Autoregulation of Blood Flow within Individual Arterioles in the Rat Cremaster Muscle

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SUMMARY. Autoregulatory responses to alterations in arterial or venous perfusion pressure were determined for individual arterioles within the rat cremaster muscle. The cremaster muscle of pentobarbital anesthetized rats (50 mg/kg, ip) was surgically exposed and maintained in a controlled tissue bath for visualization by in vivo television microscopy. Cremaster bath Po2 was controlled at either a high (approximately 70 mm Hg) or low (approximately 18 mm Hg) level. Inside diameter and red blood cell velocity were measured for individual first (1A), second (2A), or third (3A) branching order arterioles, and instantaneous blood flows within each arteriole were calculated. To measure the autoregulatory responses, we decreased arterial perfusion pressure to the microvascular bed by gradually occluding the sacral aorta. Significant autoregulation was observed in all orders of vessels, but, in general, autoregulation was more pronounced at all vessel levels when bath Po2 was low, and the autoregulatory gain was greater for the smaller vessels compared to the larger vessels. Elevation of venous pressure within the vascular bed by gradual occlusion of the inferior vena cava led to a significant vasoconstriction of the smaller vessels, suggesting that a significant myogenic component was present. The vasoconstriction response to elevated venous pressure was more pronounced when bath Po2 was high. Our data are not consistent with a purely myogenic or purely metabolic mechanism, but suggest that both mechanisms are simultaneously contributing to the local vascular regulation. (Circ Res 51: 43-55, 1982)

IT has been recognized for many years that most tissues will maintain a relatively constant blood flow when the pressure perfusing the tissue is altered, i.e., most tissues exhibit the phenomenon of autoregulation. That this capacity for autoregulation resides within the tissue, independent of extrinsic neural and humoral influences, has been demonstrated by a number of studies (Haddy and Scott, 1978; Johnson, 1978). Recent research has attempted to identify the specific mechanism(s), inherent in the tissue vasculature, that are responsible for this autoregulation. However, in spite of the long-term interest and recent accomplishments in studying this phenomenon, controversy still exists with regard to the underlying mechanism that makes autoregulation possible. In fact, whereas most investigators would agree that autoregulation involves changes in the “microcirculation” of the tissues, the behavior and contribution of the various arteriolar segments with regard to this local regulatory process have not been determined (Duling and Klitzman, 1980), since most studies of autoregulation have measured global changes in flow to a circulatory bed, and have not involved direct measurements within the microcirculation proper. Therefore, one objective of the studies reported here was to obtain direct, quantitative data from the various arteriolar segments of the microcirculation within skeletal muscle, and to determine the contribution of each of these segments to the overall autoregulatory response.

With regard to the mechanisms responsible for autoregulation in skeletal muscle tissue, two general hypotheses have been presented. The myogenic hypothesis (Folkow, 1964; Johnson, 1980) attributes the regulation of blood flow observed when perfusion pressure is altered to a direct effect of transmural pressure changes on vessel wall stress and, hence, on vascular smooth muscle reactivity. In contrast, the metabolic hypothesis (Berne, 1964; Haddy and Scott, 1968, 1971, 1978) proposes that the microvascular segments that regulate blood flow are modulated through the release of some vasoactive substance whose concentration changes with tissue metabolism. The two theories are not necessarily mutually exclusive, and both mechanisms may in fact contribute to the autoregulatory responses observed. A second objective of the studies presented here was to determine how these theories might explain the flow autoregulation observed in the cremaster muscle microcirculation.

Another aspect of considerable interest with regard to local blood flow control and autoregulation is the possible participation of oxygen in the control mechanisms. Considerable evidence has been obtained to indicate that oxygen does play a role in the regulation of blood flow within the microcirculation of skeletal muscle (Granger and Shepherd, 1973; Hutchins et al., 1974; Granger et al., 1976; Prewitt and Johnson, 1976) although some recent studies (Pittman and Duling, 1973; Duling, 1974; Gorczynski and Duling, 1978) provide evidence that the contribution of oxygen to flow control in skeletal muscle is not due to a direct
effect of oxygen on vascular smooth muscle, but rather through an indirect effect related to tissue metabolism. A third objective of our study was to determine the effects of changes in local oxygen tension on the autoregulatory responses observed in individual skeletal muscle arterioles.

Methods

Animal Preparation

Fifty-seven male Sprague-Dawley rats (Timco, Houston, TX) were utilized in this study. All animals were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal), 50 mg/kg. Supplemental anesthetic doses, equal to one-tenth of the initial dose, were administered as required, but only four of the animals required anesthetic supplements. The weight range for all animals used in this study was 84-147 g, averaging 119 ± 16.3 g (mean ± SD).

Following anesthesia, the right cremaster muscle was surgically prepared for in vivo visualization of its microcirculation. The surgical technique utilized in our laboratory is a modification of the techniques described by Majno et al. (1967) and by Baez (1973), and has been described in detail elsewhere (Harris et al., 1975). Briefly, the scrotal sac was cut longitudinally and the testicle was gently teased away from the sac. The testicle then was cleared of connective tissue to expose the underlying cremaster muscle, which was kept moist with a modified Krebs solution (described below) throughout the surgical procedure. Next, the cremaster muscle was cut longitudinally from the distal tip to the external inguinal ring, taking care to position the cut as far as possible from the major artery and vein which entered the muscle. After this incision, the testicle was gently pulled away from the cremaster muscle and was pushed through the inguinal canal into the abdominal cavity. The rat then was placed on its back on a heating pad and positioned on a Plexiglas board fitted with a cremaster bath chamber. The cremaster muscle was secured in a relatively flat position over a coverglass within the chamber by five ties of 4-0 silk suture which were placed at equal intervals around the margin of the cut muscle. The bath chamber then was filled with a modified Krebs solution (25.5 mM NaHCO3, 112.9 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2 • 2H2O, 1.19 mM MgSO4 • 7H2O, and 11.6 mM dextrose) which was heated intermittently by electrical current which passed through a coil of nichrome wire submerged in, but electrically isolated from, the bath chamber. By means of this heater system, bath temperature was controlled at 34.5°C. Bath pH was monitored and controlled at 7.40 ± 0.05 units by bubbling CO2-N2 gas mixtures through the bath solution. Bath Po2 and PCO2 were also controlled at desired levels by altering the gas bubbling rates. Nitrogen and carbon dioxide were continuously and separately bubbled into the bath, and by adjusting the composition of the gas mixture, the Po2 of the solution could be set at either a high (~70 mm Hg) or at a low (~17 mm Hg) level. When a low Po2 was desired, the bath chamber was covered with a Plexiglas plate to isolate it from the atmosphere. In all experiments, the bath was well stirred due to the bubbling of the gases. The osmolality of the bath solution was sampled before and after each experiment and was maintained in the range of 285-295 milliosmolar. We have recently reported (Morff and Granger, 1980) that the cremaster muscle, when exposed and maintained as described, has a blood flow per unit weight that is similar to other skeletal muscle tissues, and this blood flow is not altered by the surgical exposure. To visualize the microcirculation, the rat board was placed on the movable stage of a modified Lietz intravital microscope, and the cremaster muscle was transilluminated using a 100-W tungsten-halogen lamp. The image was magnified by a 20X water-immersion objective and a 16X ocular and projected into a silicon target television camera (Cohu, Inc. 4400 series). The video image was visualized on a television monitor at a total magnification of approximately 3500X. The video signal passed through a timing device (Thalner Electronic Labs model VC-405) which generated a 5-digit clock on the video image that was used for timing of the experiment. In addition, the video signal from the monitor was intercepted for recording on video tape (Sony AV-3650).

Experimental Protocol

Instantaneous blood flows within single arterioles in the cremaster muscle were calculated from instantaneous diameters and velocity measurements. Internal diameter of each vessel was measured using an image-shearing monitor (Instrumentation for Physiology and Medicine, model 907), and centerline velocity was determined using a cross-correlating velocimeter (IPM model 102). The theory of operation for the image-shearing monitor (Intaglietta and Tompkins, 1973), as well as for the velocimeter (Intaglietta and Tompkins, 1972), have been described previously. The input signals for the velocimeter were derived from two photodiodes that intercepted the image of the vessel being measured. The image-shearing monitor and velocimeter provide D.C. outputs that were connected to a strip-chart recorder to give continuous recordings of vessel diameter and centerline velocity. The image-shearing monitor was calibrated daily with a stage micrometer, and the velocimeter was calibrated weekly by measuring the velocity of red blood cells smeared on a Plexiglas wheel turning at a known velocity.

Utilizing the instantaneous diameter and centerline velocity measurements, the volumetric blood flow rate was calculated for each individual vessel, using the relationship that centerline velocity equals 1.6 times the true mean velocity. This relationship has been shown to hold true for vessels above 20 μm in diameter (Baker and Wayland, 1974; Lipowsky, 1975). Recently, LaLone and Johnson (1978) demonstrated the validity of this method for determining blood flow in the microcirculation of the cat sartorius muscle. Instantaneous values of velocity and diameter were read directly from the strip chart recording and entered into a computer (Interdata 8/16E) for off-line computation of flow within each vessel.

To study the autoregulatory responses within individual microvessels, the arterial pressure perfusing the cremaster muscle was decreased in steps by graded occlusion of the lower aorta. A small, specially constructed cuff occluder (In Vivo Metrics) was placed, through a midline abdominal incision, around the aorta just rostral to the iliac bifurcation. The occluder cuff was filled with water and the distal end connected to a 1-ml disposable syringe. By depressing the syringe, the occluder was gradually inflated to occlude the aorta and thus decrease downstream pressure. The downstream pressure was monitored by observing the pressure record from a pressure transducer (Statham P23Db) connected to a femoral artery cannula. We have recently described a feedback control system that automatically regulates this pressure (Morff and Granger, 1981).

The experimental protocol involved the determination of the blood flow vs. perfusion pressure profile for individual microvessels at several anatomical branching levels within the cremaster muscle. A given vessel was selected and oriented vertically on the image-shearing monitor. Vessel diameter, centerline velocity, and perfusion pressure were

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recorded during a 5-minute control period. Perfusion pressure then was rapidly decreased from control to a lower value and maintained at this new value for approximately 1 minute. Vessel diameter and velocity were recorded continuously during this period, and then perfusion pressure was further decreased to a still lower value. Vessel blood flow was calculated for four or five such pressure reductions, and normalized plots of flow vs. pressure were constructed for each vessel. After a pressure reduction sequence was performed for a given vessel, the aorta occlusion was rapidly released, and pressure and flow were allowed to return to control values for 10 minutes. Then another vessel was selected and the pressure reduction sequence repeated. Three branching orders of arterioles were studied in each animal preparation; first (1A), second (2A), and third (3A) order. Figure 1 is a schematic representation of the arteriolar circulation of the cremaster muscle. Typical measurement locations for 1A, 2A, and 3A arterioles are shown on this figure.

Experiments were performed with either a low (LO) or high (HI) cremaster bath P02, controlled, as described above, by altering the composition of gases bubbling through the bath. Bath PO2 and Pco2 were measured polarographically using a blood gas analyzer (Corning model 165). Bath gas values were determined at 10-minute intervals throughout each experiment, and were corrected for temperature differences between the bath and the analyzer (Andritsch et al., 1981).

In addition to the experiments described above in which arterial pressure was reduced (AP groups) a second series of experiments were performed in which arterial pressure remained unaltered, but local venous pressure was increased (VP groups). To increase venous pressure within the cremaster muscle microcirculation, an inflatable cuff occluder was placed around the lower vena cava, and inflated in a fashion similar to that described above for the aortic occlusions. This graded occlusion resulted in step increases in the venous pressure within the animal's hindquarters, including the cremaster muscle. To monitor the venous pressure, a cannula was inserted into the left femoral vein and connected to a venous pressure transducer (Statham P23BC). Blood flow and diameter vs. venous pressure curves were obtained for 1A, 2A, and 3A arterioles utilizing the same experimental protocol described for the experiments involving arterial pressure reductions. Experiments involving venous pressure elevation (VP) were performed with either low (LO) or high (HI) cremaster bath PO2. Thus, four distinct experimental groups were utilized in this study: (1) a group in which cremaster arterial perfusion pressure was reduced and cremaster bath PO2 was high (APHI group); (2) a group in which arterial pressure was reduced and bath PO2 was low (APLO group); (3) a group in which venous pressure was elevated and bath PO2 was high (VPHI group); and finally (4) a group in which venous pressure was elevated and bath PO2 was low (VPLO group).

Data Analysis

After each experiment, the diameter, velocity, and blood flow for each individual vessel studied were calculated and plotted vs. the arterial or venous pressure. The responses of each variable for each vessel studied then were averaged together for all vessels of a given branching order within each group. To alleviate differences resulting from differences in the control values of the different variables, all diameter, blood flow, and pressure data were plotted relative to the average control values, i.e., all data were plotted as a fraction of the control value for that variable.

For the APLO and APHI groups, evaluation of the group responses to decreased arterial pressure was accomplished by fitting all the data points utilizing a polynomial regression analysis. A stepwise regression search technique was used to generate best-fit polynomials (Barr et al., 1976). To select the best polynomial fit, several criteria were used: (1) all coefficients of the polynomial had to be significant at the 5% level, and (2) an increase in the order of the polynomial had to result in at least 10% increase in the coefficient of multiple correlation (R2). In order to compare the regression lines obtained for the low and high bath oxygen concentrations at each arterial branching level, an F test was performed using the full and reduced regression models. The same method was also utilized to determine whether the responses for all arterial branching orders within a given group were the same.

In order to quantify the relative magnitude of the autoregulatory responses for each pressure-flow relationship, the closed-loop gain (Gc) of the autoregulatory control system was calculated from the slope of the normalized pressure (P)-flow (F) plots using the equation

\[ G_c = \frac{|(\Delta F/\Delta P)|}{|\Delta P|} - 1. \]

Negative values of Gc indicate negative feedback control of blood flow in response to perfusion pressure alteration. Perfect autoregulation would yield a Gc value of −1, whereas values of Gc more negative than −1 indicate superperfusion, i.e., an increase of flow in response to a decrease in perfusion pressure. Positive values of Gc suggest a system behaving in a passive elastic fashion, i.e., flow falling more rapidly than pressure (Granger and Norris, 1980).

In the experiments in which venous pressure was altered, neither the diameters nor flows could be expressed as polynomial functions of pressure. Therefore, for the venous pressure elevation groups (VPLO and VPHI), statistical comparisons were performed using only the data values obtained with the highest venous pressure elevation. A Student's t-test was used to determine whether the diameter
or flow responses at the highest venous pressure were different from the control values, and to compare the responses for a given arteriolar branching order between the high bath oxygen and the low bath oxygen groups (VPHI vs. VPLO group). Analysis of variance and Schefe's method of multiple comparisons (with a total $P < 0.05$) were utilized to compare the responses of each arteriolar branching order within each group, i.e., to determine whether the responses for 1A, 2A, and 3A arterioles were different.

Results

**Contribution of Systemic Mechanisms to Vascular Responses**

In this study, the sacral aorta or vena cava were gradually occluded to alter cremaster arterial and venous pressures. If occlusion of these vessels caused alterations of central arterial pressure, reflex control mechanisms might be evoked that could alter the local responses observed in the cremaster vasculature. Since we were concerned only with local microcirculatory control mechanisms, several preliminary experiments were performed to determine whether central arterial pressure was altered during the occlusion procedures.

Gradual occlusion of the aorta resulted in step decreases in femoral artery pressure, but carotid artery pressure remained unchanged. Rapid release of the occluder resulted in a rapid return of femoral pressure to control, but carotid artery pressure was again unaffected. This experiment was repeated in several animals, and in no case was there any significant alteration in carotid artery pressure during aortic occlusion. In a similar fashion, simultaneous recordings of carotid artery pressure and femoral vein pressure showed that gradual occlusion of the vena cava resulted in gradual increases in femoral vein pressure, but neither the occlusion or release of occlusion significantly altered the pressure recorded at the carotid artery.

**Control Data**

Table 1 presents a summary of the average control data obtained for all of the measured variables in each of the four experimental groups: (1) decreased arterial pressure with high bath oxygen (APHI), (2) decreased arterial pressure with low bath oxygen (APLO), (3) increased venous pressure with high bath oxygen (VPHI), and (4) increased venous pressure with low bath oxygen (VPLO). The arterial and venous pressures shown are the mean values obtained during the initial control periods for all animals within each group. The cremaster bath gas values shown are the averages of all samples (bath gases were sampled at 10-minute intervals) obtained for all animals within each group. For the high bath oxygen groups, cremaster bath PO$_2$ averaged 68.3 ± 7.1 (mean ± SD) and 69.7 ± 7.6 mm Hg, respectively, for the arterial pressure reduction (APHI) and venous pressure elevation (VPHI) groups. The low bath oxygen PO$_2$'s averaged 18.3 ± 5.1 and 17.4 ± 4.2 mm Hg, respectively for the arterial pressure (APLO) and venous pressure (VPLO) groups. Bath PCO$_2$ was maintained at about 35-40 mm Hg, and did not differ significantly among any of the groups.

Control vessel diameters for 2A and 3A branching orders were significantly larger with low bath oxygen (APLO and VPLO groups) compared to the high bath oxygen control diameters (APHI and VPHI groups), but the control diameter of the 1A arterioles was not altered by changing bath oxygen concentration. Lowering bath PO$_2$ resulted in significantly increased RBC velocity in the 1A arterioles, while the velocities in

**Table 1**  

<table>
<thead>
<tr>
<th>Group</th>
<th>$n_A$</th>
<th>Weight (g)</th>
<th>Arterial pressure (mm Hg)</th>
<th>Venous pressure (mm Hg)</th>
<th>Cremaster bath gases (mm Hg)</th>
<th>Vessel branching order</th>
<th>Vessel diameter (μm)</th>
<th>V$_{inw}$ (mm/sec)</th>
<th>Q (nl/sec)</th>
</tr>
</thead>
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<tr>
<td>APHI</td>
<td>17</td>
<td>107 ± 6.7</td>
<td>92 ± 4.1</td>
<td></td>
<td>PO$_2$: 68.3 ± 7.1</td>
<td>1A</td>
<td>81 ± 5.7</td>
<td>4.12 ± 0.38</td>
<td>13.40 ± 2.01</td>
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<td></td>
<td></td>
<td></td>
<td>PO$_2$: 36.7 ± 3.4</td>
<td>2A</td>
<td>40 ± 4.2</td>
<td>2.65 ± 0.37</td>
<td>2.78 ± 0.49</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3A</td>
<td>21 ± 1.6</td>
<td>0.52 ± 0.09</td>
<td>0.11 ± 0.03</td>
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<tr>
<td>APLO</td>
<td>14</td>
<td>113 ± 4.8</td>
<td>93 ± 3.3</td>
<td></td>
<td>PO$_2$: 18.3 ± 5.1</td>
<td>1A</td>
<td>83 ± 4.6</td>
<td>4.98 ± 0.46</td>
<td>16.78 ± 2.39</td>
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<td></td>
<td>PO$_2$: 33.6 ± 3.6</td>
<td>2A</td>
<td>51 ± 4.4</td>
<td>2.21 ± 0.33</td>
<td>2.87 ± 0.81</td>
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<td></td>
<td>3A</td>
<td>20 ± 2.0</td>
<td>0.41 ± 0.08</td>
<td>0.13 ± 0.03</td>
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<tr>
<td>VPHI</td>
<td>14</td>
<td>101 ± 5.0</td>
<td>91 ± 3.6</td>
<td>73 ± 0.8</td>
<td>PO$_2$: 69.7 ± 7.6</td>
<td>1A</td>
<td>88 ± 5.3</td>
<td>3.89 ± 0.29</td>
<td>14.81 ± 1.79</td>
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<td>PO$_2$: 38.7 ± 4.3</td>
<td>2A</td>
<td>43 ± 3.5</td>
<td>2.71 ± 0.31</td>
<td>2.44 ± 0.54</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>3A</td>
<td>20 ± 2.3</td>
<td>0.53 ± 0.09</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>VPLO</td>
<td>12</td>
<td>104 ± 6.3</td>
<td>88 ± 2.6</td>
<td>69 ± 1.2</td>
<td>PO$_2$: 17.4 ± 4.2</td>
<td>1A</td>
<td>90 ± 4.0</td>
<td>3.98 ± 0.46</td>
<td>16.06 ± 2.60</td>
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<td>PO$_2$: 41.1 ± 6.1</td>
<td>2A</td>
<td>50 ± 5.0</td>
<td>2.65 ± 0.35</td>
<td>3.30 ± 0.75</td>
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<td>3A</td>
<td>25 ± 2.1</td>
<td>0.36 ± 0.08</td>
<td>0.11 ± 0.04</td>
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</table>

Data are shown as mean ± SD. Abbreviations: APHI = arterial pressure reduction with high bath PO$_2$; APLO = arterial pressure reduction with low bath PO$_2$; VPHI = venous pressure elevation with high bath PO$_2$; VPLO = venous pressure elevation with low bath PO$_2$. $n_A$ = number of animals in group; $n_v$ = number of vessels studied for each branching order within each group; $V_{inw}$ = red blood cell velocity at vessel centerline; Q = vessel blood flow = ($V_{inw}/1.6$) ($\pi$) (diameter$^2$)/4.

* Indicates significant difference ($P < 0.05$) comparing APLO group to APHI group, or VPLO group to VPHI group for same vessel branching order.
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2A and 3A arterioles were reduced. The control blood flows were also generally greater in all orders of vessels when the prevailing bath oxygen concentration was low.

Autoregulatory Responses—Arterial Pressure Reduction

Vessel diameter and blood flow responses to decreased arterial perfusion pressure were obtained for 1A, 2A, and 3A arterioles with high bath oxygen (APHI) or low bath oxygen (APLO). A typical strip-chart recording of diameter, centerline RBC velocity, and arterial pressure for an individual 3A arteriole during a series occlusion is shown in Figure 2. For the particular arteriole shown in this figure, centerline red blood cell velocity decreased as perfusion pressure was reduced (middle tracing), while vessel diameter increased (upper tracing) in the face of decreased arterial perfusion pressure.

Using the velocity and diameter data obtained from strip-chart recordings such as Figure 2, blood flow in each vessel was calculated as described in the Methods section, and the pooled, normalized diameter and blood flow for all vessels of a given branching order were plotted as a function of the normalized arterial perfusion pressure. Figure 3 is a plot of the data obtained with low cremaster bath oxygen (APLO group). The upper half of the figure shows the diameter of 1A (△), 2A (■), and 3A (●) arterioles plotted vs. arterial pressure, while the lower half of the figure shows the calculated blood flow for each vessel order. Because of the method utilized to alter the arterial or venous pressures, it was not possible to obtain step changes in pressure of exactly the same magnitude for each individual pressure alteration sequence, i.e., there was some variability in the independent variable (arterial or venous perfusion pressure). Therefore, because of the many data points obtained, the pressure levels were partitioned into small segments for purposes of data plotting only, and all of the data points within each segment were averaged together to yield a mean value and a standard deviation for both the dependent and the independent variable. For each variable, this grouping of data yielded four to six average data points for each vessel branching order within each experimental group. Standard deviations are shown for diameter and blood flow at each of the points, and standard deviations for arterial pressure are shown only in the upper half of the figure. The lines shown are the best-fit polynomials, determined using all data pairs of flow or diameter vs. pressure, as described in Methods. Significant changes in arteriolar diameter in response to decreased perfusion pressure were observed for all vessel orders studied. Second (2A) and third (3A) order arterioles exhibited marked dilation at reduced perfusion pressures, but dilation of 1A arterioles was not observed. Statistical comparison of the regression lines for the three different branching orders shows that they are significantly different (P < 0.05). The blood flow responses for each of the three vessel orders were also significantly different, giving the impression of better autoregulation within the smaller arterioles.

In Figure 4 is presented the diameter and blood flow responses to decreased arterial perfusion pressure for 1A, 2A, and 3A arterioles obtained with a high cremaster bath PO₂ (APHI group). The data are presented in Figure 4 in the same format used for Figure 3. Statistical analysis of the diameter and blood flow responses for each of the three branching order arterioles with high bath oxygen again demonstrated significant differences, with greater dilation, and apparently better autoregulation, in the smaller arteriolar branches. Only the 3A arterioles exhibited significant dilation in this group of experiments.

To determine the effect of the different oxygen environments on the microvascular responses, the regression data for each vessel order obtained with
Effects of Local Oxygen Environment on Autoregulatory Feedback Gain

In order to quantitate the effects of the different oxygen levels on the autoregulatory capacity of each series arteriolar segment, we calculated the closed-loop gain ($G_c$) of the blood flow autoregulation control system from the blood flow response curves, as explained in Methods. The gain curves for each vessel branching order for either high bath oxygen (solid lines) or low bath oxygen (dashed lines) are presented in Figure 5. With high bath oxygen, both the 3A and 2A vessels exhibited autoregulation ($G_c$ between 0 and $-1$), while all vessel orders showed autoregulatory phenomena when the bath $Po_2$ was low. Super-regulation ($G_c$ more negative than $-1$) was observed over the arterial pressure range of 1.00 to 0.85 times control for the 3A vessels with high bath $Po_2$, and over the range of 1.00 to about 0.70 times control for the 3A vessels with low bath $Po_2$. Superregulation was also observed in the 2A arterioles with low bath oxygen levels.

Autoregulatory Responses—Venous Pressure Elevation

Diameter and blood flow changes within 1A, 2A, and 3A cremaster arterioles were also determined in response to elevation of venous pressure with two different local oxygen conditions: low $Po_2$ (VPLO) and high $Po_2$ (VPHI). A typical strip-chart recording from an individual 3A arteriole during venous pres-
sure elevation is presented in Figure 6. The lower tracing shows that venous pressure, recorded from a femoral vein cannula, was increased in several steps from control to about 20 mm Hg. In response to this venous pressure elevation, this particular vessel responded with vasoconstriction (upper tracing) and gradually decreased red blood cell velocity (middle tracing).

From recordings such as Figure 6, blood flow was calculated for each individual arteriole, and the diameter and blood flow responses were averaged together for all vessels of a given branching order to yield the data presented in Figure 7. Figure 7 shows the diameter (upper half) and blood flow (lower half) responses to elevated venous pressure for 1A (▲), 2A (■), and 3A (●) arterioles with high cremaster bath Po₂ (VPHI group). All variables are normalized (x-control). There was no significant change in the diameter of the 1A arterioles when venous pressure was increased from control up to about 4.5X control. However, the 2A and 3A vessels exhibited significant constriction in response to increased venous pressure, the 2A vessels constricting to a diameter of about 0.78X control, and the 3A vessels reaching a minimum diameter equal to approximately 0.65X control. Blood flow decreased significantly in all vessels branching orders when venous pressure was elevated, with the response magnitude inversely related to vessel size.

Similar responses to venous pressure elevation were obtained for 1A, 2A, and 3A arterioles with low cremaster bath Po₂ (VPLO group). These responses are presented in Figure 8. There was no significant vasoconstriction of the 1A arterioles when venous pressure was elevated, but the 2A and 3A arterioles did exhibit significant constriction. The blood flows observed at the highest venous pressure (approximately 4.5X control) were significantly lower than the control blood flows for all three vessel orders, and again the responses were most pronounced in the smallest vessels.

To compare the effects of changes in bath oxygen concentration on the response to venous pressure elevation, a two-sample t-test was used to compare

![Figure APHI GROUP](image)

**Figure 4.** Arteriolar responses to decreased arterial perfusion pressure with high cremaster bath oxygen (APHI group). Diameter (upper half of figure) and blood flow (lower half of figure) responses are presented for 1A (▲, dashed lines), 2A (■, dotted lines), and 3A (●, solid lines) arterioles during gradual reduction of arterial perfusion pressure. All data are expressed as x-control. Vertical or horizontal bars indicate one standard deviation.
the maximum diameter and blood flow responses obtained with low bath $P_O_2$ to those obtained with high bath $P_O_2$. In each case where a significant response did occur, the diameter and blood flow reductions obtained with high bath $P_O_2$ were significantly greater than those obtained for the same order vessel with low bath $P_O_2$, i.e., the degree of the vasoconstriction and the magnitude of the blood flow decrease were greater for the 2A and 3A arterioles when cremaster bath $P_O_2$ was high.

**Discussion**

**General Considerations**

In isolated skeletal muscle, a step reduction of arterial pressure elicits an intrinsic vasodilation of resistance vessels and consequently tissue blood flow is stabilized in the face of a diminished perfusion pressure differential (Stainsby and Renkin, 1961; Jones and Berne, 1964.) The effectiveness of local flow autoregulation in muscle is dependent on numerous factors including tissue oxygenation, level of parenchymal metabolic activity, prevailing flow rate, and other non-metabolic factors. In the dog, flow falls 25 to 50% in resting skeletal muscle when arterial pressure is reduced from 100 to 50 mm Hg, suggesting a 0-50% recovery in tissue perfusion (Granger et al., 1976). This weak-to-moderate level of flow autoregulation is enhanced in mild hypoxemia (Granger et al., 1976) and exercise (Granger et al., 1976; Stainsby, 1962); by contrast, hypometabolic states (Goodman et al., 1978) and nonpulsatile perfusion (LaLone, 1975) are characterized by a reduction in the degree of autoregulation.

At present, two theories provide conceptual bases for understanding autoregulation of blood flow in skeletal muscle (Johnson, 1964). The metabolic theory...
states that vascular tone is modulated by vasodilator metabolites continuously released by skeletal muscle cells. According to the metabolic viewpoint, reduction of arterial pressure causes an initial passive decrease in blood flow which, in turn, leads to a rise in the interstitial concentration of vasodilator metabolite as a result of diminished convective washout. With accumulation of the metabolite, vascular relaxation occurs and muscle blood flow returns toward the control level. According to the myogenic theory, autoregulation of blood flow is a direct manifestation of the intrinsic contractile properties of vascular smooth muscle. More specifically, the frequency and force of smooth muscle contraction fall as the degree of stretch decreases (Johansson and Mellander, 1975). Thus, these intrinsic myogenic reactions can account for the vasodilation observed upon perfusion pressure reduction.

Nearly all of our present knowledge about flow autoregulation in skeletal muscle derives from macroscopic or whole organ studies. In addition to its technical simplicity, the whole organ approach has the additional advantage of providing valuable insight into the integrative behavior of populations of microvessels. By contrast, the major strength of the microscopic approach lies in its ability to probe the function of individual components of the microvascular tree. The present study represents an attempt to apply quantitative microscopic techniques to the problem of flow autoregulation in skeletal muscle of the rat.

In our studies, microvascular pressure changes were initiated by changing the resistance to flow at the level of the sacral aorta. Since we measured only the systemic pressure (and did not measure microvascular pressures), we do not know the exact intravascular pressure within a given arteriolar segment. Our analysis is therefore based on the assumption that the microvascular pressure within each segment studied varies in direct proportion to the systemic pressure when systemic pressure is altered. Bohlen et al. (1977) measured microvascular pressures within various segments in the cremaster muscle of normotensive and hypertensive rats. Their data show that, over a wide range of pressure, microvascular pressure in each segment is a constant fraction of systemic pressure, supporting the validity of our assumption.

Segmental Arteriolar Responses to Graded Reductions of Arterial Pressure in the Control State

In cremaster muscle, tissue \( P_{O_2} \) ranges between 10 and 22 mm Hg, with an average value of 17 mm Hg (Gorczynski and Duling, 1978). In a recent study, we
found that the blood flow per unit mass of cremaster muscle, biceps, and gastrocnemius were identical, suggesting that the cremaster muscle may serve as a useful model of skeletal muscle in general (Moff and Granger, 1980). In the present study, the P02 of the bath solution was adjusted to about 18 mm Hg to mimic the level of tissue oxygenation observed in cremaster muscle at rest. At this low bath P02, blood flow to the cremaster muscle—represented by flow through the 1A, 2A and 3A vessels—exhibited moderate to intense autoregulation as arterial perfusion pressure was reduced. Although flow through the 1A arteriole was autoregulated, intrinsic vasodilation was not observed in this segment of the microvasculature. Since flow through the 1A vessel also is dependent on the resistance of downstream microvessels, it follows that the smaller arterioles must be the loci of autoregulatory control. Indeed, in the control state, the second and third order arterioles exhibited intrinsic vasodilation, the extent of lumen widening being greatest in the 3A microvessels. Thus, our data suggest a gradient of sensitivity of precapillary microvessels to reduced perfusion pressure. In the early phase of the study, we also examined the behavior of 4th and 5th order arterioles. At low bath P02, many of these microvessels exhibited pronounced vasomotion; reduction of perfusion pressure usually caused a diminution of the excursions in diameter and velocity and the mean diameter increased. Because the steady state analysis is inadequate for vessels exhibiting vasomotion, the 4A and 5A microvessels were excluded from the present study.

In approximately one-third of the 3A arterioles, blood flow rose as perfusion pressure was reduced. This phenomenon, known as superregulation of blood flow, has been observed previously in individual microvessels (Johnson and Intaglietta, 1976) and whole organs (Norris et al., 1979; Granger and Norris, 1980). In terms of control system theory, superregulation implies that blood flow per se is not the directly controlled variable in the local microvascular feedback loop. In other words, a pure flow control system is at best capable of 100% compensation; overcompensation is not possible, since a return of flow to control eliminates the error signal that elicits the compensatory vascular reaction. Explanations of superregulation based on myogenic and metabolic feedbacks are considered later in the discussion.

Effect of Hyperoxia on Microvascular Responses to Reductions of Arterial Pressure

The prevailing level of tissue oxygenation is an important determinant of the intensity of intrinsic microvascular reactions to local stresses. In the pres-
ent study, we induced a relatively hyperoxic state by exposing the surface of the cremaster muscle to a PO₂ of approximately 70 mm Hg. We did not measure tissue PO₂ in this study, and hence do not know the exact tissue PO₂ extant with the low and high bath PO₂ solutions. Nevertheless, it is logical to assume that the prevailing tissue PO₂ is higher when bath PO₂ is high than it is when bath PO₂ is low, even though we do not know the absolute values. Proctor et al. (1981) and Gorczynski and Duling (1978) have measured tissue PO₂'s in the hamster cremaster muscle with various levels of superfusate PO₂, and their data demonstrated a significant change in tissue PO₂ associated with each increment of superfusate PO₂.

In our studies, the relative hyperoxia elicited a constriction of the 2nd and 3rd order arterioles. In addition, vasomotion of 4th and 5th order arterioles was rarely observed in this group. Red blood cell velocity and blood flow were generally reduced in the higher oxygen groups. This observation is consistent with that of Prewitt and Johnson (1976), who observed a reduction of arteriolar red cell velocity in arterioles of rat cremaster muscle exposed to high oxygen tension, and with those of Lindbom et al. (1980) who reported decreased capillary density and RBC velocity in rabbit tenuissimus muscle exposed to high PO₂.

Hyperoxia had several effects on the microvascular responses to reduced arterial pressure. In general, the intensity and range of flow autoregulation at each arteriolar level was diminished when the bath PO₂ was high. In addition, of the three arteriolar levels examined in this study, only the third order arteriole exhibited intrinsic vasodilatation when arterial pressure was reduced in the hyperoxic group. By contrast, 2nd and 3rd order arterioles contributed to local flow regulation in the control state, suggesting that the larger arterioles become more involved in intrinsic autoregulation of blood flow as the degree of tissue oxygenation falls.

An interesting aspect of the hyperoxia experiments concerns the apparent paradox of disparate flow responses in series coupled vessels. More specifically, how can flow autoregulation occur in 2A and 3A vessels when blood flow through the first-order arteriole is falling in direct proportion with perfusion pressure? Since flow must be conserved as blood streams through the several orders of arterioles, one possible explanation is that flow fell dramatically or ceased in some 2nd and 3rd order arterioles. We rarely observed this type of behavior in the 2nd and 3rd order vessels examined in our study. However, because we necessarily selected vessels with sharp wall outlines for accurate diameter measurements, our selection of smaller vessels may have been biased toward the thinner portion of the cremaster muscle. Thus, the flow conservation paradox could be explained by pressure-dependent decrements of blood perfusion in the thick portions of the muscle. Although we cannot exclude this possibility, it is not readily apparent why hyperoxia should diminish the autoregulatory capacity of microvessels in the thicker areas to a greater degree than in the thinner, frequently sampled portions of the muscle.

Another possibility may lie in the sampling problems inherent in microcirculatory studies. Ideally, simultaneous velocity and diameter measurements should be made on a large number of 2nd and 3rd order arterioles in the same preparation. However, at present, simultaneous velocity determinations at the varous arteriolar levels are not possible. As a consequence, the stepwise pressure reduction protocol must be performed in sequence for each microvessel in a given muscle. Under the best of circumstances, no more than 5 to 7 pressure reduction protocols may be carried out in succession in any given experiment. Thus, an optimal experiment may yield data on a single 1A vessel and two 2nd order and 3rd order vessels. Considering the large number of 2nd and 3rd order arterioles supplying the cremaster muscle, this sample size is inadequate except in those cases in which the arteriolar responses are homogeneous. It is interesting to note that the flow patterns observed in 1A, 2A, and 3A vessels in the control state do not show the large disparities characterizing the hyperoxic state. This idea is consistent with the assertion of Honig et al. (1980) that the degree of heterogeneity of microvascular flow distribution may be a direct function of tissue oxygenation.

Mechanisms of Intrinsic Control of Skeletal Muscle Arterioles

In this section, we attempt to interpret our data in the light of the myogenic and metabolic theories of local vasoregulation. Let us first consider the myogenic viewpoint. In general, the intrinsic vasodilatation elicited by reduced transmural pressure is in complete agreement with the idea that smooth muscle relaxes when the stretching force falls. If the myogenic sensitivity of the series arteriolar segments were identical, the larger arterioles should exhibit the highest degree of vasodilatation, since the magnitude of transmural pressure reduction is greatest at these sites. This type of behavior has been reported for the arteriolar tree of cat sartorius muscle (Johnson, 1980). In the present study, the vascular responsiveness to local hypotension was greatest in the smaller arterioles. However, this observation does not argue against a myogenic mechanism, because the myogenic properties of vascular smooth muscle may be most highly developed in the terminal ramifications of the arterial tree (Folkow, 1964). The myogenic theory also accounts for the intrinsic vasoconstriction observed upon elevation of transmural pressure secondary to venous pressure elevation. Finally, the phenomenon of superregulation of blood flow is compatible with a myogenic tension sensor arranged in series with the smooth muscle cells of the arteriolar wall (Johnson and Intaglia, 1976; Koch, 1964). A consequence of this intrinsic tension-regulating system, together with the Laplace relationship between wall tension, transmural pressure, and microvessel radius, is that negative slopes of pressure-flow curves are theoretically possible since wall tension, not blood flow per se, is the controlled variable (Johnson, 1980).
If the myogenic mechanism is the primary local controller of microvascular tone, how can tissue oxygenation modulate intrinsic arteriolar responses to local arterial hypotension? An obvious answer is that parenchymal metabolites alter the myogenic sensitivity of the arterioles to reduced transmural pressure. In other words, the observed degree of flow autoregulation is less in hyperoxia because the myogenic reactivity of the arterioles is reduced when tissue \( P_{O_2} \) is elevated to a high level. A corollary to this concept is that intrinsic arteriolar vasoconstriction in response to venous hypertension should be weaker in hyperoxia than in the "normoxic" state. Our data argue against this idea; in our experiments, hyperoxia augmented the intrinsic vasoconstriction elicited by elevation of venous pressure. Finally, hyperoxia reduced the frequency and intensity of superregulation at the same time that vasoconstrictor responses to venous hypertension were intensified. This observation is difficult to reconcile with the view that a myogenic mechanism is the sole or primary cause of superregulation. However, changes in tissue \( P_{O_2} \) could alter the hydrostatic pressure distribution within the microvascular network, resulting in a change in preload on the vascular smooth muscle segments that could alter their responses to transmural pressure variations. Further experiments involving microvascular pressure measurements will be required to address this problem more fully.

Flow autoregulation and its modulation by the prevailing level of tissue oxygenation can be explained in terms of a chemical feedback signal, if we assume a purely metabolic mechanism of intrinsic vasoregulation. For example, the higher metabolic rate of rat skeletal muscle (Honig et al., 1971) may account for the more intense autoregulation in the control state when a comparison is made with flow responses in canine muscle. In addition, hyperoxia probably reduces the rate of production of vasodilator metabolites and thereby diminishes the role of these chemicals in intrinsic control of arteriolar tone; the final effect is a reduction of the degree of flow autoregulation. The intrinsic vasoconstriction elicited in our experiments by venous hypertension is not readily accounted for by the metabolic theory. Indeed, according to the metabolic theory, elevation of venous pressure should reduce flow and consequently vasodilator washout. In turn, the rise in metabolite concentration should elicit an intrinsic vasodilation, a response opposite to the one we observed.

From the above discussion, it appears that our experimental results cannot be explained in full by either a metabolic or myogenic mechanism operating alone. Consider, however, the behavior of a microvasculature regulated by both metabolic and myogenic control systems operating simultaneously. With reductions in arterial pressure, the widening of the arteriolar lumen could represent the sum of two vasodilatory influences—a myogenic relaxation secondary to reduced transmural pressure and a metabolic relaxation elicited by buildup of vasodilator metabolites in the interstitial space. If, in hyperoxia, the concentration of vasodilator metabolites is reduced, the contribution of the metabolic system would be diminished or abolished, and only a portion (i.e., the myogenic component) of the intrinsic vasodilator capacity of the arteriole would be operative. As a consequence, hyperoxia would be characterized by a diminution in the ability of the muscle to autoregulate its own flow. In the control state, the intrinsic vasoconstriction elicited by venous hypertension would represent an imbalance of the myogenic vasoconstrictor and metabolic vasodilator signals, favoring the myogenic mechanism. In hyperoxia, the influence of the metabolic signal would be reduced or abolished and the full potential of the myogenic reaction unmasked. As a consequence, the arteriolar constriction elicited in response to elevation of venous pressure would be more intense in hyperoxia.

While the argument presented above represents a possible scheme for operation of an autoregulatory mechanism that is consistent with the overall picture presented by our data, some elements of our data are explicable in terms of alternate mechanisms that should be considered. For example, whereas our data on superregulation are consistent with a primary myogenic mechanism being modulated by metabolic influences, it is conceivable that elevations in venous pressure could produce a shift in blood flow resulting in a more homogeneous perfusion of the tissue and therefore in increased flow within certain individual vessels. In addition, the possibility of the existence of a veni-vasomoter reflex (although it is not well defined) should be considered as a potential contributor to the vascular responses to venous pressure elevation. With regard to the effects of tissue oxygenation on the autoregulatory potential, it is possible that changes in tissue \( P_{O_2} \) could produce direct effects on vascular smooth muscle without acting through a metabolic intermediary. Further experiments, involving intravascular pressure measurements, microvascular \( P_{O_2} \) measurements and a more thorough survey of vascular responses vis-a-vis overall network geometry will be required if microvascular control mechanisms are to be more fully understood.

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