-second intervals. Due to tissue movement during contraction, the stimulus was interrupted for \( \leq 10 \) seconds to permit measurements. At all other times, except during wash or equilibration periods, vessel diameter was recorded at 1-minute intervals. Unless otherwise indicated, reported diameter values represent averages over the 5-minute test intervals. Measurements of large arterioles were made on-line. The small arterioles generally exhibited vasomotion (rhythmic cycles of spontaneous contraction and relaxation). Thus, instantaneous and electronically averaged terminal arteriolar diameter values were obtained with an electronic caliper by continuous measurement of vessel diameter for 10-second intervals during off-line analysis of videotape. The instantaneous and averaged caliper output were recorded on an oscillograph.

Statistical evaluations of the effect of muscle contraction on arteriolar diameter were made by comparison of the averaged diameter during a given contraction frequency to the averaged recovery diameter immediately preceding that contraction period. Data were normalized as a percentage of the maximal arteriolar dilation with nitroprusside. Unless otherwise stated, individual comparisons of paired data were made with Student’s \( t \) test. One-way analysis of variance with critical \( t \) values obtained by the Dunn-Bonferroni procedure was used for multiple comparisons. Results are expressed as mean±SEM, with \( p<0.05 \) representing significance.

Drugs

Gallamine triethiodide and succinylcholine were dissolved in saline. All other drugs were prepared as described previously. All drugs were obtained from Sigma Chemical Co., St. Louis, except for UK-14,304 and prazosin (Pfizer Laboratories, Kent, England), phentolamine (CIBA Pharmaceutical Co., Summit, N.J.), and succinylcholine (Burroughs Wellcome Co., Research Triangle Park, N.C.).

Results

Effect of Skeletal Muscle Contraction on \( \alpha \)-Adrenergic Constriction of Arterioles

Large (114–125-\( \mu \)m-diameter) arteriolar \( \alpha \) constriction was significantly attenuated at all frequencies (0.5–8 Hz) of skeletal muscle contraction (Figure 2, Table 1); \( \alpha \) constriction was opposed only at frequencies \( \geq 4 \) Hz. Even at the highest contractile frequencies examined, considerable tone persisted in both \( \alpha \) and \( \alpha \) groups. Diameter changes for the effect of 6-Hz stimulation on \( \alpha \) constriction and the effect of 1-Hz stimulation on \( \alpha \) constriction are shown in Figure 3. Maintained dilation with no distinct peak was evident over the 5-minute stimulation interval. This pattern of diameter change represented in Figure 3 is typical of the attenuation of \( \alpha \) constriction during all other stimulation frequencies and of \( \alpha \) constriction during stimulation \( \geq 4 \) Hz. Note here, and in Figure 2, that 1-Hz stimulation produced \( \sim 25\% \) inhibition of \( \alpha \) constriction, whereas 6-Hz stimulation produced approximately 15% inhibition of \( \alpha \) constriction, thus demonstrating the greater attenuation of \( \alpha \) constriction during skeletal muscle contraction. Comparison of the frequency of contraction necessary to produce inhibition of 20% of maximal dilation indicated that \( \alpha \) constriction was \( \sim 10\)-fold more sensitive than \( \alpha \) constriction to “metabolic” inhibition. Recovery of \( \alpha \) and \( \alpha \) tone to within 10% of the original constricted diameter occurred within 2 minutes for all stimulation frequencies (see also Figure 1). Mean recovery

![FIGURE 2](http://circres.ahajournals.org/)

**FIGURE 2.** Plot showing effect of skeletal muscle contraction (direct stimulation of decentralized genitofemoral nerve) on intermediate level of large arteriolar \( \alpha \) (norepinephrine [NE] + rauwolscine [RWJ]) and \( \alpha \) (NE+prazosin [PRZ]) constriction. Only \( \alpha \) or \( \alpha \) constriction was induced in a given experiment. One arteriole was studied per experiment. Inhibition of adrenergic constriction during muscle contraction is normalized as percent of maximal arteriolar dilation to nitroprusside (3×10\(^{-5}\) M). Statistical significance was determined by comparison of the mean diameter during the 5-minute contraction interval to the mean diameter of the 5-minute recovery period immediately preceding that contraction interval (see Table 1) and by one-tailed Student’s \( t \) test.
TABLE 1. Effect of Skeletal Muscle Contraction on \( \alpha_1 \) and \( \alpha_2 \) Constriction of Large Arterioles

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter (( \mu \m) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>125.2±15.0</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>113.8±13.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM; \( n=7 \) for each group. C, control diameter; CD, constricted diameter (intermediate \( \alpha_1 \) or \( \alpha_2 \) constriction); NP, maximal diameter after addition of nitroprusside; Recovery, mean diameter during the 5-minute recovery (of adrenergic constriction) interval immediately preceding cremaster muscle stimulation at a given contraction frequency; Stimulation, mean diameter during the 5-minute interval of muscle contraction produced by direct stimulation of the decentralized genitofemoral nerve. (See Figure 1 for graphic representation of experimental protocol.)

\( *p<0.05 \) vs. C; \( t p<0.05 \) and \( \ddot{p}<0.01 \) vs. Recovery at corresponding contraction frequency.

time for \( \alpha_1 \) experiments was \(-50\) seconds; for \( \alpha_2 \) experiments, mean recovery time was \(-90\) seconds.

The intermediate level of constriction induced in the large arterioles, expressed as percent reduction from control diameter, was 40±3% for \( \alpha_1 \) constriction and 35±3% for \( \alpha_2 \) constriction (Table 1). Based on previous data,\(^{10,14}\) this represents 55–65% of the maximal adrenergic constriction that can be achieved by large arterioles. Control diameters for the \( \alpha_1 \) and \( \alpha_2 \) groups were not significantly different from each other, and recovery diameters within a group were not different (Table 1). Thus, arteriolar diameters before cremaster stimulation were not different either between or within the agonist groups. The amount of basal (intrinsic) tone present in the arterioles was indicated by comparison of nitroprusside-induced maximal dilation to control diameter (Table 1). The maximal large arteriolar diameter was 9% and 12% greater than control for the \( \alpha_1 \) and \( \alpha_2 \) groups, respectively. This amount of large arteriolar resting tone is comparable to that observed in other studies in this laboratory\(^{7,8,14}\) and suggests that skeletal muscle contraction did not irreversibly inhibit the intrinsic smooth muscle tone in these large arterioles. The presence of an appropriate amount of intrinsic tone in large and small arterioles (see below) was an important criterion used to judge the suitability of the preparation.

Significant dilation of small (27-\( \mu \)m-diameter) arterioles from constricted diameter was produced by all frequencies of skeletal muscle contraction \( \geq 1 \) Hz (Figure 4, Table 2). In these small arterioles, the intermediate concentration of NE produced 45±3\% reduction from control diameter (Table 2), or approximately 65\% of the maximal adrenergic constriction that can be achieved by small arterioles.\(^{14}\) Arteriolar recovery diameters before the various stimulation frequencies were not different (Table 2). The maximal small arteriolar diameter as indicated with nitroprusside (Table 2) was 289\% greater than control, indicating the presence of a substantial amount of intrinsic tone as is commonly observed in these terminal arterioles. The pattern of diameter change during muscle contraction was similar to that shown in Figure 3 for the large arterioles.

Although there was substantial dispersion among the data, regression analysis indicated significant correlation between absolute change in arteriolar diameter and stimulation frequencies that produced significant dilation of \( \alpha_1 \) or \( \alpha_2 \) constriction: 0.5–8 Hz

![Figure 3. Plot showing averaged large arteriolar diameter changes for the effect of muscle contraction on \( \alpha \)-adrenergic constriction of large arterioles.](http://circres.ahajournals.org/)

**Figure 3.** Plot showing averaged large arteriolar diameter changes for the effect of muscle contraction on \( \alpha \)-adrenergic constriction of large arterioles. **•**, Effect of 6-Hz muscle contraction on intermediate level of \( \alpha_1 \) constriction; **○**, effect of 1-Hz muscle contraction on intermediate level of \( \alpha_2 \) constriction. Maintained dilation with no distinct peak is evident over the 5-minute contraction interval. Data are normalized as percent of maximal arteriolar dilation to nitroprusside (3\( \times 10^{-5} \) M).
for $\alpha_2$ constriction of large arterioles ($r=0.664$, slope=2.54), 4–12 Hz for $\alpha_1$ constriction of large arterioles ($r=0.528$, slope=2.00), and 1–8 Hz for $\alpha_2$ constriction of small arterioles ($r=0.419$, slope=2.99); $p<0.05$ for all $r$ values.

**Effect of Skeletal Muscle Contraction on Intrinsic Tone**

Because intrinsic or basal tone was evident in the large and especially prominent in the small arterioles, dilation of vessels during skeletal muscle contraction could arise from concomitant inhibition of both adrenergic and intrinsic tone and thus complicate interpretation. Nitroprusside dilated large arterioles to ~10% greater than the initial control diameter (Table 1). Therefore, even total inhibition of large arteriolar intrinsic tone could not account for the degree of inhibition observed for either $\alpha_1$ or $\alpha_2$ tone in these large arterioles. Because of the substantial amount of intrinsic tone present in the small arterioles, an additional group of experiments was done to examine whether muscle contraction had an effect on this intrinsic tone. In the presence of prazosin alone, without NE application, skeletal muscle contraction frequencies <8 Hz did not significantly dilate terminal arterioles (Figure 5, Table 3). Maximal diameter (nitroprusside dilation) of these small arterioles was 302% of the initial control diameter (Table 3). Thus, these arterioles and the small arterioles in the experiments in which we examined the effect of muscle contraction on $\alpha_2$ constriction had similar amounts of intrinsic tone.

**Control Experiments**

The somatomotor nerves in the mixed genitofemoral nerve, which supplies the major cremaster innervation, should be selectively activated by low-voltage, short-duration stimuli. Control experiments were done during neuromuscular junction blockade or combined neuromuscular junction and $\alpha$-receptor blockade to determine if our results were complicated by concomitant stimulation of autonomic afferent and efferent nerves during stimulation of the mixed genitofemoral nerve. Although stimulus parameters were chosen to selectively activate somatomotor nerves, this remained a possibility. As shown in Figure 6, the stimulus parameters used in this study did not directly affect arteriolar diameter and, thus, produced selective stimulation of somatomotor fibers. Additional control experiments verified that an intermediate level of $\alpha_1$ (NE+rauwolscine) and $\alpha_2$ (NE+prazosin) constriction was maintained for 50 minutes (Figure 7), which approximates the period in the above protocols over which we examined the effect of skeletal muscle contraction on comparable intermediate $\alpha_1$ and $\alpha_2$ constriction.

We have previously established the potency and selectivity of prazosin ($\alpha_2$-antagonist) in the rat cremaster muscle. However, the selectivity of the $\alpha_2$-antagonist rauwolscine in this preparation had not

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**TABLE 2. Effect of Skeletal Muscle Contraction on $\alpha_2$-Adrenergic Constriction of Small Arterioles**

<table>
<thead>
<tr>
<th>Period</th>
<th>Diameter (µm)</th>
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<tbody>
<tr>
<td>C</td>
<td>26.9 ± 5.3</td>
</tr>
<tr>
<td>CD</td>
<td>14.9 ± 3.4*</td>
</tr>
<tr>
<td>NP</td>
<td>66.9 ± 14.2*</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Contraction frequency</th>
<th>Recovery</th>
<th>Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Hz</td>
<td>14.5 ± 2.9</td>
<td>16.4 ± 2.8</td>
</tr>
<tr>
<td>1 Hz</td>
<td>14.2 ± 3.4</td>
<td>21.0 ± 3.6†</td>
</tr>
<tr>
<td>2 Hz</td>
<td>13.1 ± 3.2</td>
<td>27.3 ± 4.3†</td>
</tr>
<tr>
<td>4 Hz</td>
<td>11.8 ± 2.4</td>
<td>41.6 ± 10.2‡</td>
</tr>
<tr>
<td>8 Hz</td>
<td>15.9 ± 3.9</td>
<td>44.6 ± 10.3‡</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; $n=7$ arterioles. C, control diameter; CD, constricted diameter (intermediate $\alpha_2$ constriction); NP, maximal diameter after addition of nitroprusside; Recovery, mean diameter during the 5-minute recovery (of adrenergic constriction) interval immediately preceding cremaster muscle stimulation at a given contraction frequency. Stimulation, mean diameter during the 5-minute interval of muscle contraction produced by direct stimulation of the decentralized genitofemoral nerve. (See Figure 1 for graphic representation of experimental protocol.)

* $p<0.05$ vs. C; † $p<0.01$ and ‡ $p<0.05$ vs. Recovery at corresponding contraction frequency.
been evaluated before this study. We examined the effect of rauwolscine on selective \( \alpha_1 \) (phenylephrine) and \( \alpha_2 \) (UK-14,304) large arteriolar constriction \((n=21\) vessels and rats\) by obtaining cumulative concentration–response curves for these agonists in the absence and presence of rauwolscine \((1\times10^{-7} \text{M})\). Rauwolscine decreased \((30\)-fold\) the concentration–response curve for UK-14,304 but did not affect the concentration–response curve for phenylephrine. \( EC_{50} \) values for the UK-14,304 concentration–response curve were \( 6.33\pm0.13 \) and \( 5.05\pm0.35 \) in the absence and presence of rauwolscine, respectively \((p<0.01)\); for the phenylephrine concentration–response curve, these values were \( 5.44\pm0.22 \) and \( 5.52\pm0.58 \), respectively \((p=0.40)\). Thus, \( 1\times10^{-7} \text{M} \) rauwolscine is selective for \( \alpha_2 \)-receptors on vascular smooth muscle of cremaster arterioles.

For all rats studied, the mean arterial pressure and heart rate at the beginning of the experiments were \((\text{mean}\pm\text{SEM}) \ 90\pm1 \text{mm Hg and } 438\pm5 \text{beats/min}\), respectively; at the end of the experiments, values were \( 91\pm1 \text{mm Hg and } 442\pm6 \text{beats/min}\), respectively. Regression analysis indicated no significant change in these parameters over the duration of the experiments.

**Discussion**

The major finding of this study was that \( \alpha_2 \) constriction of both large and small arterioles was significantly attenuated at all frequencies of skeletal muscle contraction \( \geq1 \text{ Hz}\), whereas large arteriolar \( \alpha_1 \) constriction was reduced only during muscle contractions \( \geq4 \text{ Hz}\). The magnitude of the increase in arteriolar diameter is correlated with skeletal muscle contraction frequency.

It is important to distinguish between the effect of muscle activity and metabolic inhibition on adrenergic constriction versus a possible effect on intrinsic tone. When in the presence of prazosin alone \((\text{no NE})\), skeletal muscle contraction frequencies \(<8 \text{ Hz}\) did not significantly dilate terminal arterioles \((\text{Figure 5, Table 3})\), indicating no effect of low-frequency muscle contraction on small arteriolar intrinsic tone. Thus, the significant attenuation of \( \alpha_2 \) \((\text{NE+ prazosin})\) constriction of arterioles during low contraction frequencies cannot be attributed to loss of intrinsic tone. It is possible, however, that a component of the inhibition of adrenergic constriction during higher frequency skeletal muscle contractions may be due to inhibition of intrinsic tone. During 2-Hz contraction frequency, small arterioles that were constricted \((\text{NE+prazosin})\) dilated to a diameter that was not significantly different from the original resting control diameter \((\text{Table 2})\). Thus, complete and selective inhibition of \( \alpha_2 \) constriction of small arterioles was produced by 2-Hz muscle contraction. In contrast \((\text{Table 2})\), muscle contraction frequencies \(>2 \text{ Hz}\) dilated \( \alpha_2 \)-constricted small arterioles.

**TABLE 3. Effect of Muscle Contraction on Small Arteriolar Intrinsic Tone**

<table>
<thead>
<tr>
<th>Period</th>
<th>Diameter (( \mu \text{m} ))</th>
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<tbody>
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<td>C</td>
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<tr>
<td>NP</td>
<td>30.5±2.7*</td>
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</table>

<table>
<thead>
<tr>
<th>Contraction frequency</th>
<th>Diameter (( \mu \text{m} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery</td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>8.4±1.3</td>
</tr>
<tr>
<td>1 Hz</td>
<td>9.9±2.3</td>
</tr>
<tr>
<td>2 Hz</td>
<td>8.5±1.8</td>
</tr>
<tr>
<td>4 Hz</td>
<td>7.2±1.3</td>
</tr>
<tr>
<td>8 Hz</td>
<td>10.1±2.5</td>
</tr>
<tr>
<td>12 Hz</td>
<td>7.8±1.1</td>
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</tbody>
</table>

Values are mean±SEM; \( n=5 \) arterioles. C, control diameter; NP, maximal diameter after addition of nitroprusside; Recovery, mean diameter during the 5-minute recovery \((\text{of resting tone})\) interval immediately preceding cremaster muscle stimulation at a given contraction frequency; Stimulation, mean diameter during the 5-minute interval of muscle contraction produced by direct stimulation of the decentralized genitofemoral nerve.

*\( p<0.05 \) vs. C; \( f p<0.05 \) vs. Recovery at corresponding contraction frequency.
rioles to diameters greater than original resting control diameter, reflecting inhibition of both adrenergic and intrinsic tone during 4- and 8-Hz muscle contraction. Because large arterioles have a relatively small amount of intrinsic tone (Table 1), any component of dilation of large arterioles that is due to inhibition of intrinsic tone during exercise should be minimal. Even if large arteriolar intrinsic tone was totally inhibited during high-frequency muscle contractions, this could not account for the inhibition of α₁- or α₂-adrenergic constriction observed at this level of the microcirculation.

Overall regulation of microvascular function involves complex interactions between local myogenic and metabolic mechanisms and extrinsic neural and humoral mechanisms. In other studies from this laboratory, it was observed that α₂ constriction was more sensitive than α₁ constriction to myogenic inhibition produced by reduced transmural pressure. Skeletal muscle contraction may raise interstitial pressure (decrease transmural pressure) and, thus, provide a stimulus for myogenic inhibition of vascular tone. Although evidence indicates that myogenic relaxation of vascular smooth muscle may be important for the vascular responses during twitch contractions, it does not contribute significantly to arteriolar dilation during twitch contractions. Therefore, the results of our study using twitch contraction should not be influenced by myogenic complications secondary to the physical effects of muscle contraction. It is possible, however, that a component of the attenuation of large arteriolar α₁ and α₂ constriction during high-frequency skeletal muscle contraction may be due to reduced intravascular pressure and myogenic relaxation subsequent to small arteriolar dilation.

Additional studies from our laboratory (S.M. Muldowney, J.E. Faber, personal communication, 1990) have demonstrated that both α₁ and α₂ constriction of arterioles is inhibited during simultaneous reduction of cremaster intravascular pressure and flow. It is important to note that arteriolar constriction produced by direct activation of the contractile apparatus with potassium chloride was not reduced despite the significant metabolic and myogenic dilator stimuli present during these conditions. These data indicate
that dilation due to metabolic or myogenic stimuli does not result from a direct effect on the contractile apparatus of vascular smooth muscle cells and suggest that the inhibition of adrenergic tone demonstrated in the present study may not arise from a generalized decrease in vascular smooth muscle contractility.

Large arteriolar $\alpha_1$ constriction (the $\alpha$-adrenergic receptor population that is selectively activated by direct neural stimulation$^9$) was maximally attenuated by 8-Hz muscle contraction (Figure 2). However, during 8-Hz stimulation, large arteriolar diameter was still less than the initial resting control diameter (Table 1), indicating that adrenergic regulation of large arteriolar diameter (i.e., blood flow) is not completely inhibited by metabolic control mechanisms. These data are consistent with previous studies of whole limb or isolated muscle preparations that demonstrated maximal conductance at 8-Hz contraction$^4$ and incomplete inhibition of adrenergic control of blood flow during skeletal muscle exercise$^{13,20,23-25}$ and are also consistent with recent evidence that blood pressure may be maintained during dynamic exercise by persistent reflex-induced vasoconstriction.$^{26,27}$

Gorczynski et al.$^{21}$ previously examined the effect of cremaster muscle contraction on resting small arteriolar diameter and capillary density. Although differences in experimental conditions (e.g., adrenergic versus nonadrenergic tone was not defined) make direct comparison of our results difficult, interesting similarities exist. They observed an increase in capillary density and change in small arteriolar diameter that was proportional to contraction frequency between 1 and 4 Hz and maximal at 4 Hz. Although the magnitude of arteriolar diameter change is different in the two studies, we demonstrate the same relation for the effect of cremaster contraction or $\alpha_2$ constriction of small arterioles. Gorczynski et al.$^{21}$ observed an early peak dilation $\sim$30 seconds after the onset of muscle contraction and a further secondary dilation to maximum steady-state diameter at 80–110 seconds. During muscle contraction, at frequencies that significantly inhibited $\alpha_1$ and $\alpha_2$ constriction, we generally observed substantial dilation by the first measurement (30 seconds after onset of muscle contraction). In some cases this represented near steady-state diameter; in others it represented a distinct early peak that was followed by further dilation to steady-state diameter (1.5–2 minutes after onset of contraction). No distinct peaks were evident in our averaged time course data (Figure 3) because of variation in the response pattern of individual arterioles. Mean averaged recovery times were also similar in the two studies. Consistent with this response of arteriolar diameters during muscle contraction, Mohrman and Sparks$^{28}$ observed biphasic resistance changes in exercising dog calf muscles.

It is not clear which metabolic signals couple skeletal muscle activity and oxygenation to microvascular smooth muscle function. Studies in which no adrenergic tone was induced have demonstrated that arteriolar dilation occurs during muscle exercise without a reduction in tissue $P_{O_2}$.$^{29-31}$ Gorczynski and Duling$^{30}$ reported that the early peak arteriolar dilation occurred independent of changes in muscle $P_{O_2}$, whereas the secondary dilation to steady-state diameter was associated with muscle hypoxia; they also proposed that the early dilation is mediated by oxygen-independent factors directly associated with muscle contraction such as transmembrane ion fluxes (e.g., $K^+$) or by-products of ATP hydrolysis (e.g., adenosine, $P_i$, and $H^+$), whereas later secondary dilation is mediated, at least in part, by factors directly associated with tissue oxidative metabolism.

Thompson and Mohrman$^{33}$ recently reported that adrenergic constriction (sympathetic stimulation at 0.5 and 1 Hz) did not limit muscle oxygen consumption at rest or during 1-Hz muscle contraction but did significantly reduce muscle oxygen consumption during 4-Hz exercise. Our data indicate that $\alpha_2$ constriction of arterioles is significantly and proportionately attenuated during muscle contractions between 0.5 and 4 Hz and that 4 Hz produces maximal small arteriolar dilation; $\alpha_1$ constriction is attenuated only during contractions $\geq$ 4 Hz. Based on the observations of Thompson and Mohrman and the present data, we suggest the following modification of the original proposal of Gorczynski and Duling.$^{30}$ During light or early stages of exercise, oxygen-independent factors directly associated with muscle contraction may attenuate neural ($\alpha_2$) constriction of small arterioles to cause small arteriolar dilation and increased capillary density. In contrast, during more intense or later stages of exercise, when muscle may become hypoxic, metabolic factors directly associated with tissue oxidative metabolism, as well as even greater concentrations of oxygen-independent factors, may oppose large arteriolar neural ($\alpha_1$) constriction and intrinsic tone at both vessel levels to produce increased muscle blood flow. The net result would be optimization of flow distribution and capillary–tissue oxygen exchange at the onset of activity or during light exercise, followed by enhanced oxygen delivery during later stages or heavier exercise, and efficient and economical maintenance of the blood flow/$O_2$ extraction ratio during increased metabolic demand. This proposal is consistent with the previous evidence that capillary recruitment and increased oxygen extraction precede hyperemia in the defense against muscle hypoxia$^{2,3,32}$ and that small and large arterioles can be regulated independently.$^{32}$ Moreover, the model is supported by our previous evidence$^{14}$ that acidosis consistent with that obtained in muscle during light-to-moderate exercise selectively attenuates $\alpha_2$ constriction.

Another study from our laboratory$^{16}$ demonstrated that large arteriolar $\alpha_1$ constriction was threefold to fourfold more sensitive than $\alpha_2$ constriction to inhibition by purinoreceptor stimulation with physiological concentrations of adenosine ($10^{-7}$–$10^{-6}$ M). Evidence indicates that adenosine contributes to local control of skeletal muscle blood flow only when muscle is likely to be hypoxic (e.g., during long-duration or...
flow-restricted exercise)\(^3\)\(^3\)\(^3\)\(^3\)\(^3\) and suggests a greater role of adenosine in functional hyperemia during muscle contractions >2 Hz.\(^3\)\(^5\) Because we initially induce intermediate adrenergic constriction of large arterioles in our experiments (i.e., flow is restricted), it is likely that the cremaster muscle tissue oxygen decreases during 5 minutes of contraction at ≥4 Hz,\(^2\)\(^4\) thereby increasing the local concentration of adenosine. Thus, we speculate that at least a portion of the attenuation of large arteriolar α\(_1\) constriction during contractions ≥4 Hz may be attributed to adenosine.

To summarize, low frequency (≤2 Hz) skeletal muscle contraction selectively attenuated α\(_2\)-adrenergic constriction of small and large arterioles. Contraction frequencies ≥4 Hz significantly attenuated α\(_1\) constriction and further reduced α\(_2\) constriction. Inhibition of intrinsic tone may contribute to reduction of adrenergic tone during the higher contraction frequencies. Our previous studies\(^7\)\(^-\)\(^9\)\(^,\)\(^1\)\(^4\) indicate that neural constriction of large arterioles, which have both postjunctional α\(_1\)- and α\(_2\)-adrenoceptors, is mediated by α\(_1\)-receptors, whereas small terminal arterioles depend on α\(_2\)-receptors. The present data suggest that the dominance of terminal arterioles by α\(_2\)-receptors and a preferential sensitivity of α\(_1\)-mediated constriction to vasodilator metabolites associated with light exercise may underlie the particular sensitivity of these precapillary arterioles to metabolic inhibition during exercise. In contrast, neural control of large arterioles by α\(_1\)-receptors and the differential sensitivity of α\(_1\) constriction to inhibition by dilator metabolites associated with light versus heavier exercise would preserve adrenergic control of large arterioles during light exercise but permit metabolic inhibition of large arterioles during heavier exercise. Such a hierarchy would maintain the most economical ratio of capillary flow and oxygen extraction to blood flow (oxygen delivery) during different levels of muscle oxygen demand and might provide a control system that minimizes loss of reflex control of resistance arterioles while maximizing local regulation of skeletal muscle oxygenation.

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References


**KEY WORDS** • vascular smooth muscle microcirculation • α-adrenergic receptors • metabolic regulation
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