Effect of Reduced Blood Flow on $\alpha_1$- and $\alpha_2$-Adrenoceptor Constriction of Rat Skeletal Muscle Microvessels

Karen M. McGillivray-Anderson and James E. Faber

Adrenergic constriction of skeletal muscle arterioles, particularly small terminal arterioles, is opposed by decreased blood flow or increased metabolic rate. Our previous studies indicate that neural constriction of large arterioles, which have both postjunctional $\alpha_1$- and $\alpha_2$-adrenoceptors, is mediated by $\alpha_1$-receptors; small arterioles depend on $\alpha_2$-receptors. Also, $\alpha_5$, but not $\alpha_7$, constriction is reduced by acidosis. Differential sensitivity of $\alpha_1$ versus $\alpha_2$ constriction to metabolic signals such as $H^+$ may underlie the sensitivity of arteriolar adrenergic constriction to metabolic inhibition. To examine this hypothesis, we studied the effect of reduced perfusion on $\alpha_1$- versus $\alpha_2$-mediated constriction of large arterioles and venules. Intravital microscopy of rat cremaster skeletal muscle was used to obtain concentration–response curves for phenylephrine ($\alpha_1$-agonist) and UK-14,304 ($\alpha_2$-agonist). Thirty percent reduction in cremasteric artery flow by venous outflow obstruction had no effect on baseline diameter, indicating no effect on “intrinsic tone.” Reduced perfusion also had no effect on arteriolar or venular sensitivity to phenylephrine or venular sensitivity to UK-14,304 but significantly attenuated arteriolar response to UK-14,304. To examine a possible mechanism for the selective inhibition of $\alpha_1$ constriction by acidosis, we determined the effect of acidosis on the partial $\alpha_1$-agonist St587. Like $\alpha_2$ constriction, St587-mediated constriction of arterioles was reduced during acidosis and was attenuated by nifedipine. These data suggest that 1) increased local concentrations of tissue metabolites during reduced perfusion may attenuate neural constriction of small arterioles because of their reliance on $\alpha_2$-receptors and sensitivity of $\alpha_2$ constriction to metabolic inhibition, 2) the selective sensitivity of arteriolar $\alpha_2$ constriction to $H^+$ and possibly to other metabolites may be due to the reliance of $\alpha_2$ constriction on nifedipine-sensitive calcium channels, and 3) venular $\alpha_2$ constriction is less sensitive to metabolic regulation. (Circulation Research 1991;69:165–173)

Sympathetic modulation of microvascular tone is achieved primarily through the action of neuronally released norepinephrine (NE) on $\alpha$-adrenergic receptors located on vascular smooth muscle cells. Adrenergic constriction of arterioles, particularly small terminal arterioles, is opposed by local metabolic control. However, the mechanisms by which neural and metabolic signals interact within the microvasculature are not well understood. Based on the sensitivity of selective agonists and antagonists, we have found in skeletal muscle microvasculature that large arterioles and venules have both postjunctional $\alpha_1$- and $\alpha_2$-adrenoceptors, whereas terminal arterioles appear to have only $\alpha_2$-receptors.1,2 Additional studies from our laboratory3 using direct nerve stimulation have demonstrated that only $\alpha_1$-adrenoceptors mediate constriction of large resistance arterioles, even though both adrenoceptor subtypes are present. In contrast, neural constriction of small arterioles is mediated by $\alpha_2$-receptors.3 Modest tissue acidosis (pH 7.1) significantly decreases $\alpha_2$-mediated constriction but does not affect $\alpha_1$-mediated constriction.4

Based on these findings, we4 proposed that the distinct neural control of large arterioles by $\alpha_1$-receptors and of small arterioles by $\alpha_2$-receptors and a greater sensitivity of $\alpha_2$ contraction to acidosis (and possibly to other local metabolic feedback signals)
may underlie the particular susceptibility of adrenergic constriction of terminal arterioles to metabolic inhibition. These interactions could be important for microvascular integration of neural and metabolic control mechanisms. The purpose of this study was to further examine the influence of the tissue metabolic environment on adrenergic constriction of microvessels. We examined the effect of a potential “mismatch” between oxygen supply and demand, created by reducing cremaster muscle perfusion, on $\alpha_2$- versus $\alpha_2$-mediated constriction of arterioles and venules. In agreement with the selective effect of acidosis on $\alpha_2$ constriction, reduced tissue perfusion selectively attenuated arteriolar $\alpha_2$ constriction.

The mechanism responsible for the selective sensitivity of $\alpha_2$-mediated constriction to metabolic inhibition is unclear. One possibility may relate to a different reliance of $\alpha_2$- versus $\alpha_2$-adrenoceptor responses on influx of extracellular calcium. $\alpha_2$- Receptors are coupled to transmembrane influx of calcium through dihydropyridine-sensitive calcium channels. In contrast, dependence of $\alpha_2$-adrenoceptor constriction on this mechanism can vary: full $\alpha_2$-agonists (i.e., phenylephrine [PE] and NE— as used in our previous study) exhibit minimal sensitivity to organic calcium channel antagonists, whereas partial $\alpha_2$-agonists are primarily dependent on influx of extracellular calcium to produce vasoconstriction.

Studies from our laboratory indicate that $\alpha_2$ constriction of cremaster arterioles is much more sensitive to calcium channel antagonists than is constriction to full $\alpha_2$-agonists. Recent evidence suggests that acidosis inhibits calcium channel conductance. If the mechanism by which metabolic vasodilator signals selectively inhibit $\alpha_2$ constriction involves inhibition of calcium influx, acidosis should decrease the effectiveness of any $\alpha$-agonist that produces constriction primarily via dihydropyridine-sensitive calcium influx. Thus, in this study we also examined the effect of acidosis and nifedipine on arteriolar constriction produced by the partial $\alpha_2$-agonist St587.

**Materials and Methods**

**Surgical Procedures**

Experiments were performed on 6–7-week-old male Sprague-Dawley rats (body weight, 165±1 [mean±SEM]; n=47) that were anesthetized with urethane and $\alpha$-chloralose (425 and 100 mg/kg i.p., respectively) as described elsewhere. Rectal temperature was maintained at 37°C, and the rats breathed room air spontaneously via tracheostomy.

The right cremaster muscle was acutely denervated via abdominal approach by transecting the right lateral cutaneous, iliohypogastric, ilioinguinal, and genitofemoral nerves. This produces complete denervation of the cremaster but has no effect on the sensitivity of the microvasculature to topically applied vasoactive agents. Denervation prevented variations in nerve activity and release of NE during anesthesia and prevented possible complications of interpretation presented by interaction of agonists with presynaptic $\alpha_2$-adrenoceptors. The cremaster muscle was suspended in a tissue bath containing a modified Krebs solution and was prepared for in situ microvascular observation as described previously. Nitrogen and CO$_2$ were bubbled through both the tissue bath and a Krebs stock reservoir to provide mixing and to maintain PO$_2$ (20–30 mm Hg), PCO$_2$ (35–45 mm Hg), and pH (7.4±0.05). Tissue bath and stock solution pH were continuously monitored, and tissue bath temperature was maintained at the normal cremaster in situ temperature (34°C). Tissue bath and stock solution PO$_2$ was monitored by an oxygen macroelectrode (model IL 113, Allied Instrumentation Laboratory, Lexington, Mass.). Tissue bath parameters were digitized (1 Hz) and computer-displayed for analysis of preparation stability.

The preparation was placed on the stage of a trinocular microscope, and the cremaster microcirculation was observed by video microscopy and a digital image analysis system (Force Computers Ltd., Munich, FRG; Datacube, Inc., Peabody, Mass.) as described in detail elsewhere. A long-pass filter (model 450FFH90, Andover Corp., Salem, N.H.) was used to prevent exposure of the cremaster muscle to wavelengths below 420 nm, which may affect vascular smooth muscle and can also inactivate dihydropyridines. Experiments were conducted in a dark room, and tissue illumination was restricted to 10–20-second intervals once per minute. The preparation was allowed to equilibrate for approximately 30 minutes and was considered acceptable according to previously described criteria.

For the reduced perfusion experiments, microvascular measurements were made on either a paired first-order arteriole (diameter, 128±5 [mean±SEM] µm; n=21) and venule (diameter, 180±5 µm; n=21) or a paired second-order arteriole (diameter, 101±9 µm; n=5) and venule (diameter, 122±8 µm; n=5) as defined previously. The adrenergic sensitivity of first- and second-order vessels does not differ significantly. Thus, results have been combined and reported as large arterioles and venules. The cremaster bath Krebs solution contained, at all times, propranolol (10$^{-6}$ M) for blockade of $\beta$-adrenergic receptors, cocaine (4×10$^{-6}$ M) or desipramine (10$^{-8}$ M) for blockade of neuronal catecholamine uptake mechanisms, and normetanephrine (10$^{-5}$ M) for blockade of nonneuronal catecholamine uptake mechanisms. Drug concentrations expressed here and elsewhere represent final bath concentrations.

The effect of reduced tissue perfusion on $\alpha$-adrenoceptor-mediated constriction of large arterioles and venules was evaluated with concentration-reponse curves (CRCs) to selective $\alpha$-agonists determined during normal tissue blood flow and tissue bath PO$_2$ (20–30 mm Hg) and during reduced cremaster blood flow and bath PO$_2$ (12 mm Hg). Bath PO$_2$ was reduced (increased N$_2$ aeration) to ensure that the bath did not act as a source of O$_2$ to the tissue during reduced blood flow. $\alpha_2$ constriction was
induced by the selective $\alpha_2$-adrenoceptor agonist UK-14,304 in the presence of the $\alpha_1$-antagonist prazosin (1 x 10$^{-8}$ M, n=12); $\alpha_1$ constriction was produced by the selective $\alpha_1$-agonist PE in the presence of the $\alpha_2$-antagonist yohimbine (5 x 10$^{-7}$ M, n=14). A single agonist was evaluated per experiment. The protocol (Figure 1) was identical for both agonists. After a 30-minute stabilization period at normal flow and tissue bath PO$_2$, vessel diameter was measured for a 5-minute control period (C1, Figure 1). An additional 15-minute control period followed, during which cremaster blood flow and bath PO$_2$ were either maintained at normal control levels, or reduced. Vessel diameter was measured during the last 5 minutes of this second control period (C2, Figure 1). A CRC was then constructed by stepwise cumulative addition of agonist to the cremaster bath. The concentration was increased in approximately half-log increments every 5 minutes. Previous studies$^{1,4}$ indicated, and present studies confirmed, that 3-minute intervals were sufficient to obtain the maximal, steady-state response to a given concentration of these agonists when they were applied in approximately half-log increments starting from concentrations less than or equal to threshold level. Previous studies$^{4,15}$ also demonstrated that large vessel sensitivity to UK-14,304 and PE is maintained over the time interval necessary to complete a single CRC. Only one CRC was obtained per experiment, and whether the CRC was determined at normal or reduced tissue flow was randomized among experiments. In those experiments in which cremaster blood flow was reduced, flow was returned to control level on completion of the CRC. After completion of the CRC, the cremaster bath was changed three times over 10 minutes with Krebs stock solution maintained at normal PO$_2$. An additional 15–20 minutes was allowed to pass to permit vessels to return to control diameter.$^{1,2,4}$ After a third (5-minute) control period (C3), maximal adrenergic constriction was determined by a 10-minute exposure to a high concentration of NE (10$^{-5}$ M), previously determined to produce maximal large vessel constriction.$^{1,2,4}$ All concentration–response data were normalized to this maximal response. The cremaster bath was then washed (two changes over 5 minutes), and papaverine (3 x 10$^{-4}$ M) was added to the bath for 5 minutes to produce complete smooth muscle relaxation for determination of maximal diameter.

Reduction of cremaster blood flow was achieved via servo-controlled stenosis (perivascular pneumatic occluder) of the right common iliac vein. The stenosis was adjusted to produce 50% reduction of right common iliac artery blood flow, as monitored by a Doppler flow probe placed on the artery. Venous rather than arterial stenosis was used to prevent a decrease in microvascular pressure and the attendant myogenic inhibition of vascular tone.$^{16}$ The imposed 50% reduction in common iliac artery flow produced a 28 ± 3% (n=8) decrease in cremaster inflow as
determined by measurement of center-line red blood cell velocity in the main cremaster arteriole by a periodic differential detector grating velocimeter and according to the following equation: \( Q_r = \frac{V}{1.6} \times (\pi D^2/4) \), where \( Q_r \) is the total volumetric blood flow in the first-order arteriole, \( V \) is the center-line red blood cell velocity, and \( D \) is the diameter of the arteriole. This calculation is based on studies showing that in glass tubes (18–90-\( \mu \)m diameter) center-line velocity of blood flow is 1.6 times the average blood velocity. Total volumetric flow in the main cremaster arteriole provides a good index of tissue perfusion. In control experiments, the iliac vein occluder and iliac artery flow probe were placed on the vessels, but flow was not reduced.

In a second experiment, 16 rats were studied for the effect of acidosis (pH 7.1) on large arteriolar (107±8 \( \mu \)m) sensitivity to the partial \( \alpha_1 \)-agonist St587. The protocol was similar to that described above and depicted in Figure 1 with the exception that tissue bath pH, not tissue blood flow, was the altered variable. A single large arteriole was studied per experiment. Bath pH was either maintained at 7.4±0.05 or changed to 7.1±0.05 (randomized among experiments) by increasing the rate of flow of \( CO_2 \) into the cremaster bath. A CRC was obtained for St587 in the presence of the \( \alpha_2 \)-antagonist yohimbine (5\( \times \)10\(^{-7} \) M). On completion of the CRC, the cremaster bath was washed three times over 10 minutes with stock Krebs solution maintained at pH 7.4, and the tissue bath \( CO_2 \) flow rate was readjusted to maintain normal bath pH. Vessel diameter was allowed to return to control; maximal adrenergic constriction and then maximal arteriolar diameters were determined as described above.

Additional experiments were done to examine whether St587 produced constriction of large arterioles (139±14 \( \mu \)m, \( n = 5 \)) in our preparation by calcium-influx–dependent mechanisms. St587 CRCs were obtained in the presence of the dihydropyridine calcium channel antagonist nifedipine (7\( \times \)10\(^{-5} \) M), added to the cremaster bath during the 30-minute equilibration period. Previous studies from this laboratory demonstrated that nifedipine has no effect on resting tone (i.e., no effect on baseline diameter). These experiments were done with the protocol described above, with the exceptions that bath pH was maintained at 7.4 throughout the protocol and nitroprusside (3\( \times \)10\(^{-5} \) M) was used to completely relax smooth muscle to obtain maximal arteriolar diameter. Nitroprusside and papaverine produce comparable maximal dilation in this preparation.

Data Analysis

On-line measurements of vessel diameter were obtained at 1-minute intervals throughout the experiment, except during wash or equilibration periods. Control diameters represent the average of five measurements taken at 1-minute intervals over the last 5 minutes of the control period (no agonists present). Unless otherwise indicated, values for agonist responses represent average diameter during the last 2 minutes of the test period. For construction of CRCs, agonist responses are expressed as a percentage of the maximal response to NE: response = [(\( D_c - D_m \)) \( \div (D_c - D_m) \)] \( \times 100 \), where \( D_c \) is the control diameter, \( D_m \) is the diameter produced by \( x \) concentration of agonist, and \( D_m \) is the diameter obtained during maximal constriction with NE (1\( \times \)10\(^{-5} \) M). CRCs were derived from nonlinear, least-squares sigmoid regression analysis of the concentration–response data.

Data were analyzed with paired and grouped \( t \) tests where appropriate. Analysis of variance and the Dunn-Bonferroni procedure were used for multiple comparisons. Least squares linear regression analysis was performed on blood pressure (left carotid artery) and heart rate data. Results are expressed as mean±SEM, with \( p < 0.05 \) representing significance.

Drugs

St587 (Boehringer-Ingelheim, Frankfurt, FRG) was prepared in Krebs solution. Nifedipine (Sigma Chemical Co., St. Louis) was prepared daily in 50% ethanol in distilled water and protected from exposure to light wavelengths <420 nm. The final concentration of ethanol in the cremaster bath on addition of nifedipine was 1.5\( \times \)10\(^{-5} \) M. All other drugs were obtained from Sigma Chemical, except for UK-14,304 and prazosin (Pfizer Laboratories, Kent, England), and were prepared as previously described.

Results

Figure 1 shows results from a representative experiment that examined the effect of reduction in cremaster blood flow on \( \alpha_1 \)-adrenoceptor constriction induced by PE in the presence of yohimbine. Cremaster blood flow was reduced by 30% in the first-order arteriole by stenosis of the common iliac vein. Stenosis was applied at the end of the first control period (C1) and removed at the end of the agonist CRC. Comparable experiments were conducted using the selective \( \alpha_2 \)-adrenoceptor agonist UK-14,304 in the presence of prazosin. Control experiments consisted of the same agonist protocol with no reduction in cremaster flow. Reduction of cremaster blood flow (by 30%) significantly decreased \( \alpha_2 \) constriction of arterioles (Figure 2). In contrast, reduced perfusion did not affect \( \alpha_1 \) constriction of venules nor \( \alpha_1 \) constriction of either arterioles or venules.

Reduced tissue perfusion had no effect on baseline diameter. Arterioles were 129±7 \( \mu \)m during C1 (\( n = 12 \)) and 127±7 \( \mu \)m during reduced perfusion (C2, \( n = 12 \)); venules were 170±9 \( \mu \)m during C1 and 171±9 \( \mu \)m during C2. Control diameters at the beginning (C1) and end (C3) of the protocol were different (Table 1), indicating that the preparation remained stable over the duration of the protocol.
the end of the protocol (Table 1) was comparable to previous studies in this laboratory that did not involve changes in cremaster perfusion. Thus, exposure of microvessels to reduced perfusion and bath P02 had no effect on maximal vasoconstrictor responses obtained on return of the cremaster muscle to control conditions. The amount of basal (intrinsic) tone present in the vessels during control periods was indicated by comparison of papaverine-induced maximal dilation to the average diameter during C3 (Table 1). Maximal arteriolar diameter was 15% and 13% greater than control for the PE and UK-14,304 groups, respectively. These data indicate that a similar amount of basal smooth muscle tone was present in the arterioles of both agonist groups throughout the duration of the experiment. The finding that stenosis had no effect on baseline diameter indicates that the degree of blood flow reduction imposed in this study (30%) does not influence the intrinsic smooth muscle tone in these large arterioles. As is

![FIGURE 2. Graphs showing effect of reduced tissue perfusion (30% decrease in cremaster artery flow via venous stenosis) on large arteriolar and venular responses to phenylephrine (α1-agonist) in the presence of yohimbine (5×10⁻⁷ M) and UK-14,304 (α2-agonist) in the presence of prazosin (10⁻⁷ M). Bath P02 was reduced during stenosis by increasing N₂ aeration. For α1 experiments, bath P02 was 23 and 13 mm Hg during control and reduced perfusion, respectively; for α2 experiments, these values were 27 and 12 mm Hg, respectively. Only one concentration–response curve was determined per experiment. Concentration–response data were normalized to maximal constriction to norepinephrine (10⁻⁷ M) during normal perfusion/bath P02 and in the absence of antagonists. Significance was assessed by analysis of variance and nonpaired Bonferroni t test.]

**TABLE 1.** Baseline Data for Flow Reduction Experiments

<table>
<thead>
<tr>
<th>Diameter</th>
<th>10⁻⁵ M NE C1 (μm)</th>
<th>C3 (μm)</th>
<th>%C3</th>
<th>Maximal C1 (μm)</th>
<th>%C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterioles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>14</td>
<td>125±6</td>
<td>126±5</td>
<td>55±4*</td>
<td>44±2</td>
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<tr>
<td>UK-14,304</td>
<td>12</td>
<td>120±8</td>
<td>118±8</td>
<td>42±6*</td>
<td>34±4</td>
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<tr>
<td>Venules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>14</td>
<td>168±8</td>
<td>169±8</td>
<td>118±8*</td>
<td>71±4</td>
</tr>
<tr>
<td>UK-14,304</td>
<td>12</td>
<td>170±10</td>
<td>167±10</td>
<td>122±18*</td>
<td>73±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Control, average diameter at beginning (C1) and end (C3) of protocol (see Figure 1); NE, diameter during 10⁻⁵ M norepinephrine (in the absence of antagonists) that was used to normalize concentration–response data. Maximal, average diameter during exposure to papaverine (3×10⁻⁷ M) at end of protocol.

*p<0.001 and #p<0.05 vs. C3 by paired t test.
commonly observed, no statistically significant tone was present in the venules.

Stenosis of the common iliac vein did not affect mean arterial pressure or heart rate. In those experiments in which stenosis was induced, mean arterial pressure and heart rate during C1 (before stenosis) were 94±3 mm Hg and 492±15 beats/min, respectively; during the C2 (10 minutes after initiation of stenosis), these values were 93±2 mm Hg and 485±16 beats/min, respectively. In addition, placement of the noninflated perivascular occluder on the common iliac vein did not affect baseline blood flow in the common iliac artery, as measured by the Doppler flow probe.

A second series of experiments was conducted to extend our previous studies of the effect of acidosis on \( \alpha \)-adrenoceptor constriction. In those studies acidosis inhibited \( \alpha_2 \) but not \( \alpha_1 \) responses induced by full agonists. In the present study, constriction produced by the partial \( \alpha_1 \)-agonist St587 was significantly decreased when the cremaster bath pH was changed from 7.4 to 7.1 (Figure 3). To determine whether St587 stimulation of \( \alpha_2 \)-receptors increases calcium influx through dihydropyridine-sensitive calcium channels, we also examined the effect of nifedipine on St587 constriction (Figure 3). St587-induced constriction of arterioles was markedly reduced by nifedipine (7×10\(^{-9}\) M). This concentration of nifedipine was chosen on the basis of previous studies from this laboratory\(^4\) that demonstrated that this concentration selectively inhibits arteriolar constriction mediated by NE stimulation of \( \alpha_2 \) but not \( \alpha_1 \)-adrenoceptors.

Acidosis had no effect on baseline arteriolar diameter: control diameters at pH 7.4 (C1) and at pH 7.1 (C2) were 110±11 and 109±12 \( \mu \)m, respectively. This is consistent with our previous studies\(^4\) that showed no effect of pH 7.1 on intrinsic tone of large or small arterioles. Other studies from our laboratory\(^10\) indicate no effect of nifedipine (7×10\(^{-9}\) M) on baseline diameter of arterioles and venules. Comparison of control diameter at the beginning (C1) and at the end (C3) of the protocol showed that no change occurred in baseline diameter over the duration of the protocol (Table 2). The amount of intrinsic tone present in the vessels was determined by comparison of diameter during papaverine or nitroprusside with the average diameter during C3. Maximal arteriolar diameter was 20%, 24%, and 16% greater than control diameter for the control (pH 7.4), acidosis (pH 7.1), and nifedipine groups, respectively (Table 2). Thus, a comparable amount of basal smooth muscle tone was present in all three groups.

For all rats studied (both the reduced perfusion and the St587 experiments, \( n = 47 \)), mean arterial pressure and heart rate at the beginning of the experiments were (mean±SEM) 99±2 mm Hg and 482±6 beats/min, respectively; at the end of the experiments, values were 100±1 mm Hg and 486±6 beats/min, respectively. Regression analysis indicated no significant change in these parameters over the duration of the experiments.

**Discussion**

According to the metabolic theory of blood flow regulation, alterations in oxygen supply or demand result in changes in the local tissue environment, which elicit changes in the caliber of resistance vessels such that blood flow remains matched to metabolic demand.\(^9\) There is evidence that terminal arterioles are more sensitive than large arterioles to changes in tissue oxygenation\(^20\) and that neural constriction of arterioles, particularly small terminal arterioles, is opposed by local metabolic control.\(^21,22\) In a previous study,\(^4\) we observed that acidosis attenuates \( \alpha_2 \) but not \( \alpha_1 \) constriction, suggesting that the predominance of \( \alpha_2 \)-receptors on terminal arterioles and the sensitivity of \( \alpha_2 \) constriction to \( \text{CO}_2/\text{H}^+ \) (and possibly other metabolic feedback signals) may provide a mechanism by which adrenergic control of terminal arterioles is more sensitive to metabolic control. A major goal of the present study was to examine this neural–metabolic hypothesis directly by determining the effect of reduced skeletal muscle perfusion (i.e., likely decreased \( \text{O}_2 \) delivery) on \( \alpha_1 \)-
versus \( \alpha_2 \)-adrenoceptor constriction. Arteriolar constriction produced by a selective \( \alpha_2 \)-agonist (UK-14,304) was significantly attenuated during reduction of cremaster blood flow. In contrast, reduced perfusion had no effect on \( \alpha_2 \) constriction of venules. Neither arteriolar nor venular constriction induced by a selective \( \alpha_2 \)-adrenoceptor agonist (PE) was affected. These findings are in good agreement with our hypothesis of selective inhibition of \( \alpha_2 \)-adrenergic constriction of arterioles by metabolic factors.

Reduction of cremaster blood flow was achieved by stenosis of the right common iliac vein. In other studies from this laboratory,\(^\text{16} \) it was observed that \( \alpha_2 \) constriction was much more sensitive than \( \alpha_1 \) constriction to myogenic inhibition (dilation) produced by reduced transmural pressure. Thus, venous rather than arterial stenosis was used to prevent a decrease in microvascular transmural pressure and differential myogenic inhibition of \( \alpha_1 \) and \( \alpha_2 \) vascular tone. It can be expected that venous stenosis increased cremaster venular and arteriolar pressures slightly. With elevated venous pressure, myogenic mechanisms favor arteriolar constriction, while metabolic mechanisms favor dilation. Myogenic reactivity of arterioles (but not venules) is markedly amplified during induced adrenergic tone.\(^\text{16,23} \) However, myogenic constriction is augmented to the same degree in the presence of \( \alpha_2 \)- or \( \alpha_1 \)-mediated arteriolar tone.\(^\text{16} \) Thus, the effect of increased venous pressure and decreased perfusion to selectively attenuate \( \alpha_2 \)-mediated constriction of large arterioles in the present study cannot be attributed to differences in interaction of myogenic constriction with \( \alpha_2 \)- versus \( \alpha_1 \)-mediated constriction. We examined the effect of reduced perfusion on \( \alpha_1 \) versus \( \alpha_2 \) constriction of large arterioles and venules, because these microvascular segments possess both receptor subtypes, whereas terminal arterioles have only \( \alpha_2 \)-receptors.\(^\text{1,2,4} \) This allowed us to compare the effect of reduced flow on \( \alpha_2 \)- and \( \alpha_2 \)-adrenoceptor constriction of the same vessel levels within the microcirculation.

Similar amounts of basal smooth muscle tone were present in the arterioles of both groups (Table 1). The combined 30\% reduction in cremaster blood flow and 50\% reduction in bath PO\(_2\) did not affect control diameters. It is unlikely that this reflects concomitant myogenic constriction and metabolic dilation subsequent to the imposed elevated venous pressure and reduced tissue blood flow, since large arterioles demonstrate little myogenic behavior in the absence of induced vascular tone.\(^\text{23} \) Rather, it appears that resting basal tone is unaffected by the metabolic stimulus (e.g., reduced oxygen delivery or reduced tissue "washout") produced in these experiments. This is consistent with our previous results\(^\text{4} \) that indicate no effect of pH 7.1 on basal tone in either large or small terminal arterioles. Under denervated conditions, basal tone in arterioles is primarily dependent on intrinsic mechanisms.\(^\text{1,2} \) The absence of an effect of reduced perfusion or acidosis\(^\text{4} \) on resting tone suggests that modest metabolic changes do not act through inhibition of arteriolar intrinsic tone but may instead depend on selective inhibition of extrinsic \( \alpha_2 \)-mediated neurogenic tone. It is possible that greater reductions in tissue perfusion or increases in metabolic activity can elicit different or stronger metabolic signals that may attenuate \( \alpha_1 \)-mediated or intrinsic tone.

The precise mechanisms that underlie integration of neural and metabolic regulation of microvascular smooth muscle remain unclear. In particular, it is not known why adrenergic constriction of the small arterioles is more sensitive to metabolic inhibition.\(^\text{24} \) Our previous data\(^\text{1-4} \) suggest that modest tissue acidosis may preferentially attenuate adrenergic constriction of terminal arterioles versus large arterioles because of inherent differences in \( \alpha_2 \)- and \( \alpha_1 \)-adrenoceptor distribution, innervation, and sensitivity to acidosis. From these data we proposed a model\(^\text{4} \) for the interaction of metabolic feedback signals with \( \alpha \)-receptors on vascular smooth muscle of skeletal muscle microvessels. According to the model, large arteriole adrenergic constriction relies preferentially on \( \alpha_2 \)-receptors, whereas terminal arterioles have predominantly \( \alpha_1 \)-receptors.\(^\text{1-3} \) Vasodilator metabolites increase in skeletal muscle during increased skeletal muscle activity or decreased oxygen availability and oppose adrenergic constriction. It is possible that certain metabolites or metabolite concentrations may also directly inhibit intrinsic tone. The reliance of large arterioles on \( \alpha_2 \)-receptors and the minimal sensitivity of \( \alpha_1 \) constriction to metabolic inhibition could preserve adrenergic regulation of large "resistance" arterioles during modest reductions in O\(_2\) supply or increases in O\(_2\) demand. In contrast, dominance of terminal arterioles by \( \alpha_2 \)-receptors and a

<table>
<thead>
<tr>
<th>Table 2. Baseline Arteriolar Data for St587 Experiments</th>
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<tr>
<td>Control (pH 7.4)</td>
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<td>Acidosis (pH 7.1)</td>
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<td>Nifedipine</td>
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Values are mean±SEM. Control, average diameter at beginning (C1) and end (C3) of protocol (see Figure 1); NE, diameter during 10\(^{-5}\)M norepinephrine (in the absence of antagonists) that was used to normalize concentration-response data. Maximal, average diameter during exposure to papaverine (3×10\(^{-4}\)M) at end of protocol.

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\( *p<0.001 \) and \( tp<0.05 \) vs. C3 by paired \( t \) test.
particular sensitivity of \( \alpha_2 \) constriction to metabolic signals may make adrenergic constriction of these precapillary arterioles that control blood–tissue exchange especially susceptible to metabolic inhibition during mismatches in \( O_2 \) supply and demand. This model is supported by the finding in the present study that \( \alpha_2 \) constriction of large arterioles was significantly attenuated during reduced skeletal muscle perfusion, whereas \( \alpha_1 \) constriction was unaffected.

The selective attenuation of \( \alpha_2 \) constriction of large arterioles during reduced perfusion may have resulted from a direct effect of a reduction in tissue \( P_{O_2} \) likely created by these conditions and/or from accumulation of vasodilator metabolites (e.g., \( H^+ \), \( CO_2 \), and adenosine). However, since we did not obtain measurements of tissue \( P_{O_2} \), our data do not provide any direct evidence concerning whether the reduction in blood flow achieved in our experiments actually produced metabolic changes due to reduced oxygen delivery per se. Independent of alterations in oxygen delivery, reduced “washout” of tissue metabolites subsequent to decreased blood flow could possibly produce relevant inhibitory metabolic stimuli such as acidosis, a condition that we have previously shown to selectively attenuate \( \alpha_2 \) constriction.4

An interesting finding in the present study was that large arteriolar \( \alpha_2 \) constriction was attenuated during reduced perfusion, whereas \( \alpha_2 \) constriction of venules was unaffected. Venular \( \alpha_2 \) constriction also was unaffected by reduced perfusion. These data are consistent with other studies from our laboratory25 in which venular smooth muscle sensitivity to adrenergic receptor stimulation or direct activation of the contractile apparatus with potassium chloride was unaffected, or even enhanced, during reduction in cremaster intravascular pressure and flow produced by stenosis of the common iliac artery or selective precapillary constriction with vasopressin; arteriolar responses, however, were markedly depressed. Other studies also demonstrate less sensitivity of venous smooth muscle contractility to metabolic regulation (e.g., acidosis,4,26 hypoxia,26,27 and flow reduction or increased skeletal muscle activity26).

The mechanism imparting the particular sensitivity of \( \alpha_2 \)-mediated constriction to metabolic inhibition is unclear. A possible difference in \( \alpha_2 \), versus \( \alpha_2 \)-adrenoceptor reserve26 in cremaster microcirculation does not appear to contribute to the selective sensitivity of \( \alpha_2 \), versus \( \alpha_1 \)-mediated constriction to acidosis.4 A number of recent in vivo whole animal and in vitro large vessel studies demonstrate that \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors use different calcium sources to produce contraction.5–9 \( \alpha_2 \)-Adrenoceptors appear to rely on transmembrane influx of extracellular calcium,5–9 whereas \( \alpha_1 \)-adrenoceptors can induce both release of intracellular calcium and influx of extracellular calcium. Consistent with this concept, previous data from our laboratory indicate that \( \alpha_2 \)-mediated NE constriction of cremaster large arterioles is strongly inhibited by verapamil \( (10^{-7} \text{ M}) \) and nifedipine \( (7 \times 10^{-9} \text{ M}) \), whereas \( \alpha_1 \)-mediated NE constriction is unaffected.10 The degree of \( \alpha_2 \)-receptor reliance on influx versus release of intracellular calcium appears to depend on agonist properties.5–7 Full \( \alpha_2 \)-agonists (e.g., PE and NE) use both intracellular release and influx of calcium, whereas vasoconstriction to partial \( \alpha_2 \)-agonists (e.g., St587) results primarily from an influx of extracellular calcium. In the present study, arteriolar constriction by St587 was significantly reduced by nifedipine, which is consistent with these findings.

Our previous results4 demonstrated that \( \alpha_2 \)-arteriolar constriction in response to full agonists (PE and NE) is not affected by acidosis (pH 7.1). In contrast, \( \alpha_2 \) constriction of both large and small arterioles was significantly attenuated by pH 7.1. There is evidence that acidosis inhibits influx of extracellular calcium.11–13 If the mechanism by which acidosis inhibits \( \alpha_2 \)-adrenoceptor–mediated constriction of arterioles involves inhibition of calcium influx, acidosis should also attenuate constriction of arterioles induced by the partial \( \alpha_2 \)-agonist St587. In the present study, St587-induced constriction was significantly reduced by pH 7.1. Thus, inhibition of \( \alpha_2 \)-adrenoceptor constriction by acidosis may depend not on the adrenoceptor type per se but rather on the degree of reliance of the agonist on calcium influx to produce constriction. These data suggest that the particular sensitivity of arteriolar \( \alpha_2 \) constriction to acidosis, and perhaps other metabolic signals, may relate to the coupling of the \( \alpha_2 \)-receptors to calcium channels whose conductance may be inhibited by tissue metabolites. It remains possible that acidosis could also alter binding affinity of the \( \alpha_2 \)-adrenoceptor.30 However, the inhibitory effect of acidosis on St587 but not the PE response appears to argue against such a selectivity against \( \alpha_2 \)-adrenoceptor affinity.

To summarize, modest reduction in blood flow significantly decreased \( \alpha_2 \)-mediated constriction of large arterioles. \( \alpha_2 \)-Receptor–mediated constriction of venules was not affected, nor was \( \alpha_1 \) constriction of either vessel type. These data suggest that decreased \( P_{O_2} \), or increased local concentrations of tissue metabolites during reduced oxygen delivery and flow, preferentially attenuate \( \alpha_2 \) constriction of arterioles. Differences in \( \alpha_2 \) versus \( \alpha_2 \)-adrenoceptor distribution among large versus small arterioles may underlie the differential sensitivity of adrenergic constriction of consecutive vascular segments to metabolic inhibition. The selective sensitivity of \( \alpha_2 \) constriction to acidosis (and possibly other metabolites) may extend from a reliance on calcium influx, which is susceptible to functional inhibition.

Acknowledgments

The authors wish to thank Boehringer-Ingelheim, Frankfurt, FRG, for the gift of St587; Pfizer Laboratories, Kent, England for the gift of UK-14,304 and prazosin; and Glaxo Inc., Research Triangle Park, N.C., for doctoral scholarship support for K. McGillivray-Anderson.
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KEY WORDS • α-adrenergic receptors • vascular smooth muscle • microcirculation • metabolic regulation • phenylephrine • UK-14,304 • St587 • calcium channel • nifedipine
Effect of reduced blood flow on alpha 1- and alpha 2-adrenoceptor constriction of rat skeletal muscle microvessels.
K M McGillivray-Anderson and J E Faber

Circ Res. 1991;69:165-173
doi: 10.1161/01.RES.69.1.165

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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