Response of the Microcirculation in Rat Cremaster Muscle to Peripheral and Central Sympathetic Stimulation

Bernard P. Fleming, Kirk W. Barron, Timothy W. Howes, and J. Kevin Smith

Microvascular diameter and flow responses to peripheral and central sympathetic stimulation were measured in different segments of the arteriolar network in rat cremaster muscle. For peripheral stimulation, a bipolar electrode was placed on the internodal segment of the lumbar sympathetic chain between ganglia L1 and L2. For central stimulation, a bipolar electrode was stereotaxically implanted in the posterior hypothalamus. Inside vessel diameter, red blood cell velocity, and volumetric flow rate were recorded in response to electrical stimulations of varying magnitude in four series-coupled segments of the arteriolar network: 1A, 2A, 3A, and 4A. Systemic arterial pressure was also monitored. The vasoconstriction and flow reduction produced by stimulation of the lumbar chain was graded with the frequency of stimulation over the range of 0.5-16.0 Hz in all arteriolar segments.

Examination of the relation between stimulation frequency and vasoconstrictor response measured as percent of control diameter indicated a sequence of responsiveness to peripheral stimulation where 4A = 3A > 2A = 1 A. No changes in diameter were recorded in the venous microcirculation at any level of stimulation. Stimulation of the posterior hypothalamus with currents of 38-300 /u,A for 60 seconds produced graded vasoconstriction in only 3A and 4A vessels. Fluorescence histochemistry for biogenic amines was used to examine the distribution of innervation to the microvasculature. All segments of the arteriolar network from 1A to 4A possessed an adrenergic innervation; no vessels of the venous network were found to be innervated. The results indicate that the pattern of response of the arteriolar network in rat cremaster muscle to peripheral and central sympathetic stimulation is segmentally differentiated and consistent with the distribution of the vasomotor innervation. (Circulation Research 1987;61(suppl II):II-26-II-31)

Neurogenic regulation of the peripheral circulation is accomplished in large part by control variations in sympathetic adrenergic discharge to the arteriolar resistance vessels of the microcirculatory network. To date, the majority of our knowledge of neural control mechanisms has been derived from hemodynamic studies on whole organs or on the responses of larger vessel segments studied in vitro. Recently, there has been an increasing interest in using microcirculatory models and methodology in studies on neurogenic control of the microvasculature. An often-described aspect of the response of the peripheral circulation to sympathetic neural stimuli is the apparent specialization of the response pattern in the various series-coupled segments of the precapillary resistance network. This differentiated pattern of response in the various segments of the arterial-arteriolar network implies a complex system of microcirculatory neural control that would have important physiologic implications.

In direct studies on the microcirculation in skeletal muscle, it has been found that, in general, there is an inverse relation between vessel diameter and percent vasoconstriction to neurogenic stimuli. It should be noted that segmental blood flow responses to neural stimuli have been qualitatively described but not directly measured in the microvasculature of skeletal muscle.

The principal hypothesis examined in this study is that the series-coupled segments of the arteriolar network in rat cremaster muscle respond to sympathetic stimulation in a differentiated manner. To test this hypothesis, vasoconstrictor and blood flow responses to stimulation of the lumbar sympathetic chain and posterior hypothalamus were recorded systematically in four series-coupled arteriolar segments. In addition, the distribution of adrenergic vasomotor nerves to the microcirculation in rat cremaster muscle was determined by fluorescence histochemistry and correlated with the functional behavior.

Materials and Methods

Animals

For experiments involving stimulation of the sympathetic chain, 20 male Sprague-Dawley rats (153 ± 28 g, mean ± SD) were used. Six male Wistar rats (183 ± 46 g, mean ± SD) were used in studies involving posterior hypothalamic stimulation. All rats were anesthetized with 2% chloralose-10% urethane in 0.9% NaCl (0.6 ml/100 g i.v.). Supplemental doses (0.1 ml/100 g) were administered as required. Rectal temperature was continuously monitored and main-
tained at 37°C. The trachea was intubated, and arterial pressure was measured by cannulation of the left carotid artery.

**Stimulation of Sympathetic Chain**

Preliminary experiments were performed to determine the appropriate placement of the stimulating electrode. The distribution of retrogradely transported horseradish peroxidase (HRP) was quantitated in the paravertebral ganglionic cell bodies of 5 Sprague-Dawley rats. HRP (type VI, Sigma Chemical Co., St. Louis, Mo.) was dissolved in distilled water in a concentration of 10%. The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The external spermatic nerve was sectioned, and the proximal stump was immediately aspirated into a segment of polyethylene tubing containing the HRP solution. The nerve was soaked in the HRP solution for 1 hour. The rats were allowed to recover for 48 hours and were then reanesthetized with ether and transcardially perfused with isotonic saline followed by fixative. The right lower thoracic and complete lumbar chain was dissected and removed. The individual ganglia were sectioned at 30-μm thickness with a freezing microtome, and tissues were prepared according to the tetramethylbenzidine method. The number of cell profiles in each ganglia from T12 to L4 containing HRP-reaction product was determined. The percent of total HRP-labelled cell bodies was calculated for each ganglion, as shown in Figure 1; 85% of the labelled cell bodies were located in ganglia L1 and L2.

Using the anatomic information shown in Figure 1, the stimulating electrode was placed on the intermodal segment between ganglia L1 and L2. The paravertebral chain was sectioned at the diaphragm above L1 and below L3. A bipolar platinum cuff electrode was implanted employing a retroperitoneal approach. A Grass Model S48 stimulator (Quincy, Mass.) was used to deliver rectangular pulses of 5–7-V amplitude, of 1–3-msec duration, and at 0.5–16.0-Hz frequency. Stimulation trains of 45 seconds were applied. Rest periods of 3 minutes were allowed between stimuli.

**Stimulation of Posterior Hypothalamus**

For electrode implantation, rats were anesthetized with ketamine (120 mg/kg) and acepromazine (12 mg/kg). A bipolar stainless steel electrode (MS 303/2, Plastic Products, Roanoke, Va.) was stereotaxically placed in the posterior hypothalamic nuclei using established coordinates. The center-to-center distance between the two wires of the electrodes was 0.2 mm. The rats were allowed to recover for 48–72 hours before experimentation on the microcirculation. Constant current pulse stimuli of 0.5–1.0-msec duration, 60-Hz frequency, and 1-minute train length were applied. Current amplitudes of 38, 75, 150, and 300 μA were used. Four-minute rest periods were allowed between stimuli. At the end of the experiment, verification of electrode placement was established by producing an electrolytic lesion at the tip of the electrode and histologically examining the tissue.

**Preparation of Cremaster Muscle for Microcirculatory Studies**

The right cremaster muscle was surgically exposed and prepared for intravital observation by slight modification of a standard technique. The muscle was spread over the viewing port of a tissue bath and secured in place by silk sutures. Warmed Krebs-Henseleit solution equilibrated with 95% N₂–5% CO₂ was continuously dripped on the muscle during preparation. The solution was composed of (in mM) NaCl 118, KCl 4.7, KH₂PO₄ 1.2, NaHCO₃ 25, MgSO₄ 1.2, and CaCl₂ 2.54. The tissue and bath were maintained at 34°C. The muscle was covered with Dow-Corning Saran Wrap. The preparation was then transferred to the microscope stage. After a 30-minute equilibration period, the experiment began. The entire protocol required approximately 2 hours to complete.

**Measurement of Microvascular Diameter and Red Blood Cell Velocities**

A Zeiss ACM Multipurpose microscope (Thornwood, N.Y.) equipped with a closed-circuit television system was employed. The following objectives were used: UD20 (N.A. 0.57), UD40 (N.A. 0.65), and 40× (N.A. 0.65). The magnified image of the microvessel was projected onto the TV monitor (3,400× with the 40× objective), and a continuous measurement of vessel inside diameter was made by an elec-
tronic image shearer. Red blood cell velocity over the central portion of the vessel lumen was measured using a periodic differential detector system. Volumetric flow rates were calculated from the average red cell velocity and vessel inside diameter using an analog computer. The average red cell velocity was obtained by dividing the measured centerline velocity by 1.60 for vessels greater than 13-μm diameter or by 1.30 for vessels smaller than 13-μm diameter.

Identification of Vessel Segments

Beginning with the major feeding arteriole and venule designated as 1A and 1V, consecutive branches were assigned an appropriate order. The vessel segments studied were identified by modification of systematic approach described by Joshua et al. The first 2A branch that supplied the anterolateral portion of the cremaster muscle was identified. Then, the first 3A segment branching off of the 2A branch was chosen. Next, 4A branching from the chosen 3A segment was identified.

Histofluorescence Technique

The adrenergic vasomotor innervation of the cremaster muscle was investigated by glyoxylic acid fluorescence histochemistry on frozen sections. The cremaster muscle was frozen, and specific vessel segments were removed by dissection. Tissue adjacent to 2A vessels was removed so that samples of only 3A and 4A vessels would be obtained. Tissue blocks were mounted on a cryostat and cut to a thickness of 15-20 μm. Tissue sections were observed on a Zeiss Multipurpose microscope fitted for incident light fluorescence excitation of catecholamines.

Results

Vasomotor Responses to Stimulation of Lumbar Sympathetic Chain

Control inside diameters of the four orders of arterioles studied were: 1A = 126.2 ± 5.9, 2A = 80.9 ± 6.7, 3A = 24.4 ± 2.1, and 4A = 10.0 ± 1.0 μm (mean ± SEM, n = 20). Mean arterial pressure was 88.6 ± 2.0 mm Hg.

In all arterioles studied, the magnitude of the vasconstrictor response to sympathetic stimulation was graded with increasing frequency of stimulation. In Figure 2, the stimulus frequency-response relations for each arteriolar segment is shown. With vasomotor responses expressed as percent of control diameter, the distal arterioles (3A and 4A) were found to be more responsive than the larger vessels (1A and 2A) at all stimulus frequencies. This result is valid for both maximal and time-averaged vasoconstrictor responses. When the responses are expressed in terms of the absolute magnitude of the diameter reduction, the sequence of responsiveness is reversed: 1A > 2A > 3A > 4A.

In general, the vasoconstrictor response was more rapid in the smaller arterioles (3A and 4A) that reached a maximum vasoconstriction in 10-15 seconds. In addition, there was a tendency for the initial vasocon-
sure. This flow cessation was usually transient in nature, and flow partially escaped toward control levels after a short period.

Vasomotor and Flow Responses to Stimulation of Posterior Hypothalamus

Control inside diameters for the four orders of arterioles studied were: 1A = 151.3 ± 5.8, 2A = 101.7 ± 0.7, 3A = 29.1 ± 4.5, and 4A = 15.4 ± 0.7 μm (mean ± SEM, n = 6). Mean arterial pressure was 115 ± 9 mm Hg.

As shown in Figure 3, the 1A and 2A segments demonstrated no significant change in diameter at any stimulus level. In the 3A and 4A vessels, the vasoconstriction increased with increasing current amplitude. Mean arterial pressure also rose with increasing stimulus to a maximal average increase of 21 mm Hg at a current of 300 μA. The time course of the vasoconstriction observed in the 3A and 4A vessels did not appear to proceed as rapidly as that observed in response to direct stimulation of the sympathetic chain. Venous responses were not measured in these experiments. The volumetric flow rate measured in the 1A segment was 487 ± 18 nl/min (mean ± SEM, n = 6). The percent reduction in 1A-vessel flow at the stimulus current of 300 μA was approximately 7%.

Histofluorescence Evaluation of Microvascular Innervation

Determination of the distribution of adrenergic innervation to the microcirculation in rat cremaster muscle revealed an innervation of the entire arteriolar network to the level of the 4A vessel. No evidence of direct venous or capillary innervation was obtained. This pattern of distribution is consistent with the presence of neurogenically induced vasoconstriction in the arteriolar network and with its complete absence in the venous vessels of the rat cremaster muscle.

Discussion

From a functional viewpoint, the peripheral circulation comprises a number of series-coupled vascular segments, each of which subserves a particular function. The functional differences between vascular segments are particularly evident in direct intravital studies of the responses of the microcirculation to vasoactive stimuli. Results presented in this study confirm and extend previous studies on the response of muscle microvasculature to stimulation of sympathetic nerves.

The vasomotor and blood flow responses to peripheral sympathetic stimulation have been previously described in the microcirculation of two skeletal muscles: cat tenuissimus and rat spinotrapezius. In both of these studies and in the results reported here for rat cremaster muscle, it was found that the smaller the arteriolar segment, the greater the percent of vasoconstriction became. In addition, in all three muscle preparations, the maximum percent of constriction occurred in arterioles with resting diameters in the range of 15–30 μm. Although the exact time course and magnitude of vasoconstrictor escape differ among the above studies, vasoconstrictor escape remained a consistent observation and was primarily a phenomenon attributable to the more distal arterioles. These direct intravital observations fit well with the response pattern proposed for cat skeletal muscle from measurements of the pressure gradient in large and small pre-capillary resistance vessels.

The mechanisms underlying the microvascular gradient in responsiveness to adrenergic stimuli have not been precisely defined. Studies on larger vessel segments have identified a number of anatomic and physiologic factors that may also contribute to the variations in segmental responsiveness observed in microcirculation. The distribution of the adrenergic innervation is one of the more important and easily definable factors. In this study and in previous microcirculatory studies, a strict correlation has been found between the distribution of the innervation and the vasomotor response. This was especially evident in the venous microcirculation of the cremaster muscle where neither innervation nor response was observed. It is difficult to accurately define other morphologic factors that are known to influence the vascular response from histofluorescence observations alone.
Such factors include density of innervation and the spatial relation between adrenergic varicosities and vascular smooth muscle. Another anatomic factor that has been shown to correlate with responsiveness to nerve stimuli is the wall thickness: luminal radius ratio. That the wall thickness: luminal radius ratio increases with decreasing diameter in rat cremaster muscle may indicate a role for this factor in producing the observed responses. A number of physiologic determinants of vasomotor responsiveness also have been defined. Gore has shown that the prevailing wall stress plays a significant role in determining the response of microvessels to adrenergic stimuli. Variations in wall stress among the different arteriolar segments in rat cremaster muscle do exist. The manner in which these differences in segmental wall stress affect the response to neural stimuli remains to be determined. The lower threshold frequency observed for the smaller arterioles (3A and 4A) shown in Figure 2 may be related to the electrical cable properties of these vessels. Calculations of membrane depolarization in the smooth muscle produced by neural activity predict that the smaller vessels reach threshold for contraction at a lower frequency of nerve discharge.

Vasoconstrictor escape is primarily observed in the more distal arterioles and may be because of the intervention of local influences produced by the reductions in blood flow and intravascular pressure during sympathetic stimuli. The influence of local metabolic factors on responses to sympathetic stimuli have been widely studied in whole organs. The role played by metabolic factors in modifying the response of resting tissues is not certain.

The number and sensitivity of α-adrenergic receptors has been shown to vary longitudinally along the arterial tree. It is not known if these results can be extrapolated to the microvasculature or, if so, what significance they would have in influencing the segmental responses to sympathetic stimuli.

In summary, the time course and magnitude of the response to sympathetic stimuli varied among the different segments of the arteriolar network in rat cremaster muscle. The venous microvessels did not respond to stimulation of the sympathetic chain. The arteriolar responses correlated with the distribution of adrenergic innervation determined by fluorescence histochemistry. The precise mechanisms underlying the different microvascular vascular responsiveness to sympathetic neural stimuli are unclear at the present time.

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