Myogenic Vasoregulation Overrides Local Metabolic Control in Resting Rat Skeletal Muscle

Gerald A. Meininger, Chris A. Mack, Karen L. Fehr, and H. Glenn Bohlen

Microvascular reactions to increases in intravascular pressure were studied in the cremaster muscle of the anesthetized rat by enclosing the animal in an airtight box with the muscle exteriorized for observation of the microcirculation. Since the cremaster was exposed to atmospheric pressure, increasing pressure within the box produced equal increases in arterial and venous pressures. Thus, intravascular pressure was altered without affecting the pressure gradient for blood flow. Raising box pressure had no effect on respiration or heart rate and did not change the systemic activity of the sympathetic system, angiotensin II, or vasopressin. Diameters and flows were measured for first (107 ± 3 μm, mean ± SEM), second (87 ± 5), third (29 ± 2), and fourth (15 ± 2) order arterioles during increases in intravascular pressure of +10, +20, and +30 mm Hg. No significant changes in the diameters of first or second order arterioles were elicited when pressure was increased. However, when box pressure was increased to +10, +20, or +30 mm Hg, a sustained constriction occurred in third (29%, 45%, and 63%, respectively) and fourth (5%, 38%, and 57%, respectively) order arterioles. Blood flow was significantly reduced in all arterioles, and perivascular PO2 was decreased adjacent to third and fourth order arterioles. Furthermore, the third order arteriole constrictor response was not abolished by local α-receptor blockade (phenolamine), indicating that it was not mediated by a local sympathetic axon reflex. Collectively, these data indicate that a potent, non-neural, pressure-dependent mechanism for vasoregulation is present in small arterioles of the cremaster. The sustained constriction in the presence of reduced blood flow and reduced periarteriolar oxygen tension indicates that the vascular response is independent of and capable of overriding flow-dependent (i.e., metabolic) control in resting skeletal muscle. The observations are compatible with the operation of a powerful myogenic mechanism in small arterioles. (Circulation Research 1987;60:861–870)

The myogenic mechanism is thought to be an important contributor to peripheral vascular tone and to the regulation of tissue blood flow and/or capillary pressure.1,2 Furthermore, it is considered to be independent of neurotransmitters, vasoactive metabolites, and hormones.1,2 The stimulus for the myogenic response is proposed to be an alteration in the stretching forces acting on the vascular smooth muscle cell2 such that a rise in transmural pressure leads to vasoconstriction and a fall leads to vasodilation.

It has been difficult to evaluate the myogenic mechanism in vivo because the methods employed to alter transmural pressure also modify the pressure gradient for blood flow. Consequently, these experimental conditions simultaneously activate the metabolic mechanism of flow control. To overcome this difficulty, an alternative method for altering transmural pressure was developed by Wiederhielm et al.3 Their method involved enclosing the body of the experimental animal in an airtight box while exteriorizing the tissue to be studied. Because the vasculature in the exteriorized tissue was exposed to atmospheric pressure, increasing the pressure within the box resulted in equal increases in arterial and venous pressure. This technique has been successfully used for studies of myogenic vascular behavior in the bat wing,3-5 the hamster cheek pouch,6 and the rat cerebral cortex.7

In the present study, one of our goals was to evaluate the use of an airtight box as a technique for studies involving the cremaster skeletal muscle. More importantly, an attempt was made to characterize the microvascular responses to increased intravascular pressure at successive network locations in the cremaster microcirculation. Our major finding was that there is a potent pressure-sensitive vasoconstrictor mechanism (i.e., myogenic) existing in small arterioles of the cremaster muscle with the power to override the local metabolic control of blood flow.

Materials and Methods

Surgical and Experimental Procedures

General. Male, Sprague-Dawley rats (Harlan, Houston, Tex.), which weighed 240 ± 12 g (mean ± SEM, n = 47), were used for all experiments. Rats were housed with a 12-hour light/dark cycle and provided with food and tap water ad libitum. For an experiment, rats were anesthetized with an intraperitoneal injection of urethane (425 mg/kg) and chloralose...
(100 mg/kg) and given supplements (20% of initial dose) as needed. After anesthesia, rats were placed on a heating pad to maintain rectal temperature (36°–37.5°C) and were tracheostomized. Cannulas were placed in the femoral artery and vein to measure pressure and into the peritoneal cavity for administration of anesthetic supplements.

Cremaster isolation. Techniques for preparation of the cremaster muscle microcirculation have been previously described.\(^8\)\(^9\)\(^10\) In brief, the right cremaster muscle was dissected free from the scrotal skin and testicle. The animal was placed on a Plexiglas board, and the cremaster was extended into a bath chamber with its circulation and innervation intact. The cremaster was positioned over an optical pedestal in the chamber, and the bath was filled with warmed (34.5°C) Krebs-bicarbonate solution. Carbon dioxide and nitrogen gas were bubbled through the bath to control pH at 7.35–7.45, PCO\(_2\) at 40–50 mm Hg, and PO\(_2\) at 20–35 mm Hg.

Description of pressure box. After preparation of the cremaster, a Plexiglas box was placed over the body of the rat (Figure 1). The box enclosure was designed to seal against the Plexiglas base board on which the rat was lying. A small slot in the box permitted the cremaster to be exteriorized into the bath chamber without being compressed by the box. The femoral artery and vein cannulas and the anesthetic supplement tube were exteriorized through the box wall. Pressure in the box was regulated and monitored by connecting the box to a compressed air supply and a pressure transducer, respectively. Fresh air was continuously circulated through the box.

Measurement of microvascular variables. The animal preparation was transferred to the stage of a Zeiss-ACM microscope for observation of the microcirculation with videomicroscopy. A 20× microscope objective gave a final magnification of 1,350× on the television monitor.

Arteriolar lumen diameters were measured with an image shearing monitor (model 907, Instrumentation for Physiology and Medicine, San Diego, Calif.). Centerline red cell velocity was measured with an optical Doppler intravital velocimeter (Microcirculation Research Institute, College Station, Tex).\(^11\) Volume flow was calculated by the equation: flow = \((v/1.6) \times (\pi r^2) \times (0.001)\), where \(v\) is centerline red cell velocity.
cell velocity (mm/sec), 1.6 is a conversion factor applied to convert centerline velocity to average cross-sectional velocity, 12 r is the lumen radius (µm), and 0.001 converts the units of flow to nl/sec. Measurements of microvascular pressure were made as previously described using a servo-null micropipette system (model 4A, Instrumentation for Physiology and Medicine). 9,10

To perform the measurements of perivascular PO2, it was necessary to suffuse the cremaster preparation. The suffusion solution consisted of warmed (34–35°C) bicarbonate buffered solution equilibrated with 5% CO2–95% N2. Microvascular diameter responses were compared from experiments in which the cremaster was suffused with those in which the bath was used and were found not to differ. Suffusion solution PO2 and periarteriolar PO2 were measured with Whalen-type oxygen-sensitive microelectrodes 13 with tip diameters from 1.5 to 3.0 µm. The PO2 measured in the suffusion solution approximately 100 µm above the cremaster surface was maintained at approximately 20–30 mm Hg by adjusting the flow rate of the solution (2–6 ml/min). All the oxygen microelectrodes operated in the picocampere current range and were calibrated prior to and after each experiment. 14,15

The arterioles examined in this study were identified on the basis of their branching order. 9 The main feeder arteriole and venule draining the cremaster were classified as the first order arteriole (1A) and venule (1V). Subsequent branches from the 1A were designated as second (2A), third (3A), and fourth (4A) order arterioles.

Experimental Protocols — Validation of Box Technique

I. Effect of box pressure on heart rate and respiration rate. Rats were sealed into the pressure box, and pressure in the box was raised in steps of 5 mm Hg, lasting 60 seconds, from atmospheric pressure to 25 mm Hg. During the last 30 seconds of a pressure step, heart rate and respiration rate were determined.

II. Effect of box pressure on systemic neurohumoral control. Neurogenic vascular tone was estimated from the fall in mean arterial pressure following administration of the ganglionic blocking agent, trimethaphan camsylate (Arfonad, Roche Laboratories, Nutley, N.J.). The ganglionic blocker was given intravenously as a bolus dose of 1.73 mg/kg followed immediately by infusion of 1.4 µg/kg/min. The maximum fall in mean arterial pressure was recorded, and the infusion was stopped. After recovery (30–40 minutes), box pressure was elevated to +30 mm Hg, and the ganglionic blocker was readministered. A comparison of the fall in arterial pressure was used to determine whether neurogenic vascular tone changed. The same fall in arterial pressure was interpreted to indicate that sympathetic activity was unaltered.

Vascular tone due to angiotensin II was estimated by using the angiotensin II receptor antagonist [sar⁴, ala⁷]-angiotensin II (Saralasin, Sigma Chemical Co., St. Louis, Mo.) in the protocol described above. Saralasin was administered as a bolus of 10 µg/kg followed by a 20-minute infusion of 10 µg/kg/min. Rats were allowed 1 hour to recover before increasing box pressure.

Vascular tone due to vasopressin was estimated by using the vasopressin antagonist Pmp¹, O-Me-Tyr²-[Arg¹]-vasopressin (Peninsula Laboratories, San Carlos, Calif.). The antagonist was given as a single bolus injection of 20 µg/kg, and a 2-hour recovery was allowed before elevating box pressure.

III. Effect of box pressure on systemic vascular pressures. Box pressure was elevated in steps of 5 mm Hg from 0 to 25 mm Hg and maintained at each step for 30–40 seconds. Box pressure was correlated with femoral artery and vein pressures and with 1A and 1V pressures, which were made near the entry point of these vessels into the cremaster.

Experimental Protocols — Myogenic Responses in Cremaster Muscle

I. Effect of box pressure on arteriolar diameter and flow. Microvessel lumen diameter (µm) and red cell velocity (mm/sec) were measured in 1A, 2A, 3A, and 4A arterioles. After a 1-minute control period, box pressure was increased +10 mm Hg for 2 minutes followed by a 3-minute recovery period. After a 1-minute rest period, the sequence was repeated with +20 and +30 mm Hg steps in box pressure. The effect of a sustained increase in box pressure was also studied in 3A arterioles that were exposed to 30 minutes of elevated box pressure (+20 mm Hg).

II. Effect of box pressure on tissue PO2. Measurements of PO2 (mm Hg) were made at periarteriolar sites at the same tissue depth as the arteriole and as near to the arteriolar wall as possible without provoking constriction or dilation. The sites were never greater than about 20 µm from the vessel. The PO2 was measured for 1 minute immediately preceding an increase in box pressure and continued for as long as tissue movements would permit. Raising box pressure sometimes caused a slow and small mechanical translocation of the tissue, which would interfere with the placement of the oxygen microelectrode. When this occurred, the measurements were discarded.

III. Effect of local sympathetic blockade. To determine whether a local sympathetic axon reflex was contributing to the vascular response to increased box pressure, 1 × 10⁻⁶M phenolamine was used as an α-receptor antagonist. This concentration completely blocked the 3A constrictor response to a topically applied ED₉₀ dose of norepinephrine (1 × 10⁻⁷M). The response of a 3A arteriole to a +20 mm Hg increase in box pressure was studied before and 25 minutes after addition of phenolamine to the cremaster bath.

Data Analyses

The relations between box pressure and heart rate, respiration rate, femoral artery and venous pressures, or 1A and 1V pressures were analyzed using simple linear regression analyses. 16 Comparisons of the changes in arterial pressure produced by the gan-
glionic, α-adrenergic, and hormonal blocking drugs were made with the paired t test. Diameter and flow responses were tested using analysis of variance procedures. Differences were considered significant at \( p < 0.05 \).

**Results**

**Validation of Box Technique**

**EFFECT OF BOX PRESSURE ON RESPIRATION, HEART RATE, AND ARTERIAL \( \text{PO}_2 \).** The control respiration rate and heart rate were 91 ± 4 breaths/min (mean ± SEM) and 410 ± 11 beats/min, respectively. Increasing box pressure in steps of 5 mm Hg from 0 to 25 mm Hg had no effect on respiration \( (n = 11) \) or heart \( (n = 19) \) rate. Arterial \( \text{PO}_2 \) was measured in 6 rats and was 83.5 ± 5.0 mm Hg (mean ± SEM) when the box was open to atmospheric pressure and increased to 88 ± 5.2 mm Hg at a box pressure of +30 mm Hg.

**EFFECT OF BOX PRESSURE ON ARTERIAL AND VENOUS PRESSURES.** Raising box pressure from 0 to 25 mm Hg was accompanied by equivalent changes in the arterial and venous pressures measured in the femoral artery and vein \( (n = 24) \) and in the 1A arteriole and 1V venule \( (n = 24) \). For example, when box pressure was elevated from 0 to 25 mm Hg, femoral artery pressure rose from 123 ± 2 to 147 ± 2 mm Hg, femoral vein pressure from 3 ± 0.5 to 27 ± 0.5 mm Hg, 1A pressure from 65 ± 3 to 96 ± 2 mm Hg, and 1V pressure from 12 ± 0.5 to 33 ± 0.5 mm Hg. In all cases, the slopes of the relations between box pressure and vessel pressure were significantly different from 0, and there was a significant correlation between the increases in box pressure and the increases in vessel pressure.

**EFFECT OF BOX PRESSURE ON SYSTEMIC NEUROHUMORAL MECHANISMS.** At 0 mm Hg, ganglionic blockade \( (n = 7) \) significantly reduced mean arterial pressure by 54 ± 5 mm Hg. This change in pressure was not different from the decrease in arterial pressure of 54 ± 4 mm Hg produced by ganglionic blockade when box pressure was elevated to 30 mm Hg. In a separate group of rats \( (n = 6) \), the competitive angiotensin II antagonist was administered and found to produce equivalent decreases in arterial pressure for box pressures of 0 (26 ± 4 mm Hg) and +30 mm Hg (24 ± 4 mm Hg). In another group of rats \( (n = 6) \), the vasopressin antagonist also showed that arterial pressure decreased by an equal amount whether box pressure was at 0 mm Hg (6 ± 1 mm Hg) or at 30 mm Hg (5 ± 2 mm Hg).

### Table 1. Summary of Resting Control Diameters, Velocities, and Flows for Cremaster Arterioles*

<table>
<thead>
<tr>
<th>Arteriole category</th>
<th>Lumen diameter (μm)</th>
<th>Red cell velocity (mm/sec)</th>
<th>Blood flow (nl/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First order ( (n = 10) )</td>
<td>107 ± 3</td>
<td>30.5 ± 2.7</td>
<td>171 ± 12</td>
</tr>
<tr>
<td>Second order ( (n = 11) )</td>
<td>87 ± 5</td>
<td>15.0 ± 2.1</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Third order ( (n = 10) )</td>
<td>29 ± 2</td>
<td>8.6 ± 1.7</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>Fourth order ( (n = 11) )</td>
<td>15 ± 2</td>
<td>5.9 ± 1.0</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

*All table values are expressed as the mean ± SEM.

**Myogenic Responses in Cremaster Muscle**

**EFFECT OF BOX PRESSURE ON ARTERIOLAR DIAMETER AND FLOW.** The resting control diameters, red cell velocities, and flows for the 1A, 2A, 3A, and 4A arterioles are summarized in Table 1. Increasing box pressure in steps of 10 mm Hg from 0 to 30 mm Hg had no effect on the lumen diameter of the 1A or the 2A arterioles (Figure 2). However, there was a tendency for diameter to increase with the increase in box pressure (Figure 2). In contrast to the 1A and 2A arterioles, the 3A and...
BOX PRESSURE INCREASED

DIAMETER (µm)

DIAMETER (µm)

DIAMETER (µm)

TIME (seconds)

BOX PRESSURE (mmHg)

BOX PRESSURE (mmHg)

BOX PRESSURE (mmHg)

THIRD ORDER ARTERIOLE RESPONSES TO INCREASED BOX PRESSURE (n=10)

FOURTH ORDER ARTERIOLE RESPONSES TO INCREASED BOX PRESSURE (n=11)

Figure 3. Diameter responses (mean ± SEM) of 3A (•) and 4A (O) arterioles to increases in box pressure of +10, +20, and +30 mm Hg as a function of time. Organization in this figure is identical to that in Figure 2. Asterisks indicate that after 2 minutes of increased box pressure, diameters were significantly (p<0.05) decreased.

4A arterioles underwent significant vasoconstriction when box pressure was increased (Figure 3). Diameters of these small arterioles began to decrease immediately following the increase in box pressure. Occasionally, a transient increase in the diameter of the 3A and 4A arterioles occurred with the onset of the increase in box pressure, after which the vessels constricted. At times, the constrictor responses of the 3A and 4A arterioles were of such intensity as to produce complete vessel closure.

Blood flow was significantly reduced in all arterioles following 2 minutes of increased box pressure (Figure 4). Compared to the 1A and 2A arterioles, the 3A and 4A arterioles exhibited a greater decrease in blood flow, which was particularly apparent at a box pressure of +30 mm Hg. In all the branch orders of arterioles, the fall in blood flow began immediately following the increase in box pressure and was not preceded by a detectable transient increase in vessel blood flow. This is apparent in an experimental record from a 3A arteriole: both red cell velocity and diameter began decreasing immediately following the onset of increased box pressure (Figure 5). Periodic oscillations in red cell velocity occurred during the period of increased box pressure. While these oscillations were not associated with changes in femoral artery pressures, respiration rate, or 3A vessel diameter, they were correlated with intermittent periods of dilation in downstream 4A arterioles.

The responses of 3A arterioles (n = 6) exposed for 30 minutes to a +20 mm Hg change in box pressure are shown in Figure 6. Following the increase in box pressure, there was a decrease in diameter (36%) and flow (64%) that persisted throughout the 30-minute period of elevated box pressure (Figure 6). During the recovery, diameter and flow increased to levels 18% and 29%, respectively, above their control resting values (Figure 6).

Effect of box pressure on periarteriolar PO₂. The effect of increasing box pressure on perivascular PO₂ was studied in seven 3A and five 4A arterioles in 6 rats. During the control period, the resting perivascular PO₂ was 19.3 ± 3.7 mm Hg for the 3A arterioles and 29.4 ± 5.7 mm Hg for the 4A arterioles. The higher perivascular PO₂ for the 4A arterioles is probably accounted for by random sampling of vessels.
that did not represent consecutively branching vascular segments. Increasing box pressure was accompanied by a pressure-dependent reduction in perivascular PO₂ for all of the sampled 3A and 4A arterioles (Figure 7). Topical application of adenosine (1 x 10⁻³M) to one of the 3A arterioles (dashed line, Figure 7) resulted in maximal dilation of the vessel and increased PO₂. The diameter and PO₂ response of this vessel to increased box pressure was abolished by the adenosine-induced vasodilation (Figure 7).

**EFFECT OF LOCAL SYMPATHETIC BLOCKADE.** The effect of local α-receptor blockade on the response of 3A arterioles to a +20 mm Hg increase in box pressure are summarized in Table 2. Local α-receptor blockade did not abolish the 3A (n = 19) vasoconstriction following a +20 mm Hg increase in box pressure, but it did attenuate (30%) the constrictor response (Table 2). Examination of the individual experiments revealed that only 10 of the 19 vessels studied dilated (approximately 41%) in response to phentolamine (see Table 2, arterioles withα-tone). Separate analysis of vessels with and without α-tone indicated that only the vessels that dilated in response to phentolamine demonstrated significant attenuation of the constrictor response to elevated box pressure (Table 2).

**Discussion**

One of the purposes of this study was to evaluate the box technique as a method for elevating transmural pressure in cremaster muscle arterioles, and this will be discussed before addressing the major aim of our study, which was to delineate the cremaster microvascular reactions to elevated transmural pressure. Our principal finding was that there is a potent myogenic-like constrictor mechanism existing in small cremaster arterioles that can overpower local metabolic control of blood flow.
Validation of Box Procedure

The pressurized box technique\textsuperscript{34} uses changes in ambient box pressure that are transmitted uniformly throughout the vascular system to alter transmural pressure in tissues exteriorized from the box. The transmural pressures in tissues within the box do not change because the increase in intravascular pressure is caused by and balanced by an increase in extravascular pressure. However, tissues outside of the box are exposed to an increased intravascular pressure that is not counterbalanced by the increased ambient pressure surrounding the rat’s body in the box. In the present study, increases in box pressure relative to atmosphere were found to produce equivalent increases in arterial and venous pressure, both systemically and in the microcirculation.

Several experiments were performed to verify that increasing box pressure did not activate systemic mechanisms of blood pressure regulation which could produce vasoconstriction independent of the local increase in intravascular pressure. It was found that increasing box pressure had no effect on heart or respiratory rate, corroborating similar findings by others in the bat,\textsuperscript{34} hamster,\textsuperscript{6} and rat.\textsuperscript{7} In addition, with the use of selective pharmacologic antagonists, it was found that increasing box pressure did not alter the systemic activity of the sympathetic nervous system or alter the amount of resting vascular tone produced by circulating levels of angiotensin II and vasopressin. Therefore, it could be concluded that any microvascular response was the result of a locally mediated response to the increased intravascular pressure.

Myogenic Responses in Cremaster Muscle

A characteristic of the constrictor response of cremaster arterioles to increased intravascular pressure was the variability of the constrictor response between separate arteriolar levels. In the larger 1A and 2A arterioles, no constrictor response to increased intravascular pressure was observed. In fact, there was a tendency for their diameters to increase when box pressure was elevated (Figure 2), which suggested a net passive distension. It is possible that these arterioles were not sensitive to the change in intravascular pressure or that they were stretched beyond their optimal length.\textsuperscript{17} It is also possible that although there was a small increase in vascular smooth muscle tone, it was insufficient to overcome or counterbalance the rise in intravascular distending pressure.

In contrast to the large arterioles, an intense vasoconstriction was observed in the 3A and 4A arterioles (Figure 3), and the intensity of the vasoconstriction increased over the range of box pressures studied. Furthermore, the constrictor response of the 3A arterioles appeared to be more pronounced than that of the 4A arterioles. It may be that 3A vessels are intrinsically more sensitive to changes in intravascular pressure or that they are positioned more favorably on their length-tension curve. It has been suggested that the gradient of responsiveness to vasoconstrictors that exists along the arterial tree may be due to an arteriole’s position on its length-tension curve.\textsuperscript{17} Another possibility is that the time course and magnitude of the “effective” pressure stimulus actually experienced by the 4A arterioles differs from the “effective” pressure stimulus occurring...
ring in the 3A arterioles. This difference could occur if the vasoconstriction in the 3A arterioles buffered the pressure change occurring in the downstream 4A vessels and may explain the slow attenuation of the vasoconstriction observed in 4A arterioles at box pressures of +20 mm Hg and +30 mm Hg (Figure 3). This type of buffering is supported by a recent study of the bat wing microcirculation in which it was reported that pressure in terminal arterioles increased by only 67% of the change in box pressure because of upstream vasoconstriction. These data indicate that a more precise understanding of the mechanisms responsible for the time course and intensity of the vasoconstrictor responses in the 3A and 4A arterioles will, at a minimum, require knowledge of the local intravascular pressure.

Blood flow was decreased at all arteriolar levels including the 1A and 2A arterioles that did not exhibit any vasoconstriction (Figure 4). This finding indicates that the observed decreases in 1A and 2A flow were the result of an increase in resistance produced by vasoconstriction in downstream arterioles. If the 1A arteriole carries the principal blood supply to the cremaster, then 1A flow can be used as an index of total cremaster flow. With the use of this flow index, it was estimated that the increase in total cremaster resistance required to reduce 1A flow was 11% at a box pressure of +10 mm Hg and 43% at +30 mm Hg. Virtually all of this increase in resistance was caused by the 3A and 4A arterioles.

One seemingly contradictory observation was that the flow responses in the successively coupled microvessels were not equivalent. For example, the decreases in flow recorded at the 3A and 4A arterioles were larger in magnitude than those recorded at the level of the 1A and 2A vessels (Figure 4). One explanation may be that our assumption about the series-coupled nature of the network is inadequate for analysis of blood flow and resistance distribution. It is also possible that collateral pathways opened when box pressure was increased, but these were not observed. Another explanation is that the population of 3A and 4A arterioles is heterogeneous. For technical reasons, 3A and 4A vessels were selected from among vessels located in the central portion of the cremaster, and sampling from the cauterized edge regions of the muscle was avoided. These edge regions of the muscle may be traumatized so that vessels in these regions do not respond as vigorously as those in the central portion of the muscle. Blood flow to these edge regions has been reported to be higher than in the central region of the cremaster muscle. A population of less responsive vessels would blunt the flow changes in the 1A and 2A vessels. If this is true, then our estimates of the change in cremaster resistance probably underestimate what could be expected in a nonisolated, intact cremaster muscle.

**Evidence Against a Metabolic Mechanism of Vasoconstriction**

In the present study, it is more likely that the vasoconstriction induced by elevating intravascular pressure resulted from a myogenic-like mechanism than from a metabolic mechanism of flow control. In our study, vasoconstriction associated with increasing intravascular pressure was accompanied by a significant fall in blood flow and in perivascular Po2 to levels below control. Furthermore, there was no transient increase in blood flow (Figure 5) or perivascular Po2 when box pressure was initially increased. Thus, the necessary stimulus (error signal) required to elicit vasoconstriction via a flow-dependent or oxygen-dependent route was apparently lacking. In fact, the decrease in flow and Po2 would have favored a local metabolic stimulus for vasodilation. These observations suggest that flow and perivascular Po2 are not the controlled variables under the experimental conditions of the present study. The findings, however, do support the occurrence of a myogenic vasoconstriction caused by the increased intravascular pressure.

A unique feature of the experiments on the 3A arterioles concerned their recovery from the box pressure stimulus, which was characterized by vasodilation beyond the control arteriolar caliber, hyperemia (Figures 5 and 6), and a perivascular Po2 overshoot. The vasodilation and hyperemia were especially apparent during recovery from 30 minutes of elevated box pressure (Figure 6). One interpretation is that the decrease in transmural pressure associated with restoration of box pressure to control elicited a myogenic relaxation. Another possibility is that the reactive responses could be due to the accumulation of a vasodilator substance(s) in the tissue during the period of sustained constriction and reduced flow. Evidence for the local presence of vasodilator signal(s) seemed apparent in some individual experiments in which red cell velocity in the 3A arterioles was observed to undergo a series of phasic surges (Figure 5). The diameter of the 3A arteriole remained reduced below control during these periods of surge flow, but downstream 4A vessels were observed to undergo a periodic dilation that correlated with the flow increase. These observations suggest that the pressure-sensitive constrictor mechanism (i.e., myogenic response) is capable of overriding local metabolic control.

**Mechanistic Considerations and Implications**

In a previous study, Morff and Granger reported that elevating venous pressure in the cremaster muscle resulted in a significant constriction of small arterioles. For example, when venous pressure was elevated to 4.5 times normal (approximately 24 mm Hg), they observed a 35% decrease in diameter and a 60% decrease in flow for 3A arterioles. This diameter response is comparable to the response of 3A arterioles exposed to a similar increase in intravascular pressure using the box technique. This quantitative similarity occurred despite the fact that in their study, the pressure gradient for flow decreased, a problem circumvented by the box technique. Presumably, decreased flow in their study should have activated the metabolic mechanism, which would then antagonize or blunt the myogenic-like constrictor response. Collectively, our
two studies suggest that vascular control in resting skeletal muscle is minimally influenced by metabolic factors. This situation may be entirely different when perfusion pressure is lowered or when tissue oxygen demand is increased, such as during exercise. For example, several studies of the interaction between myogenic and metabolic control have indicated that the metabolic influence of exercise acts to diminish the gain of the myogenic response.

Even though the present findings and those of Morff and Granger are consistent with the operation of a myogenic-like mechanism, it has been demonstrated by Henriksen et al. that a local venoarteriolar reflex is activated by venous distension to cause arteriolar vasoconstriction. They reported that the arteriolar constriction elicited by venous pressure elevation in the dog hind limb is attenuated by phenoxybenzamine, an α-receptor antagonist, suggesting that there is a local sympathetically mediated reflex. In contrast, Flemming found that surgical denervation of the bat wing did not diminish the arteriolar responses to changes in transmural pressure induced by box pressure. In our study, local α-receptor blockade with phentolamine did not abolish the constrictor response of 3A arterioles to a +20 mm Hg increase in intravascular pressure. However, the constrictor response was attenuated, and the attenuation was dependent on whether the 3A arterioles would dilate in response to α-blockade since vessels without α-adrenergic tone exhibited no attenuation. It is possible that the attenuation was the result of eliminating a contribution from a local sympathetic axon reflex or that the local sympathetic blockade was incomplete. Alternatively, it could very easily be related to a dilation induced change in a vessel’s position on its length-tension curve or to an alteration in a length-dependent component of norepinephrine sensitivity so that conditions for tension development are less than optimal. Whatever the reason, these data indicate that the pressure-dependent response is primarily myogenic.

In the present study, it was observed that elevation of intravascular pressure could occasionally produce vessel closure. However, during vessel closure, intravascular pressure will fall toward zero, and consequently, wall tension will approach zero, thereby removing the “effective” myogenic stimulus. Under these conditions, one may speculate that the stimulus for this constriction was independent of mechanical events occurring in the walls of these vessels. One intriguing explanation could be that myogenic responses may be propagated so that the constricting vessels were being driven by myogenic activity occurring in adjacent vessels still experiencing a distension stimulus.

In conclusion, our results indicate that a pressure-sensitive myogenic mechanism is present in small arterioles of resting skeletal muscle. This mechanism of vasoregulation appears to be nonneural and is capable of producing a sustained constriction in the presence of reduced blood flow and reduced perivascular oxygen tension, indicating that it is independent of and capable of overriding flow-dependent (i.e., metabolic) control.

Acknowledgments

The authors express their thanks to Helen Higginbotham and Martha Larson for typing the manuscript, to E. Zuzanna Ostrowska for her technical assistance, to David Boedecker for his excellent machine work and assembly of the pressure box, and to the Independent School District of Tyler, Tex., for their outstanding volunteer work for the American Heart Association, Texas Affiliate.

References


KEY WORDS • myogenic vascular control • metabolic vascular control • local control of blood flow • autoregulation • arterioles • microcirculation • local sympathetic reflex
Myogenic vasoregulation overrides local metabolic control in resting rat skeletal muscle.
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Circ Res. 1987;60:861-870
doi: 10.1161/01.RES.60.6.861

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

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