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Arteriolar network responses to opposing dilator and constrictor stimuli: Mechanism of sympathetic attenuation during muscle contraction

Dodd, Laurie Rose, Ph.D.
The University of Arizona, 1988
ARTERIOLAR NETWORK RESPONSES TO OPPOSING DILATOR AND CONSTRICCTOR STIMULI: MECHANISM OF SYMPATHETIC ATTENUATION DURING MUSCLE CONTRACTION

by

Laurie Rose Dodd

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PHYSIOLOGY
In Partial Fulfillment of the Requirements For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA
1988
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the dissertation prepared by Laurie Rose Dodd
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Stimuli: Mechanism of Sympathetic Attenuation During Muscle
Contraction

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SIGNED: Henrié Dodd
ACKNOWLEDGEMENTS

I would like to thank my advisor, Paul Johnson, above all for giving me freedom with guidance, for arranging laboratory meetings with many visiting scientists in microcirculation and other disciplines, whose helpful suggestions have contributed to this work, and for skillful editing of my seminars and manuscripts, from which I have learned a great deal.

I would also like to thank Timothy Secomb for abiding my too frequent interruptions of his work to kindly offer assistance and to David Kreulen for making my journey into the realm of pharmacology hospitable, as well as for giving me many of the pharmacological agents which I used to design and perform these experiments.

Finally, I thank my husband, Michael Breslow, for sharing with me the more important things in life.
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Evidence suggests different sections of the arteriolar network supplying muscle can respond independently and this may provide a mechanism for the localized distribution of blood flow. This hypothesis was tested in the microcirculation of the cat sartorius muscle by measurement of arteriolar diameter changes during muscle contraction and sympathetic nerve stimulation in each consecutive section of the network. The diameter changes were referenced to the initial distribution of resistance across the network, as determined from arteriolar pressure measurements and morphometric data. This led to an estimate of the change in network resistance. Unlike previous reports, the most distal arterioles dilated little during muscle contraction and our resistance estimate indicates these vessels play an insignificant role in functional hyperemia. The more proximal, third order arterioles dilated proportionately more than other arteriolar orders and made the largest single contribution to resting resistance. Similarly, these vessels were the largest single site of resistance change during sympathetic stimulation. Together, these findings suggest the third order arterioles play a dominant role in regulating flow to the capillaries that each supplies.

Antagonism of sympathetic control during muscle contraction has been attributed to direct inhibition of vascular smooth muscle contraction and to inhibition of sympathetic neurotransmission. Evidence to support the latter mechanism comes from the observation
that functional dilation is reduced with exogenous norepinephrine as compared to sympathetic stimulation. However, exogenous norepinephrine may bind to both alpha-1 and alpha-2 adrenergic receptors, whereas that released by sympathetic stimulation may bind primarily to alpha-1 receptors. Since this difference could be significant, functional dilation after systemic injection of norepinephrine or phenylephrine, a selective alpha-1 agonist, was compared to that during sympathetic stimulation. In contrast to the findings with norepinephrine, functional dilation after phenylephrine did not differ from that observed during sympathetic stimulation. This indicates the dilator substance(s) released during exercise may selectively inhibit alpha-1 mediated vasoconstriction but less effectively inhibit vasoconstriction mediated by alpha-2 receptors. Furthermore, these findings suggest that the vasodilator mechanism may act primarily at the level of the vascular smooth muscle, without appreciable pre-junctional inhibition of sympathetic nerves.
CHAPTER 1

OVERVIEW OF BLOOD FLOW REGULATION IN MUSCLE

Central Versus Local Regulation

The function of the circulatory system is to deliver blood to the various organs and tissues of the body. This is a complex task because tissue requirement for nutrients supplied by the blood can change on a moment to moment basis. Local demand for the blood supply must be integrated with the immediate demands of other tissues in the body. The sympathetic division of the autonomic nervous system provides this integrative control by regulating cardiovascular function at the level of the heart and the peripheral vasculature. There are two sympathetic actions on the heart. First, to enhance the force of myocardial contraction, which increases stroke volume and second, to increase heart rate. These two factors, rate and stroke volume determine cardiac output. The sympathetic effect on the peripheral vasculature, primarily the small arteries and arterioles is to cause vascular smooth muscle contraction and therefore vasoconstriction. This increases total peripheral resistance. Cardiac output and peripheral resistance are the primary determinants of arterial blood pressure, the driving force that propels blood through the circulatory system. So the sympathetic nervous system provides an essential function to regulate arterial pressure and this is necessary for adequate perfusion of the tissues and organs of the body. Although sympathetic nerves exert a vasoconstrictor influence
on the peripheral vasculature, tissue demand for increased flow can exert an opposing vasodilator influence that overrides sympathetic vasoconstriction (reviewed by Mitchell and Schmidt 1983; Shepherd 1983; and Shepherd and Vanhoutte 1985). Examination of the cardiovascular adjustments made in response to rhythmic exercise demonstrate the elegance of the multifactorial control system. But first, a review of sympathetic anatomy and control.

**Sympathetic Anatomy and Control**

Sympathetic nerve activity originates principally in the rostral region of the medulla oblongata and in the hypothalamus (Weiner and Taylor 1985). Preganglionic sympathetic nerves that control vasomotor outflow in the hindlimb muscles of the cat leave the spinal cord between T12-L4 with maximum outflow between L1-L3. These nerves travel down the sympathetic chain synapsing in segmental paravertebral ganglia. Postganglionic sympathetic fibers innervating the hindlimb vasculature exit from the chain primarily between L5-L7 and enter the respective ventral roots (Sonnenschein and Weissman 1978). In the dog, 91 percent of the sympathetic nerves enter hindlimb muscles with the mixed motor nerve, the remainder enter with the adventitia of blood vessels (Clonninger and Green, 1955). Sympathetic axons are unmyelinated C fibers, 0.25 - 0.5 micron in diameter (Bevan et al. 1980). Preganglionic firing rates average one to two per second but spontaneous rate and pattern of firing is variable (Polosa, 1968). Preganglionic firing rates exceed
postganglionic firing rates because not all preganglionic action potentials elicit action potentials in postganglionic neurons (McLachlan and Hirst, 1980). Moreover, variable firing rates may reflect baroreceptor feedback superimposed on the tonic firing pattern.

The nerve endings do not form an anatomically well-defined synaptic structure. Distributed along the terminal segment of the axons, every 5-15 microns (Uehara and Suyama, 1978), are thickenings or varicosities. These are the site of neurotransmitter vesicular storage and release by exocytosis (Weiner and Taylor 1985). Usually the transmitter is norepinephrine (Euler and Liechajko, 1961). Norepinephrine is synthesized from tyrosine, intermediate steps form dopa and dopamine (Weiner and Taylor 1985).

Release of norepinephrine is triggered by an action potential. Once released, norepinephrine diffuses to the effector cell, sometimes as far as two to three hundred nanometers but often the distance is only 15-25 nm. (Bevan et al., 1980). Sympathetic innervation is limited to the adventitial-medial border (Fuxe and Sedvall, 1965; Fleming et al., 1987).

An action potential in the smooth muscle cell may spread to neighboring non-innervated cells via gap junctions. So the effector site may comprise a bundle of smooth muscle cells (Uehara and Burnstock, 1970). The actions of norepinephrine are varied and depend in part on the receptor type to which it binds. In the vasculature of skeletal muscles, beta-2 receptors elicit smooth
muscle relaxation while alpha receptors elicit smooth muscle contraction (this will be discussed further in a later section) (Bevan et al. 1980). In resting skeletal muscle, tonic sympathetic discharge of 1-2 pulses per second (Folkow, 1952) maintains considerable vasoconstrictor tone.

There are three mechanisms by which the actions of norepinephrine are terminated, 1) re-uptake into the nerve terminals, 2) diffusion away from the junction and 3) metabolic degradation. The norepinephrine diffusing beyond the junction will enter the bloodstream or be taken up at extraneuronal sites. Two enzymes, monamine oxidase and catechol-O-methyl-transferase degrade norepinephrine (Weiner and Taylor 1985).

In some species, including the cat, cholinergic neurons innervate skeletal muscle vasculature (Burnstock 1980). Vasodilator cholinergic neurons are activated in anticipation of exercise but do not contribute significantly to the hyperemia that occurs during exercise (Shepherd 1983).

Pressure and Flow Response to Exercise

The primary determinants of blood flow to an organ or a tissue are arterial pressure and the caliber of the small arteries and arterioles that supply it. The gradient of pressure between artery and vein provides the driving force for flow, while adjustments of small artery and arteriole diameter controls the resistance to flow and regional delivery of blood. This relationship is obtained from
analogy to Ohm's Law $Q = \Delta P / R$, where the blood flow $Q$ is equal to $\Delta P$, arterial pressure minus venous pressure divided by $R$ the resistance. Resistance is a function of several variables, the most significant being vessel diameter.

In the transition between rest and rhythmic exercise, dilation of the arterioles supplying a contracting muscle results in a several fold increase in muscle blood flow (Renkin et al. 1966; Donald et al. 1970; and Tyml 1987). The augmentation of blood flow during exercise matches increased metabolic demand, where oxygen consumption is proportional to exercise intensity and shows a linear relationship to flow over a wide range (Goodman et al. 1978). This link between blood flow and tissue metabolism is believed to be controlled locally (reviewed by Renkin 1984; Hudlicka 1985).

If it were not for other circulatory adjustments, arterial pressure could fall as a result of vasodilation in the exercising muscles. Sensory neurons within the contracting muscles initiate an afferent reflex, enhancing sympathetic activity to the heart and peripheral vasculature (Mitchell and Schmidt 1983). Elevated heart rate and cardiac contractility increase cardiac output, while vasoconstriction in the noncontracting muscles and many of the visceral organs helps to maintain arterial pressure despite the decrease in peripheral resistance in the exercising muscles. Some of these sympathetic effects are also controlled by central command, by changes in circulating catecholamines and other hormones, and by resetting of the baroreceptors (Mitchell and Schmidt, 1983).
Sympathetic activity probably increases to contracting muscles as well as to those at rest. Although increased sympathetic activity can limit exercise hyperemia, the overriding influence during exercise is vasodilator. This phenomenon has been termed functional sympatholysis (Shepherd 1983).

Magnitude of Exercise Hyperemia

The magnitude of exercise hyperemia depends on several factors, including the fiber type composition of the muscle (Bockman et al. 1980; Laughlin and Armstrong 1982b), the metabolic rate of the animal (Honig and Gayeski 1982), the intensity of the exercise (Laughlin and Armstrong 1982b), the total muscle mass involved, and the type of exercise; i.e. isotonic or isometric (Lind 1983). Experimental procedures often require an anesthetized animal. Exercise is simulated by electrical stimulation of the mixed, motor nerve or the ventral root. This does not cause a physiological pattern of motor unit activation in which alpha motor neurons innervating different motor units have unique firing frequencies and patterns of activation (Burke, 1981).

Anesthesia reduces muscle blood flow. In anesthetized rats, muscle flow is one-half to one-third that in resting conscious rats (Laughlin and Armstrong 1982a). This result is consistent with other studies, if resting flows are compared between anesthetized dogs (Renkin et al. 1966) and conscious dogs (Pendergast et al., 1985). This difference is probably due to firing of motor units in the
conscious animals.

In many reports, there is a larger increase in muscle blood flow in conscious exercising animals than in anesthetized animals, where muscle contraction is electrically induced. Renkin et al. (1966) reported an approximate flow increase of 5 to 25 milliliters per minute per 100 grams of tissue during maximal electrical stimulation. In conscious dogs, flows increased from resting values of 13 to maximal values of 70 ml/min/100 gr. (Donald et al. 1970; Pendergast et al., 1985). However, in one report, flows in contracting hindlimb muscles of the anesthetized rat (Mackie and Terjung 1983) were as large as those observed in conscious, exercising rats (Laughlin and Armstrong, 1982b). These values were often as large as 150 to 300 ml/min/100 grams. This result is not limited to the rat, treadmill exercise flows of 130 ml/min/100 gr. have been reported in miniature swine (Armstrong et al. 1987). In gracilis and soleus muscles of anesthetized cats minimum and maximum flows range from 2 to 36 ml/min/100 gr. (Bochman et al. 1980).

Burton and Johnson (1972) reported resting blood flow in the cat sartorius of 3 ml/min/100 grams. The cat sartorius is a mixed muscle composed of 30 percent slow oxidative fibers, 46 percent fast oxidative and 24 percent fast glycolytic fibers (Ariano et al. 1973). Hammerson (1968) reported 281 capillaries per square millimeter in muscle cross-sections.
MUSCLE MICROCIRCULATION

Determinants of Flow Regulation

Muscle blood flow responses to exercise represent dilation of the many arterioles supplying a contracting muscle. Microcirculatory studies allow direct observation of arteriolar responses. Adequate sampling of the arteriolar population is important so that responses observed can be understood in relation to overall muscle flow responses. The strength of the microcirculatory approach is that it permits a better understanding of the various regulatory mechanisms and how they are integrated to determine blood flow. At the level of the microcirculation, there is an interplay between central sympathetic control, circulating vasoactive substances, myogenic and local metabolic control.

Anatomy of the Vasculature

Vessels in the circulatory system form tubes lined with a single layer of flattened endothelial cells. Adjacent endothelial cells are connected by tight junctions (Rhodin 1967). Arteries are the thickest walled vessels having three morphologically distinct layers; the intimal layer composed of endothelial cells, the media made up of elastin fibers and a smooth muscle layer several cells thick, and an outer adventitial layer composed of connective tissue. As arteries become smaller they lose the elastin fiber layers and the smooth muscle layer becomes thinner. The smallest vessels on the arterial side, the arterioles range between 6-100 microns in
diameter. The larger arterioles contain no more than two cell layers of smooth muscle while the smallest arterioles are wrapped in a discontinuous, spiralling arrangement of single vascular smooth muscle cells and have lost the outer connective tissue layer (Rhodin, 1967). The smallest vessels of the system, capillaries and post-capillary venules, are 4-12 microns in diameter and composed solely of an endothelial cell layer. In larger venules, 50-100 microns in diameter, a sparse vascular smooth muscle investment reappears. There is a thin layer of smooth muscle and an adventitia in veins (Rhodin, 1968).

Architecture of the Vascular Network

The circulatory system forms a branching series of vessels. On the arterial side, the diameters of daughter branches are generally smaller than those of the parent vessels. As vessel diameters become smaller, their lengths are also shorter and with successive branching, the numbers of vessels rapidly increases (Weideman 1984). The terminal branching order of arterioles gives rise to the capillary network. In most muscles used for microcirculatory study, between 3 to 12 capillaries branch from the final arteriolar segment (Weideman 1984). However, in the hamster tibialis, as many as 20 capillaries arise from the terminal arteriole (Damon and Duling 1984). Capillaries run adjacent to the muscle fibers in an approximate one to one ratio, with average lengths of 500-1000 microns and collect into venules, which form a converging system of
vessels paralleling the arteriolar portion of the system except that their diameters are 10-20% larger. The arteriolar network of vessels form several orders of branching before terminating at the capillary bed. Most of what is known about the detailed structure of the vascular network comes from analysis of very thin, nearly two-dimensionally organized muscles (Weideman 1984).

The sartorius muscle from a one kilogram cat has a thin central region from which measurements are taken. It is supplied by two arteries, the femoral and the circumflex (Koller et al., 1987). These arteries supply four to five 80-100 micron arterioles. Determination of normal vessel diameters is not a trivial problem because exteriorized muscles are susceptible to surgical trauma and to environmental exposure. These values are from muscle with no microscopically observable damage and covered with Saran Wrap to prevent exposure to the atmosphere. If a centrifugal ordering system is used, then the largest arterioles are labelled 1A, indicating first order arteriole. The next branching portion of the network consists of vessels that interconnect with one another. In the sartorius they have been labelled 2A but some investigators divide 2A vessels into two orders; those interconnecting 1A arterioles and those which interconnect between 2A arterioles (Marshall 1982). The sartorius 2A arterioles supplied by 1A vessels are larger, 40-60 microns than the 15-25 micron 2A arterioles supplied by other 2A vessels. Interconnecting or arcading arterioles are a common feature in most muscles studied (Weideman 1984). A function of the
Interconnections may be to equalize perfusion pressure in this distributing portion of the network and to provide alternate or collateral perfusion pathways (Schmid-Schonbein et al. 1987). The next branching order of vessels, the 3A's have resting diameters between 7-15 microns. These vessels are also referred to as transverse arterioles due to their orientation in relation to the muscle fibers. Third order arterioles supply the terminal arborations of the network. In the sartorius there are three further branching orders, 4A through 6A prior to the capillary network. There are no arterial to venous shunts in the sartorius and only 2A arterioles anastomose. The 4A-6A arterioles branch at an approximate 3:1 ratio and their diameters range from 6-10 microns (Koller et al., 1987). Diameters in daughter branches of these vascular segments are often similar to the parent vessel. The 6A arterioles, or terminal arterioles give rise to 4 to 12 capillaries. In the sartorius, a 3A transverse arteriole supplies 200 to 400 capillaries. There are no precapillary sphincters as such, the last site of smooth muscle investment is within the 6A arteriole as judged by vessel constriction to sympathetic stimulation (Boegehold and Johnson, 1988a). Therefore, regulation of flow in capillaries must minimally include the 4-12 capillaries supplied by a 6A vessel. See figure 1 showing arteriolar network of the cat sartorius muscle (modified from Koller et al., 1987).

Most other thin muscles used for microcirculatory investigation are similar to the sartorius down to the level of the transverse
Figure 1. The arteriolar network of the cat sartorius muscle. First (1A) and second (2A) order, arcade arterioles supply third (3A) order arterioles, indicated by tic marks in panel A and enlarged in panel B; 3A arterioles supply the terminal fourth (4A), fifth (5A) and sixth (6A) order arterioles in the tree-type portion of the network. Modified from Koller et al., 1987.
arteriole, except that vessel diameters may vary. The rat and hamster cremaster and the rat spinotrapezius have one or two branching orders downstream to the transverse arterioles. Many fewer capillaries are supplied by a 3A vessel in these muscles. The arteriolar network of the tenuissimus, another commonly examined muscle in the cat and rabbit is similar to that of the cremaster except that the arcading segment of vessels does not exist. A single central artery small enough to be classified 1A, about 100 microns in diameter, gives rise directly to transverse arterioles. These in turn supply as many as eight terminal arterioles (Weideman 1984).

**Hemodynamics**

To understand the importance of arteriolar diameter changes in controlling tissue blood flow, it is necessary to consider hemodynamics. The flow of blood through the vascular system, a series of interconnected tubes, is determined by several factors. These factors reflect properties of the structure of the vasculature, such as vessel diameters and lengths, as well as the fluid properties of blood, which determine viscosity (Zweifach and Lipowski 1984). As previously stated, tissue flow follows the analogy to Ohm's law, $Q = \frac{\Delta P}{R}$. In general, arterial and venous pressures remain relatively constant, due to pressure receptors and the sympathetic nervous system (Mitchell and Schmidt 1983). So resistance, which can change manyfold, represents the predominant mechanism for control of tissue blood flow. As suggested above the
determinants of resistance are inherent in the structure of the vasculature and the fluid properties of blood. Consider first a single blood vessel. Flow will be determined by the driving force, the pressure drop along the length of the vessel and by the resistance to flow. If the vessel diameter is narrow, flow will be restricted more than if the vessel is large. Similarly, if the vessel is long, flow resistance will be more than if the vessel is short. These properties are due to the ratio of surface area to volume which determines the frictional dissipation of energy at the vessel wall. Furthermore, the viscosity of the fluid, determined by friction within the fluid, will determine the ease of flow through the vessel. The determinants of flow are expressed quantitatively in the Poiseuille-Hagen relationship:

$$Q = \frac{\pi}{128} P \left( \frac{d^4 \eta}{l} \right)$$

where

- \( Q \) = viscosity of the fluid
- \( d \) = vessel diameter
- \( \eta \) = vessel length

Although formulated for a single vessel and a Newtonian fluid, this relationship provides a good starting point for understanding flow regulation in the vascular network. Note the direct inverse proportionality to viscosity and vessel length but the fourth power relationship to diameter, making diameter by far the most significant determinant of flow.

Organ or tissue blood flow, in contrast to flow in a single
vessel, is determined by the resistances of the many vessels that comprise the vascular network. Consider the arteriolar portion of the network, assuming for the moment that viscosity is constant. Comparing two first order arterioles, resistance through each vessel will be determined by respective lengths and diameters. But connected in parallel, total resistance through both arterioles will be less because there are two pathways for flow. The Poiseuille-Hagen relationship, rearranged to represent resistance is expressed as follows:

\[ \frac{P}{Q} = R = \frac{(128\eta l)}{\pi d^4} \]

For many vessels, representing parallel pathways for flow:

\[ R = \frac{(128\eta l)}{\pi d^4 N} \]

where \( N \) = number of vessels and \( d \) and 1, average diameter and length

(Lamport, 1955; Intaglietta and Zweifach, 1971).

The resistance to flow in the second order arterioles could be calculated similarly and so on for each segment of the arteriolar network. As the arterioles divide to form branches, vessel diameters become smaller and resistance in each daughter vessel is increased as compared to the parent. But the increased number of parallel pathways and their shorter lengths keep overall resistance through the more peripheral segments of the network from increasing precipitously. Conversely, the resistance to flow in the network as a whole is equal to the summed resistances of each segment because they
are connected in series.

Another factor influencing resistance to flow is blood viscosity. Since blood is not a Newtonian fluid, it does not have constant viscosity. Blood viscosity changes with flow velocity and hematocrit, the fraction of blood volume composed of red cells. Velocity of flow does not affect viscosity except possibly on the venous side, (Baeckstrom et al., 1971), where cells tend to clump together under low shear stress (Reinke et al., 1986). Hematocrit, however, is a function of vessel diameter and directly proportional to viscosity. As vessels become smaller, hematocrit decreases. So viscosity in the microcirculation is less that would be expected on the basis of systemic hematocrit. This phenomenon was discovered by Fahraeus (1931) and is due to the more rapid velocity of flow along the centerline of a vessel. Red cells are carried by the centerline flow and thus travel through the circulation faster than the plasma layer flowing along the walls of the vessel. This flow property becomes increasingly more significant as vessels become smaller, because the red cells travel faster than the plasma. Therefore, the number of cells in the vessel at any point in time is reduced. In the cat mesentery, microvessel hematocrit decreases to 8-9 percent while systemic hematocrit is 35 percent (Johnson et al. 1971; Lipowsky and Zweifach 1977). In the smallest vessels, less than 9 microns, viscous resistance increases again because the red cells deform to pass through (Lipowsky and Zweifach 1977). The effects of vessel diameter and hematocrit on viscosity do not have a large impact on
the resistance (Papenfuss and Gross 1986) but can be incorporated into the formulation of the Poiseuille-Hagen relationship. A couple of other factors influence resistance in a manner not accounted for by the Poiseuille-Hagen relationship. Blood vessels are not rigid, perfectly symmetrical cylinders. Arterioles have some compliance and in smaller vessels such as terminal arterioles and capillaries, endothelial cell nuclei which project into the lumen may hinder flow. Despite these limitations experimental measurements of the relationship between pressure and vessel length have been used to determine a near fourth power relationship to diameter, demonstrating the general validity of the Poiseuille-Hagen relationship despite simplifying assumptions (Lipowsky et al. 1978). The resistance of the network as a whole may be more complicated however. The resistance at branch points, between vascular segments is not included in the relationship. If the ratio between parent and daughter vessel is large, three or more, this can represent a significant resistance to flow in the daughter vessel (Zweifach 1974; Zweifach and Lipowsky, 1984).

Resistance in the vascular network dissipates energy and reduces flow. The energy is in the form of pressure, force per unit area. The greater the resistance, the more pressure will be dissipated and the less work can be done to move the blood through the vasculature. So the drop in pressure between two points in the vasculature represents a loss of energy due to resistance, or friction and the energy used to move the blood. Returning to the
Ohm's law analogy, $\Delta P = QR$, it is apparent that resistance can be determined by measuring pressure differences and flow. Alternatively, if resistances in different segments of the vasculature are compared, for example first order arterioles compared to second order arterioles. Then it can be assumed flow in each cross-section of the vasculature will be the same, i.e. summated flow in first-order arterioles will equal summated flow in second order arterioles and only pressure gradient needs to be measured. The Poiseuille-Hagen relationship and the pressure gradient approach provide two methods for evaluating the resistance to flow within a tissue. The first involves measurement of vessel dimensions, the second measurement of vessel pressures. Measurements of this sort have been designed to reveal the distribution of resistance in the vascular network. Pressure differences between artery and arteriole, arteriole and post-capillary venule, venule and vein have shown the arteriole to post-capillary venule portion of the network contributes the largest resistance component.

Within the arteriolar network the greatest resistance occurs in the transverse and terminal arterioles (Fronek and Zweifach, 1975; Zweifach et al., 1981). Although resistance in individual capillaries is greater than in single arterioles, the large number of them, 3 to 12 per terminal arteriole, reduces overall resistance through the capillary segment of the network (Lipowsky et al. 1978). The bottleneck to flow in the vasculature occurs primarily in arterioles 8-25 microns in diameter (Zweifach 1974). Consequently,
modulation of diameter in these vessels will have the most profound effect on tissue blood flow. Significant to this finding, is the observation in skeletal muscle that arterioles of this size have greatest resting tone (Koller et al., 1987; Boegehold 1986). They dilate to the largest extent when exposed to dilator stimuli, which is important for control of flow during exercise. It should be noted however, vessel constriction will reduce flow in downstream vessels irrespective of resting tone. This is important for our consideration of neural regulation in muscle because it means that sympathetic vasoconstrictor control can occur in principle anywhere in the arterial network.

**VASCULAR RESPONSES TO MUSCLE CONTRACTION AND SYMPATHETIC STIMULATION**

*Muscle Contraction*

A several fold increase in metabolic energy utilization accompanies exercise (Goodman et al., 1978). As one means of providing this energy, muscle cells augment the rate of nutrient and oxygen uptake from the surrounding interstitial fluid. Without close regulation of interstitial fluid composition, increased nutrient uptake would lead to interstitial depletion of these essential substances and the energy requirements of exercise would not be met. The circulatory system regulates interstitial fluid composition by delivering nutrients and removing metabolites, the waste products. This exchange, primarily a diffusion process, is proportional to blood flow rate, diffusion distances and the surface area for
exchange (reviewed by Renkin 1984).

The links between exercise, metabolism and blood flow have been investigated to a limited extent in the microcirculatory preparation, which allows direct observation of the arteriolar network, in thin, transilluminated skeletal muscles. Such studies have shown arteriolar dilation is graded to exercise intensity, (Gorczinski et al., 1978), with the smallest vessels responding most rapidly and to the greatest extent (Gorczinski et al., 1978; Marshall and Tandon, 1984). These events lead to increased flow velocity (Dawson et al., 1987; Tyml, 1987), to flow recruitment in capillaries that have no flow or minimal flow at rest (Gorczinski et al., 1978; Tyml, 1987), and to closer packing of red cells (Johnson et al., 1971; Klitzman and Duling, 1979; Tyml, 1987). Arteriolar dilation occurs rapidly, within 5-20 seconds, after the onset of muscle contraction (Gorczinski et al., 1978). Such timely adjustments improve exchange capacity, shorten diffusion distances and increase concentration gradients between blood and interstitium, thereby providing for the increased metabolic requirements of exercise.

It has been known for many years that the vasodilator response to exercise does not depend on the muscle innervation (Donald et al., 1970). This observation, along with the close and requisite link between flow and exercise intensity has led to the hypothesis that contraction-induced vasodilation is locally controlled by a feedback mechanism coupled to interstitial fluid composition (Goodman et al., 1978). Most simply, if arterioles are responsive to nutrient and
metabolic product concentrations, then smooth muscle tone will be modified so interstitial composition remains within the limits prescribed by muscle cell metabolic requirements. There has been extensive research to ascertain the validity of this hypothesis. Results have been mixed. Of the specific vasodilator metabolites investigated thus far, it appears none alone can account completely for exercise hyperemia. It has been suggested that simultaneous changes in several substances collectively may produce the hyperemia. Several reviews on this subject summarize the most likely vasodilator candidates (Hilton et al. 1978; Shepherd 1983; Renkin 1984; Hudlicka 1985; Hudlicka and Khelly 1985). These include decreased tissue $P_{O_2}$, decreased pH, increased potassium concentration, increased inorganic phosphate, hyperosmolarity and increased adenosine concentration. Of these substances, the most consistent results have been reported for tissue $P_{O_2}$, potassium and adenosine.

In order to accept a potential vasodilator candidate, several criteria should be met (Hudlicka and Khelly 1985). The substance should occur naturally in the muscle. Traces of the substance should be identified in the tissue or in the venous blood. There should be a quantitative relationship between the concentration of the substance and the vasodilation it produces. An intra-arterial infusion of the substance should produce dilation. If a substance which inhibits the formation of the vasodilator substance is given, it should attenuate the dilator response. Finally, the concentration required to produce
dilation should be similar to the concentration that is measured. At this time, it would be technically very difficult, if not impossible, to perform all of the experiments required to fulfill these criteria.

Gorczinski and Duling (1978) elevated tissue $P_02$ by suffusing solutions of differing $P_02$ (up to 70 mmHg) over an isolated cremaster muscle. They concluded the vasodilator response to muscle contraction was inhibited by 40 percent. In a similar experiment, Lindbom (1986) reported a 27 percent inhibition of hyperemia by increasing suffusate $P_02$ from 0 to 150 mmHg in the rabbit tenuissimus muscle. Gorczinski and Duling (1978) and Proctor et al. (1981) reported a sustained, reduced tissue $P_02$ during muscle contraction. However, Lash and Bohlen (1987) report only a transient reduction in tissue $P_02$ during muscle contraction in the rat spinotrapezius muscle.

Klabunde (1986) reported dipyridamole, a substance which inhibits the degradation of adenosine, potentiated hyperemia in the dog gracilis muscle only when the muscle became ischemic. Therefore, for most regimens of rhythmic exercise it did not appear that adenosine was a significant vasodilator. Moreover, Laughlin et al. (1985) reported that adenosine deaminase, a substance which promotes adenosine degradation, had no effect on hyperemic flows in rats running on a treadmill. However, Mohrman (1987) reported fluid samples that were equilibrated with the rat cremaster muscle during contraction contained concentrations of adenosine that elicited comparable dilations when topically applied to the resting muscle.

A number of investigators have reported elevated potassium in
the venous effluent of exercising muscles (Scott et al. 1970; Mohrman and Sparks 1974; Hnik et al. 1976; and Hirche et al. 1980). Interstitial concentrations of potassium are about double those measured in the venous blood during tetanic contraction (Hnik et al. 1976; Hirche et al. 1980) and these concentrations are sufficient to produce a sizable hyperemic response (Scott et al. 1970; Mohrman and Sparks 1974). However, in one report (Lash and Bohlen, 1987) interstitial potassium concentrations did not change significantly during an isometric muscle contraction.

In humans, indomethacin, an arachidonic acid inhibitor, markedly reduced the hyperemic response to rhythmic exercise (Cowley et al. 1985) which suggests a vasodilator role for prostaglandins. This result is consistent with the hypothesis that the dilator mechanism is paracrine in nature.

There is some evidence to suggest a neural mechanism may be involved (Armstrong et al. 1985) but its role is unclear because denervated muscles become hyperemic in response to muscle contraction.

Other mechanisms that may contribute to exercise hyperemia include the myogenic response and flow dependent dilation. Increased tissue pressure during muscle contraction has been hypothesized to reduce the transmural pressure across the arteriolar wall resulting in myogenic dilation. Gorczynski et al. (1978) reported gentle tugging on the cremaster muscle to simulate the movement produced by muscle contraction did not elicit vasodilation, but Tyml (1987)
reported gentle mechanical stretching did cause vasodilation in the frog sartorius muscle. If small arterioles dilate first during muscle contraction, flow velocity will increase in larger arterioles and arteries upstream. Increased flow velocity elicits vasodilation in these larger vessels by an endothelium-dependent mechanism (Smiesko et al. 1985). Dilation in the larger vessels will further augment the hyperemic response.

**Sympathetic Stimulation**

Increased sympathetic vasomotor activity produces a large fall in skeletal muscle blood flow, graded to the stimulus frequency (Renkin and Rosell, 1962a). Although the increased resistance due to sympathetic stimulation is sustained for prolonged stimulation periods, Renkin and Rosell (1962a) observed in cat and dog hindlimb muscles, a small fall in resistance after 1-2 minutes of stimulation. This became more pronounced after 4 minutes, despite continued sympathetic stimulus. Kjellmer (1965) also reported, in whole organ studies of cat hindlimb muscles, a slight decrease in steady state resistance to sympathetic stimulation suggesting a secondary dilation of blood vessels during the stimulus period. This phenomenon is prominent in several vascular beds, as for example, the intestine (Folkow et al., 1964), and has been termed sympathetic escape. In skeletal muscle, escape is modest as blood flow remains low throughout the stimulus period. Direct observation of sympathetic escape in skeletal muscle microcirculation was first reported by
Eriksson and Lisander (1972). Arterioles in the cat tenuissimus showed secondary dilation if the duration of stimulation exceeded two minutes. These authors earlier reported that the maximal constrictor response occurred in arterioles 30 microns in diameter (1969). Smaller arterioles were less responsive to sympathetic stimulation.

Folkow et al. (1971) presented the first strong evidence that sympathetic escape in skeletal muscle is primarily limited to the terminal portions of the arteriolar network. Larger arterioles remained constricted, thus preventing significant flow increase during the stimulus period. In these experiments pressure measurements were taken in the isolated cat hindlimb by cannulation of three blood vessels. A small artery in the gastrocnemius muscle was cannulated and its upstream portion clamped so that a distal, forward pressure could be obtained. This varied from 11 to 70 percent of mean femoral artery pressure. Subsequent intravital casting of the vasculature, followed by KOH dissolution showed collateral connections downstream from the catheter, with dilated diameters of 80-100 microns. A small vein was cannulated in a similar manner. Resistance between femoral artery and vein and small artery and small vein could be calculated by taking the pressure differences, divided by the flow (which was measured at the femoral artery using an electromagnetic flow meter). Responses to sympathetic stimulation clearly demonstrated a secondary fall in distal resistance while proximal resistance remained elevated. A
similar study by Lundvall and Jarhult (1976a) substantiated these results. This phenomenon has been more extensively studied in microcirculatory preparations in the rat spinotrapezius (Marshall, 1982), in the rat cremaster (Fleming, 1985) and in the cat sartorius (Boegehold and Johnson, 1988a). In each of these preparations escape was prominently observed in the terminal portions of the arteriolar network, the transverse arteriole and vessels distal to it. Marshall (1982) and Fleming et al., (1987), using histochemical fluorescence techniques, have identified the presence of adrenergic innervation throughout the arteriolar network of the rat spinotrapezius and cremaster muscles. Fuxe and Sedvall (1965) reported adrenergic fluorescence including small arterioles in several hindlimb muscles of the cat.

Various studies have indicated the terminal arterioles are most sensitive to local vasodilator stimuli. The decreased blood flow during sympathetic stimulation might be expected to result in concentration of metabolites. In support of this interpretation Boegehold and Johnson (1988b) observed a marked attenuation of escape if the $P_{O_2}$ over the muscle was elevated. Tissue and periarteriolar $P_{O_2}$ was measured using oxygen microelectrodes. During sympathetic stimulation periarteriolar $P_{O_2}$ remained near control but tissue $P_{O_2}$ was reduced. So a metabolic mechanism linked to the decrease in tissue $P_{O_2}$ may account for sympathetic escape. In contrast, Marshall (1982) observed escape in the rat spinotrapezius muscle despite elevated superfusate $P_{O_2}$. 
Distributed Network Control of Flow

The fact that both resistance and resting vascular tone are not uniformly distributed throughout the arteriolar network (Fronek and Zweifach, 1975) indicates that some sites may be more important than others in blood flow regulation. The notion of distributed flow regulation has been recognized for many years. Krogh suggested for skeletal muscle, that flow regulation to the organ as a whole was regulated independently of flow distribution among the capillaries. This was based on the concept that constriction and dilation of arterioles regulated flow while the opening and closure of individual capillaries governed flow in capillaries. The latter was based on the observation that the capillary bed may not be totally perfused under resting conditions. Thus an increase in whole organ flow might be accompanied by recruitment of non flowing capillaries, faster velocity of flow in already open capillaries or to some combination of the two. Further studies established that capillaries did not possess contractile ability and precapillary sphincters were presumed to control recruitment of capillaries while changes of arteriolar dimensions were presumed to control whole organ flow.

In support of this notion Renkin and Rosell (1962a) measured the extraction of radiolabelled rubidium from blood perfusing isolated muscles of cat and dog hindlimb muscles. In low concentrations, rubidium is exchanged for potassium by muscle cells in a nearly unidirectional fashion. Therefore, rubidium extraction measures capillary transport, a parameter which is dependent on two
factors, the permeability of the blood-tissue diffusion barrier and the surface area available for exchange. Renkin and Rosell observed the response to sympathetic denervation and to muscle contraction. In their experiments, the response to sympathetic denervation indicated differential control of the capillary and arteriolar portions of the network. Resistance fell but rubidium extraction did not increase, which indicates arteriolar dilation but no capillary recruitment. In contrast, muscle contraction which also decreased resistance, lead to increased rubidium extraction, by 2-2.5 fold (Renkin and Rosell 1962b; Renkin et al. 1966). So unlike sympathetic denervation exercise produced dilation of arterioles and precapillary sphincters.

The classic view that capillary recruitment occurs by dilation of precapillary sphincters and can control flow per individual capillary has not been supported by current anatomical and physiological observations which indicate the last site of vasoconstrictor control is the terminal arteriole (Stingl 1976). Although no precapillary sphincters have been observed in skeletal muscle (Hammerson 1969; Eriksson and Myrhage 1972), it has been proposed the precapillary sphincter be given a functional definition (Weideman et al. 1976). Minimally, flow in several capillaries supplied by the terminal arteriole is regulated as a unit. Prewitt et al. (1982) observed 23 percent of arterioles with diameters less than 20 microns, were closed in the resting gracilis muscle of the rat. In contrast, Gorczinski et al. (1978) reported in the hamster cremaster muscle that much of the 2-fold increase in capillary
recruitment they observed during muscle contraction was due to opening of side branches from flowing capillaries. So rather than opening of terminal arterioles, perhaps the increased capillary pressure gradient during exercise opens flow paths that due to length or branch geometry have higher resistance (Lindbom and Arfors 1985). More recently, Delashaw and Duling (1987) have demonstrated, in the tibialis muscle of the hamster, recruitment and de-recruitment of groups of capillaries obtained by altering $P_0^2$ over the muscle. This control was exerted at the terminal arteriole or further upstream.

The physiological significance of this result is uncertain however, because tissue oxygen tension is usually low. Lindbom et al. (1980) showed arterioles in rabbit tenuissimus constrict in response to high levels of oxygen. The greatest constriction, found in the terminal arterioles, reduced capillary density in proportion to $P_0^2$. At a suffusing solution $P_0^2$ of 35 mmHg the number of capillaries with flow decreased by 34 percent of that observed with a $P_0^2$ of 5 mmHg. Capillary density decreased by 64 percent at a suffusate $P_0^2$ of 65 mmHg and by 98 percent with a $P_0^2$ of 100 mmHg. Others have observed similar results (Prewitt and Johnson, 1976; Sullivan and Johnson, 1981). Tissue $P_0^2$ in skeletal muscle is usually about 20 mmHg. Since atmospheric $P_0^2$ is around 150 mmHg, the tissue oxygen level is normally much lower. When the muscle is covered with a material which prevents atmospheric oxygen from reaching it, tissue $P_0^2$ will fall and terminal arterioles dilate. Under physiological
oxygen tensions, various investigators have reported the majority of capillaries perfused at rest (Burton and Johnson 1972; Hudlicka et al. 1982; Dawson et al. 1987; Tyml 1987). Even in the hamster tibialis, virtually all capillaries are perfused if tissue oxygen tensions are not artificially elevated (Damon and Duling 1984). These investigators report however, that flow is non-uniform or discontinuous. It has been suggested that capillary recruitment, such as indicated by Renkin et al. (1966) may be due to recruitment of slow flow channels, having minimal exchange capacity at rest.

Few reports of direct observations of hyperemic responses to muscle contraction appear in the literature. Gorczinski et al. (1978) reported a doubling of flowing capillaries in response to 4 hertz contractions in the hamster cremaster muscle. Tyml (1987) reported only a 31 percent increase in capillary density in response to 6 hertz stimulation of the frog sartorius muscle. Maximal dilation by nitroprusside increased capillary density in rat gracilis muscles by 51 percent (Prewitt et al. 1982).

Another issue has been whether there are non-nutritive, thoroughfare channels in muscle. These could be due to arterial-venous anastomoses that by-pass capillary circulation, but arterial-venous shunts have not been found in skeletal muscles (Weideman 1984). Since capillary flows are non uniform and fast flowing capillaries have been observed in many muscles, whole organ flow could increase and be shunted through these vessels without appreciably improving exchange capacity.
In summary, it has been recognized for some time that
distributed microvascular control of blood flow might allow for
separate control of volume flow and capillary perfusion. Whole
organ studies supported this conclusion but attempts to verify the
sites of regulation by direct observation have led to modification of
the original hypothesis. It is not clear whether capillaries are
recruited passively, due to favorable pressure gradients or actively
due to terminal arteriolar dilation. The significance of such
recruitment is also unclear because many investigators report
observing flow in most capillaries in resting muscle. The
postulation of capillary recruitment based on measurement of improved
exchange capacity could be reconciled with these observations if
capillaries with low flow contribute little to exchange. In this
case, recruitment of low flow capillaries could be due to dilation
anywhere in the upstream portion of the arteriolar network.
Alternatively, observation of flow in most capillaries may be due to
surgical trauma, as microcirculatory preparations are often hyperemic
by about 2-fold above presurgery flows (Bockman et al., 1980; Renkin,
1984; Proctor and Busija, 1985), but this is not necessarily the
case (Fronek and Zweifach, 1977; Morff and Granger, 1980). Another
caveat, relevant to most microcirculatory studies, is the fact that
anesthetics generally effect vascular tone. Using a transparent
indwelling chamber, Colantuoni et al. (1984) were able to study
arterioles in conscious and anesthetized hamsters. Vasomotion which
is most prominent in terminal arterioles was usually inhibited by
anesthetics. This was the case for alpha-chloralose, the anesthetic often used for cats, but mean arteriolar diameters were unchanged soon after giving the anesthetic.

The observation that flow can increase independently of exchange capacity suggests non-nutritive shunting. Lindbom and Arfors (1984) have shown blood flow in the rabbit tenuissimus can be preferentially shunted into the connective tissue. It is not known if this could be of significant proportion in other muscles.

A growing body of data, obtained from microcirculatory observations demonstrate distributed microvascular control of blood flow, but the same vascular segments appear to regulate capillary flow and exchange area (Eriksson and Lisander, 1972; Goczninski et al. 1978; Lindbom et al. 1980). There is an attempt to integrate direct observations with whole organ measurements but this is often difficult due to experimental limitations. Frequently, insufficient data can be obtained from direct observation for meaningful comparison to whole muscle parameters, which represent the responses of numerous vessels. As more data are obtained in a particular muscle preparation, integration with whole muscle measurements becomes more feasible.

Muscle Contraction and Sympathetic Stimulation

During sympathetic stimulation sustained constriction of large arterioles and probably small arteries maintain a greatly reduced blood flow despite sympathetic escape in the terminal portion of the
arteriolar network. In contrast, Folkow et al. (1971) reported muscle contraction during sympathetic stimulation decreased resistance first in the distal portions of the network, followed by reduced resistance in the proximal portion of the network. This indicates that the functional sympatholysis which occurs during muscle contraction results in dilation of all arterioles, though this has not been observed directly in the microcirculation. Functional sympatholysis is not an all or none response. The blood flow resulting when the vasoconstrictor influence of sympathetic stimulation and the vasodilator influence of muscle contraction are in opposition depends on the relative intensity of these conflicting stimuli and on the experimental conditions. The term functional sympatholysis was introduced by Remensnyder et al. (1962) who noted only a small reduction in blood flow when sympathetic nerve activity was increased to contracting hindlimb muscles of the dog. In a similar report by Kjellmer (1965) on isolated calf-muscles of the cat, resistance increased initially when a sympathetic stimulus from 0.5 to 16 hertz was imposed during a 1 hertz muscle contraction. The reduced flow was transient, by 8-10 minutes resistance returned to near normal exercise values. This initial vasoconstriction to sympathetic stimulation during exercise was also shown by Donald et al. (1970) in conscious dogs running on a treadmill. A 30 second, 6 hertz stimulus of the lumbar sympathetic chain decreased flow by nearly 50 percent. So if the sympathetic stimulus is brief, sympatholysis does not develop. The ability of a sympathetic stimulus
to influence functional hyperemia was further investigated in a more recent study (Klabunde, 1987a) using the vascularly isolated dog gracilis muscle. A sympathetic stimulus sufficient to reduce blood flow by 50 percent in resting muscle was imposed. This was followed by tetanic muscle contractions of 1-10 second duration at 40 hertz. Muscle tetany produced a hyperemic response; but the absolute change in flow did not differ between control and sympathetically constricted muscles. Peak conductance, the absolute magnitude of the flow response, was reduced in sympathetically stimulated muscles because flow was reduced prior to tetany. So muscle contraction elicited a change in flow which was independent of the initial flow, whether or not it was altered by sympathetic stimulation.

The sympatholysis that develops after the initial constrictor response abates does not continue indefinitely. Rowlands and Donald (1968) observed in dog hindlimb muscles contracting at 2 hertz that a sympathetic stimulus between 0.2-6 hertz lasting for 10-30 minutes attenuated the hyperemic response. If the sympathetic stimulus is particularly potent, it can overwhelm the capacity for functional hyperemia altogether. Costin and Skinner (1971) reported severe systemic hypoxia, which reflexly activates the sympathetic system, can completely abolish exercise induced vasodilation. Atropine, which blocks sympathetic cholinergic responses, has no effect on sympatholysis (Rowlands and Donald 1968).

In summary, it has been shown in conscious animals exercising on a treadmill and in anesthetized animals that functional sympatholysis
takes some time to develop, on the order of several seconds to a minute. This is the case whether the sympathetic stimulus is imposed during ongoing muscle contraction or when muscle contraction is imposed during an ongoing sympathetic stimulus. After this initial vasoconstrictive response, if the two opposing stimuli continue, vasodilation ensues and there is the potential for complete sympatholysis. That is, functional dilation occurs as though the sympathetic stimulus was not present. However, if both stimuli continue for an extended period of time, at least 10 minutes, the vasoconstrictor influence of sympathetic activity reduces, but does not abolish the hyperemic response. Only when sympathetic activity is particularly severe can vasoconstriction override functional hyperemia.

**Mechanism of Sympatholysis**

There is much which is not yet understood regarding the mechanism of functional sympatholysis. There are two possible effector sites, the first being neural and due to prejunctional inhibition of norepinephrine release, the second being at the vascular smooth muscle. With respect to the latter, there are several possible mechanisms, there may be a modification of adrenoceptor sensitivity, or reduction of intracellular calcium concentration, which would lead to myosin light-chain dephosphorylation and smooth muscle relaxation. Another possibility is that the muscle membrane hyperpolarizes as would result if
potassium conductance increased (Bulbring and Tomita, 1987). Current evidence suggests prejunctional inhibition and direct smooth muscle effects both contribute to sympatholysis. Prejunctional inhibition of sympathetic activity will reduce norepinephrine release. Comparison between the effects of sympathetic stimulation and exogenously administered norepinephrine have been used to detect prejunctional inhibition in in vivo studies. It has been observed in various muscle preparations that functional dilation is less during exogenous administration of norepinephrine than during sympathetic stimulation, suggesting prejunctional inhibition.

Rowlands and Donald (1968), who observed in the dog hindlimb that sympatholysis was most prominent during the first few minutes of muscle contractions at 2 hertz, also reported that peripheral resistance was increased after infusion of norepinephrine throughout the period of contractions. In another report on the isolated dog gracilis muscle (Burcher and Garlick, 1973) norepinephrine was infused in a dose that produced the same magnitude of vasoconstriction as 2 hertz sympathetic stimulation. Under this condition, vasoconstriction to the exogenously administered norepinephrine was inhibited by approximately 30 percent during 1 hertz muscle contraction, whereas sympathetic vasoconstriction was reduced by approximately 90 percent. When the vasoconstrictor response to infused norepinephrine was matched to 5 hertz sympathetic stimulation, escape from norepinephrine was approximately 25 percent at 1 and at 2 hertz muscle contraction, whereas escape from
sympathetic stimulation was approximately 50 percent at 1 hertz and about 90 percent at 2 hertz muscle contraction. In a later study (Beaty and Donald, 1977) using the same muscle preparation, vasoconstriction with exogenous norepinephrine was matched to a sympathetic stimulation of 3 hertz. After the muscle had contracted at 2 hertz for 30 minutes, the vasoconstrictor response to norepinephrine was reduced to 30 percent, while the constrictor response to sympathetic stimulation was 22 percent of control. It has been interpreted from these studies that prejunctional inhibition of norepinephrine release plays a prominent role only during the first few minutes of exercise. As exercise continues, more of the response appears to be due to a direct effect on the vascular smooth muscle. One possible limitation of these studies is that they were conducted under constant flow. The response during autoperfusion, in which flow would increase during exercise might be very different.

The arteriolar response to muscle contraction and to norepinephrine vasoconstriction has been directly observed in the hamster cremaster muscle (Klitzman et al., 1982). As graded doses of norepinephrine solution were suffused over the muscle, the vasodilator response to one minute muscle contraction at 1 hertz was progressively reduced as the arterioles became more constricted. This result is consistent with the observations of Burcher and Garlick (1973) for muscle contraction at 1 hertz.

A number of experiments followed those cited which had the goal of identifying the tissue metabolites that inhibit sympathetic
neurotransmission. In vitro studies reported that adenosine, adenine nucleotides, hyperosmolarity, and hydrogen and potassium ions depressed tritiated norepinephrine release at concentrations less than those required to produce a direct effect on vascular smooth muscle (Shepherd and Vanhoutte 1981). Understanding the in vivo significance of these results is limited by the difficulty of determining interstitial metabolite composition. Venous effluent concentrations are probably lower than interstitial concentrations due to rapid degradation, muscle reuptake and the tissue-blood diffusion barrier. Also, venous concentration will depend on flow rate. If an in vivo experiment depends on intra-arterial infusion of a metabolite to assess the vasoreactivity of a metabolite, equilibration time and concentration reaching the effector site can be estimated at best. As well, in vitro experiments are limited to large vessels, usually arteries. Microcirculatory responses may differ. Given these limitations, the discrepancies between in vitro and in vivo experiments may be reconciled.

Skinner and Costin (1969), using the isolated, perfused dog gracilis muscle, evaluated the effect of hypoxia and potassium on sympathetic vasoconstriction. Infusions of as little as 6-8 mM potassium potentiated sympatholysis induced by hypoxia. In these studies arterial Po2 was reduced to only 16 mmHg, a value hardly physiological, making these experiments difficult to interpret. The isolated dog gracilis muscle has been used as a model to measure venous metabolite concentrations during muscle contractions. However,
of it should be noted that flow was held constant, so venous concentrations could be expected to be elevated. Of the metabolites tested by arterial infusions, within the measured range in the venous effluent, only potassium, when increased to 10 mM and Po2 when decreased to 30 mmHg attenuated sympathetic vasoconstriction. Burcher and Garlick (1975) measured Po2, phosphate, potassium and osmolarity. Beaty and Donald (1977) also measured a reduction in venous pH, 7.4 to 7.1, but reduced arterial pH had no effect on sympathetic vasoconstriction, nor did infused ATP. In a more recent study, Klabunde (1987b) also using the dog gracilis, infused adenosine and its stable analogue, 2-chloroadenosine in physiological concentrations of 10^-2 M (Mohrman 1987) with no effect on sympathetic vasoconstriction. Slightly higher concentrations of adenosine produced maximum dilation but this was deemed a direct effect on the vascular smooth muscle. Furthermore, Shinozuka et al. (1987) reported only a 20 percent inhibition of tritiated norepinephrine release from rat caudal artery exposed to 10^-2 M 2-chloroadenosine. McGillivray and Faber (1987) reported a pH reduction to 7.1 in the solution suffusing a cremaster muscle markedly reduced vasoconstriction to the alpha-2 adrenergic agonist KBHT-933, in 100 micron arterioles in the rat cremaster muscle. Thus, increased potassium and possibly decreased pH are currently the best in vivo candidates for a metabolic mechanism of prejunctional inhibition of sympathetic nerves. However, it is not certain that either of these substances change sufficiently during exercise to result in
sympathetic inhibition.

Experiments with contracting muscle derive much of the evidence for prejunctional inhibition by comparing hyperemia between norepinephrine infusion and sympathetic stimulation. Burcher and Garlick (1973) acknowledged that the difference in functional hyperemia between norepinephrine infusion and sympathetic stimulation supports the hypothesis of prejunctional inhibition only if infused norepinephrine mimics sympathetic stimulation. Infused norepinephrine may not mimic the effect of sympathetic stimulation. The presence of more than one alpha adrenoceptor has been confirmed in various studies due to the differing effects of various adrenergic antagonist and agonist drugs (Ruffulo, 1984). In general, alpha-1 receptors appear to be junctionally located and alpha-2 receptors, extrajunctionally located (Wilffert et al. 1982; Matthews et al. 1984). Yamaguchi and Kopin (1980) reported in pithed rats, that pressor responses produced by sympathetic stimulation were more effectively blocked by alpha-1 antagonists than by alpha-2 antagonists. In contrast, pressor responses to norepinephrine were completely abolished by alpha-2 antagonist concentrations which had no effect on the sympathetically induced pressor response. The authors cited results from one of their previous papers that changes in blood pressure produced by these procedures was predominantly due to vasoconstriction and not to changes in heart rate. So an exogenous source of norepinephrine may bind predominantly to alpha-2 receptors, while sympathetic release of norepinephrine binds
preferentially to alpha-1 receptors. Similar studies have been performed in the cat (Langer et al. 1985).

Alpha-2 receptors are also prejunctionally located and inhibitory. Neuronally released norepinephrine may bind to these receptors, reducing further norepinephrine release (Langer, 1981). There may also be another adrenergic receptor type, which has been referred to as the gamma receptor, that is purportedly located in the junction (Hirst and Neild, 1980 and 1981).

The presence of alpha-2 receptors is species dependent but they are most often found in medium sized arteries, smaller arteries are predominantly alpha-1 (Bulbring and Tomita, 1987). Whole animal studies indicate alpha-2 receptors are also present on resistance vessels. Moreover, Faber (1988) reported in the microcirculation of the rat cremaster, the presence of alpha-2 receptors in small, 27 micron diameter arterioles.

The two alpha receptors differ physiologically. Alpha-1 receptor binding leads to membrane depolarization, so called electromechanical coupling, and voltage dependent opening of calcium channels. Phosphatidylinositol acts as an intracellular messenger to release an intracellular store of calcium. Alpha-2 receptor binding causes an excitatory junctional potential and so called pharmacomechanical coupling, which appears to depend on an extracellular source of calcium, probably entering through a voltage independent calcium channel (Bulbring and Tomita, 1987). Due to these differences, alpha receptor function or signal transduction may
be modified differently in functional sympatholysis. In support of this conclusion, McGillivray and Faber (1987) reported in rat cremaster a pH decrease to 7.1 in the suffusing solution selectively inhibited alpha-2 mediated constriction but had no effect on constriction mediated by alpha-1 receptors.

In summary, further studies are needed to evaluate whether experiments using responses to norepinephrine and to sympathetic stimulation to discriminate between pre- and post-junctional sympatholysis were limited by the existence of different alpha receptor binding sites. If so escape from the vasoconstrictor effect of alpha-2 receptors may differ from alpha-1 receptors.

Recently a number of studies have provided evidence that norepinephrine is not the only transmitter released from adrenergic terminals. ATP is probably co-released with norepinephrine (Cheung 1982; Sneddon and Burnstock 1984; Suzuki 1985; Kuge1gen and Starke 1985). Moreover, norepinephrine may stimulate ATP release locally from non-neural tissue (Burnstock and Kennedy 1986; Sedaa et al. 1987). Neuropeptide Y is also a good candidate for co-release with norepinephrine (Lundberg and Tatamoto, 1982; Hanko et al. 1986; Pernow et al. 1988). So the complexity of vasomotor regulation is greater than generally appreciated in physiological studies of functional sympatholysis.
CHAPTER 2

OBJECTIVES OF THE PRESENT STUDY

In the first part of this study arteriolar diameter changes during muscle contraction were characterized throughout the network supplying the cat sartorius muscle. Then the experiments were repeated with increased vascular tone resulting from sympathetic nerve stimulation. Previous reports, in which arteriolar responses to muscle contraction have been directly observed, indicate that the most distal arterioles dilate proportionately more than upstream arterioles. This suggests the small arterioles may play a predominant role in regulating flow changes during exercise. However, such measurements have been taken only for a portion of the arteriolar network and do not rule out the possibility that measurements across the entire network would lead to a different interpretation. Moreover, it is not possible to assess the contribution that arteriolar diameter changes have on resistance to flow without having a measure of the initial distribution of resistance in the network. In the sartorius muscle we are able to measure diameter responses at each level of the network and previous studies from this laboratory provide both pressure and morphometric data from which the initial resistance distribution can be estimated. From this it is possible to evaluate how diameter changes effect resistance and to determine the relative contribution to resistance
at each consecutive level in the network. The benefit of such analysis is that it will enhance understanding of flow regulation and in particular, it is expected to provide insight on how differential arteriolar responses can localize flow distribution within a muscle.

Comparing the dilation during muscle contraction with different levels of initial vascular tone, adjusted by stimulating the sympathetic nerves, is expected to provide further insight on the interaction between opposing constrictor and dilator stimuli. It is known that sympathetic vasoconstriction is well maintained in the more proximal arterioles of the network, while smaller arterioles dilate in response, it is believed, to an ischemic signal. Whole organ studies show that muscle contraction during sympathetic stimulation antagonizes sympathetic control and hyperemia approaches that observed in the absence of sympathetic stimulation. This suggests, the more proximal arterioles, which resisted the dilator influence of sympathetically induced ischemia, do not resist the dilator influence of muscle contraction. But this has not been investigated directly in the microcirculation.

Two mechanisms, based on data from whole organ and in vitro small arteries, have been proposed to account for arteriolar dilation to muscle contraction during sympathetic stimulation. The hypotheses state that dilator substance(s) have a direct effect on vascular smooth muscle to inhibit tone and, the dilator substance(s) inhibit sympathetic neurotransmission. The aim of the second part of this study was to test these hypotheses in our microcirculatory
preparation.

Much of the evidence to support the latter hypothesis comes from the observation that functional dilation is reduced after infusion of norepinephrine as compared with sympathetic stimulation. However, an exogenous source of norepinephrine may bind to both alpha-1 and alpha-2 adrenergic receptors, whereas sympathetic vasoconstriction may be selectively mediated by alpha-1 receptors. Since this difference could be significant, we compared functional dilation after systemic injection of norepinephrine or phenylephrine, a selective alpha-1 agonist, to that during sympathetic stimulation. We reasoned that if the dilator substances pre-junctionally inhibit sympathetic nerves, then functional dilation should also be reduced after injection of phenylephrine. Moreover, if functional dilation differs between norepinephrine and phenylephrine injection, this would provide evidence that the dilator substances selectively inhibit one receptor subtype more than the other.

Finally, we recognize that the data indicating selective innervation of alpha-1 receptors has not been verified in a microcirculatory preparation. Therefore, we repeated the above experiments after administration of alpha-1 and alpha-2 blocking agents to determine the presence and innervation of each receptor subtype.
CHAPTER 3

ARTERIOLAR NETWORK REGULATION OF RESISTANCE DURING SYMPATHETIC STIMULATION AND MUSCLE CONTRACTION IN THE CAT

Introduction

Arterioles are a major site of resistance to blood flow in skeletal muscle (Fronek and Zweifach, 1975; Zweifach et al., 1981, House and Johnson, 1986a). Diameter adjustments within the arteriolar network contribute to the regulation of flow. These diameter changes not only influence total flow to the muscle but also provide a means of altering the distribution of blood flow to different muscle regions. Localized regulation of flow distribution has been demonstrated by Laughlin and Armstrong (1982) with rats running on a treadmill. Blood flow, as measured by distribution of injected microspheres, was much higher in muscle regions with contracting oxidative fibers, as compared to regions with inactive glycolytic fibers. This can be explained in part by the dilation of the supplying arterioles to the area of contracting muscle. Gorczynski et al. (1976) reported, in a microcirculatory preparation, that an arteriole passing across a few contracting muscle fibers dilated only in the vicinity of the active muscle.

Consecutive sections of the arteriolar network may also behave differently from each other during contraction of an entire muscle. Gorczynski et al. (1976) and Marshall and Tandon (1984) reported that the smaller arterioles dilated to a proportionately greater extent
than the larger arterioles. In this instance, it is assumed all arterioles are exposed to a comparable environment of vasodilator substances. This finding has led to the hypothesis that small arterioles are more responsive to tissue metabolites than large arterioles and, that this difference may be significant in the regional regulation of blood flow in muscle.

The response of all levels of the arteriolar network to muscle contraction has not been studied. One of the purposes of this study was to document these changes in the six orders of arterioles which supply the cat sartorius muscle. Since the pattern of response might well depend upon the initial level of vascular tone, we repeated these studies during elevated sympathetic tone. Finally, to assess the contribution of each order to flow regulation, we utilized the data on arteriolar diameter together with previous measurements of arteriolar pressure and vascular length (House and Johnson, 1986a; Koller et al., 1987), to calculate hindrance of the various orders and its changes during muscle contraction.

Methods
Surgical Procedure
After sedation by intramuscular injection of ketamine hydrochloride (Ketaset, Bristol Laboratories), 15 mg/kg, cats of either sex (n=22) weighing between 0.9 -1.2 kg were anesthetized via jugular vein catheterization with alpha-chloralose, 38 mg/kg of body weight. Surgical anesthesia was maintained with supplemental alpha-
chloralose, 30 percent of the original dose, given as needed. Catheterization of the carotid artery was performed for arterial pressure recordings using a Statham pressure transducer.

The left sympathetic chain was isolated as described by Boegehold and Johnson (1988a). A bipolar silver wire electrode made of coated, 0.005 inch diameter wire (A-M Systems), glued inside a silastic tubing cuff, was wrapped around the chain between L4-L6. A similar electrode was placed on the most lateral branch of the femoral nerve, which innervates the sartorius muscle. Calibrated Grass stimulators were used to deliver electrical shocks to each nerve.

Surgical isolation of the left sartorius muscle proceeded as described by House and Johnson (1986a) leaving neural and vascular supplies intact. During surgery the muscle was held at in situ length and kept moist with Isolyte (American McGaw) warmed to 37 °C. After surgery, the animal was transferred to a microscope stage where the muscle was fastened at in situ length, dorsal side up over a Plexiglas pedestal through which warm water circulated to maintain a 35 °C surface temperature. A saline-rinsed sheet of polyvinyl film (Saran Wrap, Dow Corning), sealed at the edges with vaseline, protected the muscle from exposure to the atmosphere and prevented drying.

In vivo observations

The preparation was left undisturbed for a one hour equilibration period prior to transillumination using a Leitz 100
watt mercury-arc light source and a Leitz Ortholux II microscope for
direct observation of the microcirculation in the thin central region
of the muscle. A long working distance Leitz um 32 (numerical
aperture = 0.2) objective gave a final video magnification of \( {\times}1200 \).
The microscope image was recorded using a silicon intensified target
(SIT) video camera and a Sony super beta cassette recorder. Tape
replay allowed stop-field analysis of internal-wall vessel diameters
using calibrated video calipers. Time was recorded to 1/100 of a
second on the video image. Designation of vessel branching orders
followed a centrifugal scheme similar to that described by Wiedeman
(1962). In the sartorius six branching orders have been identified,
with the largest, first-order vessels closest to the supplying
arteries, femoral or lateral circumflex and the smallest, sixth-order
vessels supplying the capillary bed (see mapping of the arteriolar
network in figure 1). For some of the data reported, the second-
order, arcading arterioles were further subdivided into two groups;
large second-order arterioles, which were supplied by first-order
arterioles and small second-order arterioles, which were supplied by
other second-order arterioles (Marshall and Tandon, 1984). As much
as possible, the arterioles observed in each of the six branching
orders were in series with one another. Arteriolar networks were
selected for investigation only if there was no evidence of
hemorrhage or of venous stasis. Experiments were terminated if
either of these events occurred during the observation period, which
was sometimes the case with repeated protocols of muscle contraction.
Experimental Protocols

Two protocols were performed on each arteriole. The first step in each was to measure control diameter for a period of 2 minutes. In one protocol this was followed by muscle contraction for 2 minutes, induced by stimulation of the femoral nerve at an intensity of 4 volts, for a duration of 0.1 msec and a frequency of 4 Hz. After muscle contraction, a minimum 15 minute recovery period was allowed, during which time diameter and flow returned to control values. In the second protocol, following a 2 minute control period, the sympathetic nerve was stimulated for 5 minutes at an intensity of 12 volts, pulse duration of 5 msec and a frequency of 8 Hz. During the last 2 minutes of sympathetic stimulation, muscle contraction was induced with the same stimulus parameters used in the first protocol. Since the arterioles appeared to respond consistently in repeated trials of these two protocols, no particular order was observed. In many of the experiments, there was a third protocol which consisted of motor nerve stimulation for 2 minutes at 30 Hz. This was usually performed at the end of the experiment, otherwise a 30-60 minute recovery period preceded the next protocol.

Control experiments for these protocols included, 5 minutes of continuous sympathetic nerve stimulation at 8 Hz for evaluation of sympathetic escape (Boegehold and Johnson, 1988a) and stimulation of the sympathetic chain using the motor nerve parameters to determine whether significant sympathetic nerve activation resulted.
In 4 second order arterioles, the response to 4 Hz muscle contraction was observed after administration of the alpha-1 adrenergic blocking agent, prazosin (Cambridge et al., 1977; Timmermans et al., 1980). This was done to determine whether 30 Hz femoral nerve stimulation elicited maximal dilation.

Diameter evaluation and statistical analysis

The diameter of each arteriole was measured in stop-frame analysis, recorded with time to the nearest second and entered into the computer (IBM PC). Then the diameter was observed in real time or in slow motion until a perceptible change occurred, at which time the tape was stopped or reversed as necessary to record the new diameter and time. From this, average diameter over any specified time period could be calculated. Further analyses were performed by transferring the data to the Lotus 123 spread sheet. Statistical analyses include mean, standard deviation, standard error of the mean and paired t-tests (two tail unless otherwise noted) to compare the change with each protocol to control diameter.

Resistance calculations are described in Appendix A.

Results

The mean blood pressure during control periods was 109 \( \pm \) 11 mmHg (SD) and did not change during muscle contraction. With sympathetic stimulation, blood pressure initially increased to 127 \( \pm \) 14 mmHg (SD) but fell to 119 \( \pm \) mmHg (SD) after three minutes. During combined
sympathetic stimulation and muscle contraction, blood pressure returned further toward control levels.

The mean arteriolar diameters (+SEM) for each branching order at rest and during two minutes of 4 Hz muscle contraction are shown in figure 2. Arterioles in each order dilated significantly with muscle contraction, p<.02, with the third order arterioles showing the largest proportional dilation (table 1). Dilation was monophasic in approximately 50 percent of the vessels, reaching plateau with no further dilation during the two minute period. In the remaining vessels, dilation was multiphasic. Approximately 25 percent of the 3rd-6th order arterioles showed periodic vasomotion, at a frequency of 2-4 cycles per minute, superimposed on the dilator and plateau portions of the curve. Vasomotion was rarely observed during the control period.

The response to 4Hz and 30 Hz muscle contraction is shown in figure 3. The magnitude of dilation with 30 Hz muscle contraction demonstrates that there is a high level of resting tone throughout the arteriolar network of the cat sartorius muscle. As with 4 Hz muscle contraction, the largest proportional changes were observed in the third order arterioles.

Despite the sizable dilation with 30 Hz muscle contraction, it is quite possible that diameters attained were submaximal due to activation of sympathetic vasoconstrictor nerves. Table 2 shows that 4 Hz muscle contraction activated sympathetic nerves, as indicated by the modest but significant vasoconstriction observed when the
sympathetic chain was stimulated using the parameters applied at the motor nerve. The probability that 30 Hz muscle contraction results in somewhat less than maximal dilation is further supported by the effect of muscle contraction after administration of the alpha-1 adrenergic blocking agent prazosin. Prazosin caused an initial dilation similar to that observed with 4 Hz muscle contraction. Under this condition, 4 Hz muscle contraction resulted in further dilation beyond that observed with 30 Hz muscle contraction, table 3.

With sympathetic stimulation, an initial constriction was observed in all arteriolar orders (figure 4). The fourth, fifth and sixth order arterioles dilated secondarily during continued sympathetic stimulation and, after 150 to 180 seconds their mean diameters were not significantly different from control (paired t-test). The third order arterioles secondarily dilated to a lesser extent so that diameters during 150 to 180 seconds of sympathetic stimulation remained significantly less than initial resting diameters (p<.02). Little secondary dilation was observed in the first and second order arterioles, which after 150 to 180 seconds were still significantly constricted (p<.02). The proportional changes in diameters with three minutes sympathetic stimulation are shown in table 1.

Since muscle contraction was induced by motor nerve stimulation during minutes three to five of sympathetic stimulation a control protocol was followed to determine whether dilation during this period was due in part to the secondary dilation discussed above.
The diameter of second and third order arterioles did not change significantly during this time.

Muscle contraction during sympathetic stimulation caused dilation in all arteriolar orders (figure 4), similar to the findings during muscle contraction with resting vascular tone. The proportional dilation during sympathetic vasoconstriction was as great or greater in all arteriolar orders than that observed with muscle contraction (table 1). Similarly the absolute magnitude of dilation was as great or greater than that observed with normal resting tone and muscle contraction (figure 5). However, because the vessels were initially constricted, the absolute diameters attained with muscle contraction during sympathetic stimulation were somewhat less than those attained without sympathetic stimulation (table 1).
Figure 2. Arteriolar network diameter changes (microns) during 4 Hz muscle contraction (MC) for first through sixth order arterioles (1A-6A). Arrows on x-axis indicate MC interval, shaded regions show SEM.
Table 1. Arteriolar network responses to muscle contraction (MC) and sympathetic stimulation (S) for first through sixth order arterioles (1A-6A). Control diameter is the average of the 2 minutes prior to MC and the 2 minutes prior to S.

<table>
<thead>
<tr>
<th>ARTERIOLAR ORDERS</th>
<th>1A</th>
<th>2A</th>
<th>2A</th>
<th>3A</th>
<th>4A</th>
<th>5A</th>
<th>6A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>large</td>
<td>small</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>86.2</td>
<td>50.9</td>
<td>24.6</td>
<td>11.1</td>
<td>8.1</td>
<td>7.7</td>
<td>6.8</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(8.1)</td>
<td>(3.3)</td>
<td>(2.4)</td>
<td>(0.9)</td>
<td>(1.1)</td>
<td>(0.6)</td>
<td>(0.5)</td>
</tr>
<tr>
<td><strong>4 Hz MUSCLE CONTRACTION</strong></td>
<td>99.7</td>
<td>62.0</td>
<td>38.1</td>
<td>20.0</td>
<td>13.7</td>
<td>9.7</td>
<td>8.7</td>
</tr>
<tr>
<td>mean 90-120 sec</td>
<td>(8.1)</td>
<td>(3.3)</td>
<td>(3.9)</td>
<td>(1.8)</td>
<td>(2.4)</td>
<td>(0.9)</td>
<td>(0.6)</td>
</tr>
<tr>
<td>percent of control</td>
<td>117</td>
<td>123</td>
<td>158</td>
<td>182</td>
<td>165</td>
<td>126</td>
<td>130</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(7)</td>
<td>(5)</td>
<td>(11)</td>
<td>(10)</td>
<td>(15)</td>
<td>(6)</td>
<td>(4)</td>
</tr>
<tr>
<td><strong>8 Hz SYMPATHETIC STIMULATION</strong></td>
<td>65.0</td>
<td>34.3</td>
<td>13.5</td>
<td>6.5</td>
<td>5.9</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>mean 150-180 sec</td>
<td>(9.0)</td>
<td>(4.1)</td>
<td>(3.0)</td>
<td>(1.2)</td>
<td>(1.2)</td>
<td>(1.2)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>percent of control</td>
<td>76</td>
<td>69</td>
<td>53</td>
<td>61</td>
<td>78</td>
<td>79</td>
<td>94</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(6)</td>
<td>(9)</td>
<td>(11)</td>
<td>(10)</td>
<td>(13)</td>
<td>(13)</td>
<td>(9)</td>
</tr>
<tr>
<td><strong>4 Hz MUSCLE CONTRACTION DURING 8 Hz SYMPATHETIC STIMULATION</strong></td>
<td>84.1</td>
<td>49.1</td>
<td>34.2</td>
<td>16.1</td>
<td>12.0</td>
<td>9.8</td>
<td>7.5</td>
</tr>
<tr>
<td>mean 90-120 sec</td>
<td>(7.7)</td>
<td>(3.1)</td>
<td>(4.4)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td>(0.8)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>percent of 8 Hz S</td>
<td>139</td>
<td>160</td>
<td>312</td>
<td>288</td>
<td>178</td>
<td>181</td>
<td>123</td>
</tr>
<tr>
<td>(SEM)</td>
<td>(12)</td>
<td>(26)</td>
<td>(53)</td>
<td>(41)</td>
<td>(19)</td>
<td>(19)</td>
<td>(9)</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>22</td>
<td>8</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure 3. Comparison of arteriolar network responses to 4 Hz and 30 Hz muscle contraction (MC) for second through sixth order arterioles (2A-6A). Error bars indicate SEM, where not seen bars are within symbols, n indicated in parentheses. Diameter change with 30 Hz MC, as percent of control (+SEM), was 192±12, 204±29, 218±24, 223±27 and 182 for 2A-6A arterioles, respectively.
Table 2. Arteriolar (second order) response to sympathetic nerve stimulation (S) using the motor nerve parameters—4 Hz, 0.1 msec pulse duration is shown in 2A; 2B shows the response of the same vessels to sympathetic stimulation with the usual parameters—8 Hz, 5 msec.

<table>
<thead>
<tr>
<th></th>
<th>A.</th>
<th>B.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>4 Hz S 0.1 msec</td>
</tr>
<tr>
<td>Diameter (SEM) n=5</td>
<td>38 (6)</td>
<td>34* (6)</td>
</tr>
</tbody>
</table>

a_p < 0.015 and b_p < 0.005 for a one-tail, paired t-test.
Table 3. Arteriolar response to muscle contraction (MC) after prazosin administration.

<table>
<thead>
<tr>
<th>Diameter (micron)</th>
<th>Control</th>
<th>Prazosin</th>
<th>Prazosin &amp; 4 Hz MC</th>
<th>Dilation Percent of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>(SEM) n=4</td>
<td>56.5</td>
<td>80.3</td>
<td>127.8</td>
<td>239</td>
</tr>
</tbody>
</table>

Second Order Arterioles
Figure 4. Arteriolar network diameter changes during 8 Hz sympathetic stimulation (S) and 4 Hz muscle contraction (MC) for first through sixth order arterioles (1A-6A). Arrows on x-axis indicate S and MC intervals, shaded regions show SEM.
Figure 5. Comparison of dilation to 4 Hz muscle contraction (MC) with normal vascular tone and during 8 Hz sympathetic stimulation (S) for first through sixth order arterioles (1A-6A), difference between initial diameter, just prior to MC, and diameter after 2 minutes MC. Error bars indicate SEM.
Reports in the literature indicate that during muscle contraction dilation is proportionately greatest in the small arterioles. Gorczynski et al. (1976) found this to be the case with three groups of arterioles in the hamster cremaster muscle, having average resting diameters of 20, 11 and 6 microns. Marshall and Tandon (1984) observed a similar variation among arterioles at three branching levels in the rat spinotrapezius muscle, with resting diameters of 22-50 microns, 13-18 microns and 7-13 microns.

However, these measurements did not include all orders of the arteriolar network. In the cat sartorius, in which all orders were studied, dilation during muscle contraction was proportionately larger in third order arterioles than in more proximal or more distal arterioles. In fact, in comparing third through sixth order arterioles, dilation was proportionately less in the more distal vessels. This was true for both 4 Hz and 30 Hz muscle contraction and for 4 Hz muscle contraction during sympathetic nerve stimulation. Therefore, our data do not support the hypothesis that there is a greater response of the most distal vessels.

However, our data do indicate, as do the previous findings (Gorczynski et al. 1976; Marshall and Tandon, 1984) that arterioles in consecutive sections of the network behave differently from each other during muscle contraction.

The importance of any arteriolar order in controlling flow cannot be adequately tested by measuring diameter change alone. It
is also necessary to know the vascular resistance of that section of
the network. To assess the contribution of each order to resistance,
under control conditions, data previously obtained from the resting
sartorius muscle were used to construct a pressure profile of the
various sections of the vascular network. House and Johnson (1986a)
measured pressures in the femoral artery, mid-second and mid-third
order arterioles, large vein and first, second, third and fourth
order venules. We estimated from these data the apportioning of
resistance among artery, first, second and a portion of the third
order arterioles, as well as for the venous section of the network.
To estimate the apportioning of resistance among the vascular
segments located between the third-order arterioles and fourth-order
venules, we used data on vessel diameters and lengths obtained by
Koller et al. (1987) in this muscle and applied the Poisseuille-Hagen
relationship.

The composite pressure profile is shown in figure 6, in which
the solid portion of the line indicates pressure data and the dashed
portion, values derived from the morphometric data (see appendix for
further explanation of calculations). This curve indicates the
greatest pressure drop is across the third and fourth order
arterioles, with a lesser drop across first and second order
arterioles and very little pressure drop across the fifth and sixth
order arterioles. Though the fifth and sixth order arterioles have
small diameters, their great number and short length reduces
calculated resistance to a low level.
Comparing figure 5 and table 1, it is evident that the dilation to muscle contraction was proportionately greatest in those arterioles where the pressure drop was greatest. Conversely, in those arterioles where the pressure drop was least, there was proportionately less dilation with muscle contraction. The effect these diameter changes had on network resistance is shown in figure 7A. The control curve was obtained from the pressure profile (figure 6) by equating one mmHg pressure drop with one arbitrary resistance unit. Changes in resistance with muscle contraction were determined from the ratio of experimental to control diameters taken to the fourth power. In constructing these curves, capillary and venous resistance was assumed constant.

During 4 Hz muscle contraction, the resistance was redistributed radically with first and second order arterioles becoming more important than the distal vessels. This was also the case for 30 Hz muscle contraction and for 4 Hz muscle contraction during sympathetic stimulation. These changes in arteriolar network resistance predict an increase of 2.7 fold in muscle blood flow with 4 Hz muscle contraction and a 4.0 fold increase with 30 Hz muscle contraction. The predicted flow increase with 4 Hz muscle contraction during sympathetic stimulation was 1.8 fold. In the cat sartorius muscle, House and Johnson (1986b) reported an approximate flow increase of 2.0 fold in fourth order venules measured 10 seconds after 3 Hz muscle contraction lasting a duration of 1 minute, which is in good agreement with the predicted value for 4 Hz muscle.
Figure 6. Estimated pressure distribution across the vascular network of the sartorius muscle; first through sixth order arterioles (1A-6A), capillaries (CAP), small venules (SV), large venules (LV) and femoral vein. Points marked by closed circles were derived from pressure measurements (House and Johnson, 1986), open circles from morphometric data (Koller et al., 1987).
contraction. With 30 Hz muscle contraction, the arterioles approached full dilation, predicting the maximal flow increase is approximately 4.0 fold. This value is low in comparison to previous reports (Donald et al., 1970; Mackie and Terjung, 1983; Pendergast et al., 1985) and may result from the assumption that capillary and venous resistance did not decrease during muscle contraction.

We also estimated the changes in resistance with sympathetic nerve stimulation. Like previous reports (Marshall, 1982; Boegehold and Johnson, 1988a), we found there was an initial constriction to sympathetic stimulation throughout the arteriolar network. Secondary dilation in the fourth through sixth order arterioles was so great that after three minutes of sympathetic stimulation these diameters did not differ significantly from control. Third order arterioles dilated secondarily as well, but remained significantly constricted as did the larger first and second order arterioles, which showed little secondary dilation. Though constriction was well maintained in the first and second order arterioles, proportional constriction after three minutes of sympathetic stimulation was greatest in the third order arterioles. Figure 7B shows the effect of 3 minutes of sympathetic stimulation on estimated resistance. Similar to the findings with muscle contraction, increasing sympathetic vascular tone had the greatest effect on resistance in and near the third order arterioles. These changes in arteriolar network resistance predict a reduction of muscle blood flow to 20 percent of the control value, which is in approximate agreement with previous reports of
Figure 7. Estimated resistance distribution (arbitrary resistance units) across the arteriolar network, the effect of muscle contraction (MC) is shown in 7A and the effect of sympathetic stimulation (S) is shown in 7B. Refer to figure 6 for description of vessel order abbreviations.
whole muscle flow changes during sympathetic stimulation (Kjellmer, 1965; Folkow et al., 1971). However, in the cat sartorius muscle, Boegehold and Johnson (1988a) reported, after three minutes of 8 Hz sympathetic stimulation, a calculated reduction of blood flow to 60 percent of the control value in the first order arterioles.

Overall, we observed that consecutive sections of the arteriolar network behave differently in response to both vasodilator and vasoconstrictor stimuli. Our observations and analysis support the hypothesis that the regional differences in arteriolar responses may be significant in regulation of blood flow. We found the largest changes in resistance with the various protocols was in and near the third order arterioles. Interestingly, third order arterioles serve anatomically as the gateway from the large arcading arterioles to the smaller arterioles which supply the capillary network. The exquisite responsiveness of these vessels to muscle contraction and to sympathetic nervous control, along with the large contribution to resting resistance, suggests the third order arterioles play a dominant role in regulating blood flow to the approximately 400 capillaries each supplies. It is possible that optimum length-tension characteristics are present in and near the third order arterioles and that this accounts for the greater responsiveness in these vessels (Gore and Davis, 1984).

The analysis of arteriolar network responses to muscle contraction and to sympathetic nerve stimulation also provide information on the interaction between opposing vasoconstrictor and
vasodilator stimuli. The large reduction in blood flow during sympathetic stimulation results in a fall in tissue $P_{O_2}$ (Boegehold and Johnson, 1988b). If resting tissue $P_{O_2}$ is maintained during sympathetic stimulation by suffusing the tissue with an oxygenated solution, secondary dilation can be nearly abolished (Boegehold and Johnson, 1988a). This suggests secondary dilation may result from increased interstitial concentration of a dilator substance released with low $P_{O_2}$. Whatever the mechanism, the more proximal arterioles appear resistant to this dilator under resting conditions and sympathetic control predominates over metabolic control (Folkow, 1971; Lundvall and Jarhult, 1976a; Boegehold and Johnson, 1988a).

A number of reports indicate that sympathetic dominance over metabolic control is lost during muscle contraction (Remensnyder et al., 1962; Kjellmer, 1965; Folkow et al., 1971). In agreement with these findings, we observed that during sympathetic stimulation, arterioles throughout the arteriolar network dilated to muscle contraction. Due to secondary escape, the diameter of fourth through sixth order arterioles did not differ from control after three minutes of sympathetic stimulation. Sympathetic stimulation did not effect the dilation to muscle contraction in these vessels. Dilation of the third order arterioles was less than that observed in the absence of sympathetic stimulation, but was still appreciable. However, with the first and second order arterioles, dilation brought the diameters only to their resting control values. From this, it appears that sympathetic control is least attenuated in the larger,
first and second order arterioles. In the third order arterioles, however, the dominance reverses, and metabolic control prevails over sympathetic control.

Gorcinski and Duling (1978) found that raising tissue $P_{O_2}$ over a contracting muscle inhibited dilation of small arterioles by 40-50 percent, while Boegehold and Johnson (1988a) reported that raising tissue $P_{O_2}$ during sympathetic stimulation inhibited secondary dilation of small arterioles by 60-73 percent. Even more striking was a report by Lindbom (1986), who found that elevating tissue $P_{O_2}$ inhibited hyperemia with muscle contraction by only 27 percent, but inhibited reactive hyperemia by 80 percent. In the present study, secondary escape from sympathetic stimulation was greater in the distal arterioles, but no differential effect was observed with muscle contraction at 4 Hz or at 30 Hz. Moreover, muscle contraction dilated the more proximal arterioles which had remained constricted during sympathetic stimulation. These observations are consistent with the hypothesis that exercise produces a dilator stimulus not present in the low flow state produced during sympathetic stimulation.
CHAPTER 4

ANTAGONISM OF ADRENERGIC VASOCONSTRICTION BY MUSCLE CONTRACTION: EVIDENCE FOR SELECTIVE INHIBITION OF ALPHA-1 MEDIATED VASCULAR TONE

Introduction

Arteriolar dilation during muscle contraction is believed to be due to the release of vasodilator substances, from the contracting muscle fibers, which act directly upon the smooth muscle of the arteriole (for reviews see Shepherd, 1983; Renkin, 1984; Hudlicka and Khelly, 1985). In addition, it is proposed that the dilator substances inhibit norepinephrine release from sympathetic nerves (Burcher and Garlick, 1973; Lorenz and Vanhoutte, 1975). Burcher and Garlick (1973) reported in the isolated dog gracilis muscle, that dilation with muscle contraction is significantly greater during sympathetic stimulation than during infusion of norepinephrine. Also, isolated venous and arterial segments show significantly greater relaxation to purported exercise dilator substances during transmural electrical stimulation of the sympathetic nerves than when norepinephrine is added to the bath (Lorenz and Vanhoutte, 1975).

While these findings are consistent with the hypothesis that release of norepinephrine is prejunctionally inhibited, Burcher and Garlick acknowledged an alternative hypothesis, namely the norepinephrine released from sympathetic nerve terminals may be delivered to different alpha adrenergic receptors than the
norepinephrine introduced intravenously. Evidence supporting this suggestion has been subsequently reported, indicating the presence of at least two different subtypes of alpha receptors, having differential innervation. The alpha-1 receptors are predominantly located in the junctional region of sympathetic nerve terminals and where present, the alpha-2 receptors are predominantly extrajunctional (Yamaguchi and Kopin, 1980; Dahlof, 1981; Langer, 1981; Wilffert et al., 1982; Matthews et al. 1984; Van Zwieten, 1986). Moreover, studies indicate that in low doses, an exogenous source of norepinephrine binds preferentially to extrajunctional alpha-2 receptors, which is in contrast to the selective alpha-1 adrenergic stimulation associated with sympathetic stimulation (Yamaguchi and Kopin, 1980).

If the difference in functional dilation observed during sympathetic stimulation and infusion of norepinephrine is due to differences in the alpha subtype stimulated, this would suggest that the vasodilator substances released during muscle contraction antagonize alpha-1 mediated vasoconstriction more effectively than alpha-2 mediated vasoconstriction. If so, vasoconstriction induced by a selective alpha-1 agonist, administered exogenously, should be attenuated during muscle contraction to the same degree as sympathetic stimulation, in contrast to an exogenous source of norepinephrine.

To test both hypotheses, experiments were performed in a microcirculatory preparation where it was possible to directly
observe arteriolar dilation during muscle contraction with different levels of initial vasoconstriction. Initial vascular tone was augmented by infusion of an alpha-1 selective adrenergic agonist, infusion of norepinephrine, or by electrical stimulation of the sympathetic chain.

Methods

Animal model and surgical procedure

The cat sartorius muscle, which is thin and easily transilluminated was chosen for direct observation of arteriolar diameter changes during muscle contraction and with the differing vasoconstrictor stimuli.

Cats of either sex (n=22) weighing between 0.9 - 1.2 kg were sedated by intramuscular injection of ketamine hydrochloride (Ketaset, Bristol Laboratories), 15 mg/kg of body weight, followed by cannulation of the jugular vein for administration of alpha-chloralose at 38 mg/kg. Supplemental doses of alpha-chloralose, 30 percent of the original dose, were given as needed to maintain surgical anesthesia. Arterial pressure recordings were obtained via the carotid artery using a Statham pressure transducer.

The left sympathetic chain was isolated as described by Boegehold and Johnson (1988a). A bipolar silver wire electrode made of coated, 0.005 inch diameter wire (A-M Systems) glued inside a cuff of silastic tubing, 2.5 mm in diameter, was wrapped around the chain between L4-L5. The ends of the cuff were sealed with petroleum jelly
to electrically isolate the bared portion of wire and the incision was promptly sutured to minimize surgical trauma. Another incision exposed the portion of the femoral nerve innervating the sartorius muscle, the most lateral of three branches, for placement of a bipolar electrode identical to the one used on the sympathetic chain.

The left sartorius muscle was exteriorized as described by House and Johnson (1986) leaving vascular and neural supplies intact. During surgery the muscle was held at in situ length and kept moist with Isolyte (American McGaw) warmed to 37°C. After surgery, the animal was placed on a microscope stage with the muscle fastened at in situ length, dorsal side up over a Plexiglas pedestal through which warm water was circulated to maintain a 35°C surface temperature. A saline rinsed sheet of polyvinyl film (Saran Wrap, Dow Corning) was placed over the muscle and sealed along the edges with petroleum jelly to protect it from exposure to the atmosphere and to prevent drying.

In vivo observations

After a one hour equilibration period the sartorius muscle was transilluminated using a Leitz 100 watt mercury-arc light source with a Leitz Ortholux II microscope for direct observation of the microcirculation in the thin central region of the sartorius muscle. A long working distance Leitz um 32 objective (numerical aperture = 0.2) was used with final video magnification of X1200. The microscope image was recorded using a silicon intensified target
(SIT) video camera and a Sony super beta cassette recorder. Tape replay allowed stop-field analysis of internal-wall vessel diameters using calibrated video calipers with time to 1/100 of a second recorded on the video image for reference to the experimental procedure. The criteria for selecting arterioles for investigation included absence of hemorrhage or venous stasis in the region studied.

Vessel branching orders were assigned by a method similar to that described by Wiedeman (1962). Using this method there are usually six branching orders in the sartorius, with the largest, first order, arterioles arising from the femoral and lateral circumflex arteries, and the smallest sixth order arterioles supplying the capillaries. The majority of the vessels examined in this study were second order arterioles; the remainder were classified as first order. It was determined in control experiments that these vessels maintain stable constriction to sympathetic nerve stimulation for a period of at least five minutes.

Experimental Protocol

The response to two minutes of muscle contraction was observed by stimulating the femoral nerve at an intensity of 2-4 Volts, for a duration of 0.1 msec and a frequency of 4 Hz. A recovery period of at least fifteen minutes between procedures allowed diameter and flow to return to control values. Then the response to five minutes of sympathetic stimulation was observed by stimulating the chain at an
intensity of 4-10 volts, for a duration of 5 msec and a frequency of 2, 4 or 8 Hz. After three minutes of sympathetic stimulation, the combined response to sympathetic stimulation and muscle contraction was observed for two minutes. Vasoconstrictor substances were injected in a bolus through the jugular vein catheter, producing constriction which was fairly well maintained over a four to five minute period. The dose given was determined experimentally to match constriction to 2, 4 or 8 Hz sympathetic stimulation, after allowing two minutes before measuring the constricted diameter. Between the second and fourth minute after administration of the vasoconstrictor, the response to muscle contraction was obtained. Vasoconstrictor agents used were phenylephrine (Sigma) and methoxamine (Burroughs-Wellcome, both selective alpha-1 agonists (Ruffulo, 1984; Langer et al., 1985), norepinephrine (Lemphephed, Winthrop-Breon) and vasopressin (Pitressin, Parke-Davis). In two experiments propranolol (Sigma) was administered intravenously (1 mg/kg) after other procedures were completed. Then the responses to sympathetic stimulation and to the infusion of the vasoconstrictor substances were observed. Thirty minutes were allowed for recovery between injection of each vasoconstrictor agent to permit diameter and flow to return to control values.

Diameter responses to the different procedures were measured as follows: control diameter during the two minutes prior to each procedure, vasoconstriction with sympathetic stimulation during the third minute and just prior to the onset of muscle contraction.
Vasoconstriction with injected substances was measured two minutes after the bolus, and diameter changes with muscle contraction were measured during the last few seconds of the two minute contraction.

Presence and Innervation of alpha adrenoceptor subtypes

To determine the contribution of alpha-1 adrenoceptors to sympathetic vasoconstriction in the first and second order arterioles, prazosin (Pfizer), a selective alpha-1 antagonist (Cambridge et al., 1977; Timmermans et al., 1980; Massingham et al., 1981) was administered intravenously in 0.9 percent saline solution (275 micrograms/kg body weight) or suffused over the muscle and under the Saran Wrap (25 micrograms/cc) in either Krebs-Henseleit solution (composition mM: 118.4 NaCl, 25 NaHCO$_3$, 4.8 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$) or a solution used by Tesfamariam and Halpern (1987) for in vitro studies in small arteries (composition mM: 119 NaCl, 24 NaHCO$_3$, 4.7 KCl, 1.6 CaCl$_2$, 1.17 MgSO$_4$, 1.18 KH$_2$PO$_4$, 5.5 Dextrose). Prior to use both solutions were warmed to 35 C and bubbled with 5% CO$_2$ - 95% N$_2$ for a final pH of 7.4. The effectiveness of prazosin to block alpha-1 adrenoceptors was tested by intravenous administration of phenylephrine given in a dose determined earlier in each experiment to produce the same final diameter as 8 Hz sympathetic stimulation. Prazosin produced complete block to this dose of phenylephrine, after which the response to 3 minutes of 8 Hz sympathetic stimulation was obtained for comparison.

A second protocol was followed to verify the presence of alpha-2
adrenoceptors. Following prazosin treatment, the intravenous dose of
norepinephrine determined earlier in each experiment to match
vasoconstriction to 8 Hz sympathetic stimulation was given. Under
this condition the constriction should be mediated by alpha-2
receptors. This was confirmed by administration of the same dose of
norepinephrine after yohimbine (Sigma), a selective alpha-2
antagonist (Starke et al., 1975a; Timmermans et al., 1980; Shepperson
et al., 1981), which produced nearly complete inhibition of the
constriction to norepinephrine. When the prazosin was given
intravenously yohimbine (3 mg/kg body weight) was also; alternatively
prazosin and yohimbine (1 mg/cc) were suffused over the muscle.

Since constriction to sympathetic stimulation was not completely
abolished by prazosin, a final protocol was followed to determine
whether this constriction was due to junctional alpha-2
adrenoceptors. For this study the response to 8 Hz sympathetic
stimulation was observed after prazosin and yohimbine. When the
alpha antagonists were given intravenously, hypotension resulted. In
two experiments this was restored partially by infusion of whole
blood from a donor cat (Hirst and Lew, 1987).

Statistical Analysis

Paired Student t-tests (two-tail, unless otherwise noted) were
used to compare responses of each arteriole to sympathetic
stimulation during muscle contraction or to the infused
vasoconstrictor during muscle contraction. Paired t-test was also
used to compare the response to sympathetic stimulation before and after administration of the alpha adrenergic antagonists. Tables and figures show means ± standard error.

**Results**

In most animals, data collection from a single arteriole required about 5 hours (range 2 - 10 hours). The duration of the study on any animal was usually determined by the condition of the muscle, and by stability of blood pressure, which averaged 110±15 mmHg (SD). During the experiment, the resting diameter tended to vary, both increasing and decreasing somewhat, though by the end of the experiment diameter was often within 2 to 3 microns of its initial value (12 out of 19 experiments). Diameter averaged 50 microns and over the course of the experiment remained within ±5 microns of this value. When responses of a vessel to muscle contraction were compared between different vasoconstrictor treatments, only those in which control and constricted diameters agreed to within 2 to 3 microns were used.

A number of control experiments were performed to determine the validity of comparing the diameter responses of a vessel between different protocols. One issue was whether the constriction with sympathetic stimulation and with the intravenously administered vasoconstrictor agents was sufficiently stable and long-lasting to assess accurately the dilation to muscle contraction. Figure 8a-c
shows there was little dilation during 5 minutes of sympathetic stimulation, and a small dilation between the second and fourth minutes after administration of either norepinephrine or phenylephrine. The secondary dilation after norepinephrine was greater than that with phenylephrine presumably due to a shorter half-life. Consequently, with norepinephrine and phenylephrine some of the dilation observed during muscle contraction, measured four minutes after injection, was due to the diminished effectiveness of the vasoconstrictor. Though not shown, constriction with injected vasopressin was well maintained over a 5 to 6 minute period, but with injected methoxamine the constriction diminished at a rate similar to that with norepinephrine or perhaps somewhat more quickly.

When muscle contraction was repeated on a single vessel, diameters attained were generally consistent to within 2 to 3 microns. This was also the case with repeated sympathetic stimulation and with administration of norepinephrine, phenylephrine and vasopressin. However, care was necessary to avoid exposing the arterioles to concentrations of norepinephrine or phenylephrine beyond those needed to produce vasoconstriction matched to 8 Hz sympathetic stimulation. Higher doses of these agents diminished arteriolar responsiveness to the vasoconstrictor as has been previously reported (Atkinson et al., 1987). Even with sympathetic stimulation, reduced vasoconstriction was observed at frequencies greater than 8 Hz in repeated trials.

In 9 experiments, norepinephrine, phenylephrine and vasopressin
were given in doses which produced the same constriction as 8 Hz sympathetic stimulation, and following that we stimulated the sympathetic chain again at 8 Hz. The sympathetic response was not reduced. The mean diameters for both controls and 3 minutes constricted diameters differed by less than 2 microns, which was not significant. Infused methoxamine, however, tended to lessen subsequent vasoconstrictor responses to methoxamine and to sympathetic stimulation. Thus, methoxamine administration was considered a terminal protocol.

Changes in blood pressure with vasoconstriction

The initial response to sympathetic stimulation was an increase of mean blood pressure by 22±4 mmHg (SEM). With systemic injection of phenylephrine and norepinephrine blood pressure increased by 50±4 and 51±4 mmHg (SEM), respectively. The increase with methoxamine was similar to that with phenylephrine and norepinephrine, but was less with vasopressin, 34±7 mmHg (SEM). In all cases, these peak changes in blood pressure decreased by as much as 50 percent during the experimental procedure.

Dilation to muscle contraction with resting tone and with sympathetic stimulation

Figure 9 shows the response of 10 arterioles to two minutes of muscle contraction before and during sympathetic stimulation at 2, 4 and 8 Hz. Increasing sympathetic tone reduced initial diameter but did not inhibit the dilation with muscle contraction, which remained
as great in absolute terms in the sympathetically constricted vessels as it was in those with normal tone. However, the absolute diameter attained during muscle contraction was reduced because the initial diameter was less.

Dilation to muscle contraction after norepinephrine or phenylephrine injection

Figure 10a shows the response to two minutes of muscle contraction, comparing dilation of an arteriole during sympathetic stimulation to that during infusion of norepinephrine. The data are shown in separate histograms for each level of sympathetic stimulation as it was usually not possible to perform all six protocols in each arteriole studied. Dilation to muscle contraction was significantly less during norepinephrine than during 4 or 8 Hz sympathetic stimulation. Figure 10b compares dilation to two minutes of muscle contraction during sympathetic stimulation to that during infusion of phenylephrine. In contrast to the results with norepinephrine, dilation to muscle contraction in the presence of phenylephrine did not differ from that during sympathetic stimulation. Dilation with muscle contraction after norepinephrine and phenylephrine injection shown in figure 10 was not corrected for the dilation that would have occurred without muscle contraction (see figure 8). Thus, the 30 - 40 percent reduction of functional dilation with norepinephrine is a conservative estimate. Though it appears functional dilation with phenylephrine is slightly greater than with sympathetic stimulation, this difference is lost if the
diameter is corrected for the dilation shown in figure 8. In a few experiments methoxamine was infused in a dose of 200 - 300 micrograms/kg, producing constriction similar to 8 Hz sympathetic stimulation. Like phenylephrine, dilation to muscle contraction was as great or greater than that observed during a matched sympathetic stimulus.

Dilation to muscle contraction after vasopressin injection

Figure 11 compares the dilation to muscle contraction during 8 Hz sympathetic stimulation to that with a matched initial constriction to vasopressin. As with norepinephrine, functional dilation with vasopressin was significantly less than that during sympathetic stimulation.

Effect of alpha adrenergic blocking agents on vasoconstrictor responses

Prazosin completely blocked constriction to phenylephrine when the latter was administered in a dose which before blockade had produced a response equivalent to 8 Hz sympathetic stimulation. However, figure 12 shows that prazosin blocked less than 50 percent of the constriction to 8 Hz sympathetic stimulation. Administration of prazosin caused all arterioles to dilate initially, most of them by a magnitude as great as that observed with 4 Hz muscle contraction. Subsequently, 9 out of 15 arterioles returned to the control value. The data in figure 12 includes only those 9 vessels. There was no difference in arteriolar responses when prazosin was
suffused over the muscle or infused systemically, so these data were pooled. Neither technique however, was without other effects. Suffusion of the Halpern solution or of Krebs-Henseleit between the Saran Wrap and the muscle sometimes reduced vessel tone. The majority of the experiments were done with systemic infusion, but this reduced systemic blood pressure to about 80 mmHg.

The constriction to norepinephrine, injected in a dose matching the response to 8 Hz sympathetic stimulation, before and after prazosin is shown in figure 13. In these studies, some of the arterioles remained dilated after the prazosin and are included in the analysis. The diameter attained with norepinephrine treatment was not changed after prazosin, indicating the presence and strong effect of alpha-2 adrenoceptors in these vessels.

To test whether the constriction to sympathetic stimulation after prazosin block was due to alpha-2 receptors, constriction to 8 Hz sympathetic stimulation was compared after prazosin and then after prazosin plus yohimbine (figure 14). Again, the data includes arterioles which did not return to control diameter after prazosin. Yohimbine given with prazosin blocked constriction to a dose of norepinephrine matched to 8 Hz sympathetic stimulation in four out of six vessels. In the other two vessels, constriction was about 25 percent of that observed prior to administration of the adrenergic blocking agents. The vascular diameter during sympathetic stimulation was the same with yohimbine plus prazosin as with prazosin alone. This finding suggests that alpha-2 receptors do not
contribute significantly to sympathetic vasoconstriction. After systemic infusion of prazosin and yohimbine, blood pressure was further reduced from 80 mmHg to about 50 mmHg. In two experiments up to 40 cc of blood infused from a donor cat restored blood pressure to around 80 mmHg.

Dilation to muscle contraction during sympathetic stimulation before and after prazosin treatment

Dilation to muscle contraction during 8 Hz sympathetic stimulation was studied after prazosin treatment to determine whether blocking alpha-1 receptors changed the response to contraction. As in other protocols, the diameters just prior to contraction were matched. Since vasoconstriction to 8 Hz sympathetic stimulation was reduced after prazosin, it no longer matched the control response to 8Hz sympathetic stimulation. Also, after prazosin treatment constrictor response to sympathetic stimulation was quite variable. To match initial diameters, we had to use control responses to sympathetic stimulation ranging from 0-4 Hz. When initial diameters were matched in this way, prazosin treatment did not affect dilation to muscle contraction during sympathetic stimulation.
Figure 8. Vasoconstriction to systemic injection of norepinephrine, 25±4 (SEM) μg/kg body weight (shown in 8B), or phenylephrine, 150±30 (SEM) μg/kg (8C), compared to sympathetic nerve stimulation (8A). Error bars indicate SEM, shaded regions show the period of time when the response to muscle contraction is usually observed.
Figure 9. Effect of 2, 4 and 8 Hz sympathetic stimulation (S) on functional dilation to 4 Hz muscle contraction (MC). The solid line indicates diameter during the third minute of S, the dashed line indicates dilation in the same arterioles during the second minute of MC, with continued S. Error bars indicate SEM.
Figure 10. Comparison of functional dilation after norepinephrine (NE, shown in column A) or phenylephrine (PE, shown in column B) injection with that during sympathetic stimulation (S). The open portion of each bar indicates constriction during 3 minutes of S or two minutes after injection of NE or PE, mean dose μg/kg body weight. The solid portion of each bar indicates the dilation to two minutes muscle contraction in the presence of each vasoconstrictor stimulus. Error bars show SEM, p values indicate significance using a 2-tail, paired t-test.
Figure 11. Comparison of functional dilation after vasopressin (VP) injection (mean dose 0.2 units/kg body weight) with that during sympathetic stimulation (S). The open portion of each bar indicates constriction during 3 minutes of S or 2 minutes of VP. The solid portion of each bar indicates the dilation to 2 minutes of muscle contraction in the presence of each vasoconstrictor stimulus. Error bars show SEM, p indicates significance using a 2-tail, paired t-test.
Figure 12. Vasoconstriction to 8 Hz sympathetic stimulation (S) after prazosin administration. The solid line shows constriction with 3 minutes S, the dashed line the constriction in these same vessels after prazosin blockade of alpha-1 adrenoceptors. Error bars indicate SEM, the p value shows significance using a paired, one-tail, t-test.
Figure 13. Vasoconstriction 2 minutes after norepinephrine (NE) injection (in a dose matching the response to 8 Hz sympathetic stimulation) before (open circles) and after (closed circles) prazosin administration (with blockade of alpha-1 adrenoceptors). Error bars indicate SEM.
Figure 14. Vasoconstriction to 8 Hz sympathetic stimulation (S) after prazosin and yohimbine administration, comparing the response to 3 minutes S control (solid line), and after prazosin (dashed line) or after prazosin plus yohimbine (dotted line), given in doses that blocked alpha-1 and alpha-2 receptors. Error bars show SEM, p values indicate significance using a one-tail, paired t-test.


Discussion

Pressure changes in whole organ preparations of skeletal muscle indicate that functional hyperemia is greater during sympathetic stimulation than after injection of norepinephrine (Burcher and Garlick, 1973; Beaty and Donald, 1977). We have demonstrated, in our microcirculatory preparation of skeletal muscle, that differences in arteriolar responses can account for the whole organ findings. Muscle contraction during sympathetic stimulation resulted in significantly greater vasodilation than did muscle contraction after intravenous administration of norepinephrine.

To test the hypothesis that the reduced functional dilation after injection of norepinephrine might be due to the agonism of alpha-2 adrenergic receptors, we compared the dilation to muscle contraction after intravenous administration of the selective alpha-1 agonist, phenylephrine. In contrast to the findings with norepinephrine, dilation to muscle contraction after injection of phenylephrine was not different from that observed during sympathetic stimulation. Substantiating this finding, functional dilation with another alpha-1 selective agonist, methoxamine, also did not differ from that with sympathetic stimulation.

We performed experiments with prazosin and yohimbine, selective antagonists of alpha-1 and alpha-2 adrenoceptors, to confirm that intravenous administration of norepinephrine stimulates alpha-2 receptors while intravenous administration of phenylephrine selectively stimulates alpha-1 receptors. Vasoconstriction to
phenylephrine was blocked after prazosin treatment, indicating phenylephrine selectively agonized alpha-1 adrenoceptors. In contrast, constriction to injected norepinephrine was not affected by prazosin, indicating vasoconstriction to norepinephrine may be mediated predominantly by alpha-2 adrenoceptors, which is consistent with previous findings for low doses of exogenous norepinephrine (Yamaguchi and Kopin, 1980). These results indicate that the dilator mechanism elicited by muscle contraction antagonizes alpha-1 induced vasoconstriction much more than alpha-2 induced vasoconstriction.

Further evidence that the vasoconstriction induced by one adrenergic subtype can be selectively antagonized by a vasodilator substance is provided by Faber and coworkers. McGillivray and Faber (1987) reported that reduced pH selectively inhibited vasoconstriction mediated by alpha-2 but not by alpha-1 adrenoceptors in a microcirculatory preparation of the rat cremaster. In contrast, Faber et al. (1988) reported atrial naturetic factor selectively inhibited alpha-1 mediated vasoconstriction, but not that of the alpha-2 receptor.

A number of investigators suggest that alpha-1 receptors are located in the junctional region of sympathetic nerve terminals and where present, the alpha-2 receptors are extrajunctional (Yamaguchi and Kopin, 1980; Wilffert et al., 1982; Matthews et al., 1984; and Langer et al., 1985). Such experiments have compared the systemic blood pressure response to sympathetic stimulation after selective adrenergic blockade. To determine whether this differential
distribution of adrenergic receptor subtypes was present in the second order arterioles of the sartorius, we compared the arteriolar response to sympathetic stimulation after treatment with prazosin and after prazosin plus yohimbine. Though prazosin blocked constriction to phenylephrine, no more than fifty percent of the constriction to 8 Hz sympathetic stimulation was inhibited. Addition of yohimbine did not appear to further inhibit vasoconstriction to sympathetic nerve stimulation, suggesting alpha-2 adrenoceptors are not innervated. However, using this approach to determine the innervation of alpha-2 adrenoceptors can be criticized. Since yohimbine also blocks prejunctional alpha-2 adrenoceptors, norepinephrine release could increase during 8 Hz sympathetic stimulation. If so, this might cause more constriction with sympathetic stimulation than would be observed if prejunctional receptors were not blocked. Whether this is the case is difficult to assess without a blocking agent selective to either pre- or postjunctional alpha-2 receptors. Furthermore, Hamed et al. (1986) dispute the view that alpha-2 receptors are extrajunctional based on experiments using a dog hindlimb model in which one limb was sympathetically denervated and the contralateral was left intact. After injection of prazosin or yohimbine there was no change in resistance in the denervated hindleg. In contrast, both agents decreased resistance in the innervated hindlimb, suggesting that both receptor subtypes are innervated (see also Gardiner and Peters, 1982; Elsner et al., 1984). Kahan (1987a) reported, in the same animal model, that some of his experiments supported innervation
of alpha-2 receptors, while others did not. Therefore, the question of junctional versus extrajunctional location of alpha-2 adrenoceptors is yet to be resolved.

The issue of whether alpha-2 receptors are innervated has significant bearing on the interpretation of the present study. Previously, the finding that dilation during muscle contraction was greater during sympathetic stimulation than during infusion of norepinephrine was interpreted as evidence that norepinephrine release from sympathetic nerves is inhibited during muscle contraction (Burcher and Garlick, 1973). This interpretation is based on the assumption that infused norepinephrine acts on the same receptors as that released by sympathetic nerves. This is certainly not the case if alpha-1 receptors are preferentially innervated. Thus, if we consider the hypothesis that alpha-2 receptors are extrajunctional, then it is not necessary to attribute any of the dilation with muscle contraction to inhibition of norepinephrine release from sympathetic nerves, as we found the dilation to muscle contraction after phenylephrine injection comparable to that during sympathetic stimulation.

In vitro measurements on isolated small arteries and veins indicate that several of the purported dilator substances (potassium, adenine nucleotides, hyperosmolarity, hydrogen and potassium) reduce tritiated norepinephrine release from transmurally stimulated sympathetic terminals by as much as thirty percent and associated with this is vessel relaxation of similar magnitude (Lorenz and
Vanhoutte, 1975; Shepherd and Vanhoutte, 1981). Assuming that alpha-2 receptors are not innervated, we can reconcile the difference between the in vitro studies and of the present study by supposing the vasodilator released from muscle during contraction is not one of those tested in vitro.

Alternatively, if alpha-2 receptors are innervated, then the similarity in dilation to muscle contraction with sympathetic stimulation and phenylephrine injection is coincidental and norepinephrine release probably is inhibited. Further investigation is needed to resolve this issue. For example, the efflux of norepinephrine from working muscle could be measured. In one study (Beaty and Donald, 1977), norepinephrine content in venous effluent did not differ at minutes three or twenty of muscle contraction with constant flow perfusion of the dog gracilis. However, more frequent measurements would be desirable, and currently a very sensitive method of measuring norepinephrine using high-performance liquid chromatography is available (Kahan, 1987).

The sensitivity of alpha-2 adrenoceptors to circulating norepinephrine (figure 13), suggests a mechanism for modulation of the hyperemic response to exercise. Moreover, it may be relevant that after injection of vasopressin, which presumably also exerts its effects humorally (Bennett and Gardener, 1986; Liard, 1986), that dilation to muscle contraction was inhibited as much as with injected norepinephrine. It seems possible that under extreme conditions, circulating levels of norepinephrine or vasopressin might
be sufficient to reduce exercise hyperemia through stimulation of alpha-2 adrenergic or vasopressin V₁ receptors.

The mechanism by which the vasodilator substance released with muscle contraction might selectively inhibit alpha-1 mediated vasoconstriction is yet to be explored. In many tissues alpha-1 adrenoceptors induce signal transduction via phosphatidylinositol (PI), whereas alpha-2 adrenergic contraction stimulates adenylate cyclase (Fain and García-Sainz, 1980). This may also be true for vascular smooth muscle (Bulbring and Tomita, 1987). If so, alpha-1 and alpha-2 vasoconstriction are mediated by different pathways, which possibly converge at the sarcoplasmic reticulum. The difference in functional dilation between norepinephrine and phenylephrine observed in this study suggests the dilator mechanism interferes with a step in the alpha-1 mediated pathway prior to convergence with the alpha-2 adrenoceptor pathway. This could be at the level of the receptor or by inhibition of intracellular signal transduction. The difference in functional dilation between phenylephrine and vasopressin suggest inhibition of the alpha-1 receptor, as the vasopressin V₁ receptor also stimulates PI as a second messenger. However, in the liver there appear to be differences in V₁ versus alpha-1 signal transduction independent of PI (García-Sainz, 1987). Intracellular signal transduction could be inhibited if the dilator substance activates protein kinase C which may phosphorylate the alpha-1 receptor, blocking its biological effects (Danthuluri and Deth, 1984).
Sympathetic vasoconstriction has been discussed up until this point only in relation to the alpha adrenoceptor. Yet, we observed alpha adrenergic blockade reduced constriction to sympathetic stimulation by only about fifty percent. One explanation is the postulated gamma adrenergic receptor (Hirst and Neild, 1980, 1981; Suzuki and Kuriyama, 1980; Kuriyama and Makita, 1983; Cheung, 1982). Evidence for the gamma adrenergic receptor comes from constriction to norepinephrine after administration of alpha blocking agents or from observation of excitatory junctional potentials resistant to alpha blockade. However, in the present study, little or no vasoconstriction to norepinephrine remained after administration of prazosin and yohimbine (see Itoh et al., 1983). Hirst and Neild reported similar findings with exogenous norepinephrine, but suggested that the gamma receptor requires a high junctional norepinephrine concentration to be stimulated. Alternatively, Bevan and Su (1971, 1973) and Bevan and Purdy (1973) suggested that prazosin may not fully inhibit sympathetic vasoconstriction to norepinephrine due to the accumulation of norepinephrine in the junction, overcoming the prazosin block. Another explanation for the residual constriction with alpha blockade is the co-release with norepinephrine of another vasoconstrictor. The two best candidates for co-release are ATP (for review see Burnstock, 1986; see also Kugelgen and Starke, 1985; Muramatsu, 1986; Burnstock and Warland, 1987) and neuropeptide Y (Lundberg et al., 1982, 1985; Hanko et al., 1986; Pernow et al., 1988). Regardless of the mechanism for the
remaining sympathetic constriction, our data show that this is as susceptible to antagonism by muscle contraction as that mediated by alpha-1 receptors.

A consideration of other factors which might influence the interpretation of this study will now be presented. The experiments were designed to interrupt the normal function of the cardiovascular system and its control as little as possible. For this reason the muscles were innervated and most experiments were performed without beta adrenergic blockade or adrenalectomy. Two pilot studies performed with beta adrenergic blockade showed a fall in blood pressure to 70 mmHg and a shift to more constricted control and experimental diameters. This however, did not change the pattern of responses; dilation to muscle contraction comparable with sympathetic stimulation and phenylephrine but less with norepinephrine.

Sympathetic denervation in anesthetized animals indicates sympathetic tone is a major component of resting vascular tone in skeletal muscle (Renkin and Rosell, 1962b, Honig et al., 1970; Irion et al., 1980), with lesser contributions from other vasoconstrictor mechanisms. It has been generally assumed that the dilator influence of muscle contraction attenuates sympathetic tone (Folkow et al., 1971; Remensnyder et al. 1962; Kjellmer, 1965; Burcher and Garlick, 1973; Lorenz and Vanhoutte, 1975). However, we observed (figure 9) that the change in arteriolar diameter with 4 Hz muscle contraction was the same with or without sympathetic stimulation. Thus, the additional constriction due to sympathetic stimulation was not lost
during muscle contraction. In our companion study, in which we examined arterioles throughout the network of the sartorius, most arterioles did show a somewhat larger dilation during sympathetic stimulation than when vascular tone was normal. Muscle contraction clearly attenuates vascular tone, but in the second order arterioles in this study, it is not clear that alpha-1 mediated tone is attenuated. Though the large proportional dilation we observed suggests that it is, the possibility that myogenic or other vasoconstrictor mechanisms are antagonized preferentially is not ruled out. When alpha-2 adrenoceptors and vasopressin V1 receptors contribute to constriction (figures 10a and 11), our results show that during muscle contraction the added vasoconstriction was not lost. Moreover, the dilator substance(s) were less able to attenuate the vascular tone that was initially present.

In summary, the current finding indicate that the dilator substance(s) released during muscle contraction may cause selective inhibition of alpha-1 mediated vasoconstriction, rather than inhibiting the transmission of excitatory input to the vascular smooth muscle. This finding is in concert with recent observations that constrictor stimuli may elicit vascular smooth muscle contraction via separate pathways. These new findings shed light on how the vasoconstriction mediated by different agonists results in finely modulated regulation of muscle blood flow.
CHAPTER 5
CONCLUSION

The advantage of the microcirculatory preparation is that it permits direct observation of vascular responses in individual vessels. However, to relate changes observed in individual vessels to circulation in the organ, it is necessary to have detailed information about the network which the vessels comprise. Several previous studies in this laboratory have identified the branching levels in the arteriolar network of the cat sartorius muscle and provided morphometric and pressure data, analysis of which made it possible to estimate the change in resistance within the arteriolar network during muscle contraction and sympathetic nerve stimulation in the first study of this dissertation. Our finding is that the third order arteriole, which marks the gateway from the large arcading arterioles to the smaller arterioles which supply the capillary network is the most responsive of the arteriolar orders to both sympathetic vasoconstriction and to functional vasodilation. The third order arterioles also make the largest single contribution to the resting resistance to blood flow in the sartorius muscle. Thus, in response to both sympathetic stimulation and muscle contraction, the third order arteriole is the site where the largest change in resistance to flow takes place. These findings suggest that these vessels play an important role in regulating blood flow to
the capillary network that each supplies.

During sympathetic stimulation, fourth, fifth and sixth order arterioles secondarily escaped. However, first, second and third order arterioles remained significantly constricted. Muscle contraction during sympathetic stimulation resulted in dilation of all arterioles, and the attenuation of sympathetic control in the more proximal first, second and third order arterioles. Since the proximal vessels remained constricted during sympathetic stimulation but dilated to muscle contraction during sympathetic stimulation, this suggests the vasodilator substance(s) released in exercise may differ from those which cause escape from sympathetic nerve stimulation.

To examine the effector site which results in attenuation of sympathetic control during muscle contraction, we compared functional dilation of second order arterioles exposed to exogenous adrenergic agonists. Compared to sympathetic stimulation, functional dilation was reduced after a bolus injection of norepinephrine, which binds to both alpha-1 and alpha-2 adrenoceptors. In contrast, after injection of phenylephrine, a selective alpha-1 adrenergic agonist, there was no difference in functional dilation when compared with sympathetic stimulation. Administration of the alpha-1 blocking agent, prazosin, reduced vasoconstriction to 8 Hz sympathetic nerve stimulation, indicating alpha-1 receptors are stimulated by sympathetic nerve activity. However, administration of prazosin plus yohimbine, an alpha-2 blocking agent, did not further decrease
vasoconstriction to sympathetic nerve stimulation. This suggests a predominantly extrajunctional location of alpha-2 receptors, which were shown to be present by the constriction to norepinephrine after administration of prazosin. If this analysis of alpha receptor innervation is correct, this indicates the dilator stimulus produced by exercise acts primarily at the level of the vascular smooth muscle, to inhibit contraction. Alternatively, if alpha-2 receptors prove to be innervated, the present findings support a dual effector site hypothesis, with inhibition of the vascular smooth muscle and prejunctional inhibition of sympathetic release of norepinephrine.

Though prazosin blocked only 50 percent of the constriction to sympathetic stimulation, dilation to muscle contraction under this condition did not differ from that prior to prazosin administration. Thus, the prazosin resistant constriction was no less susceptible to the dilator substance than were the alpha-1 receptors.

The finding that functional vasodilation was less after norepinephrine injection than after injection of phenylephrine indicates that the vasodilator substance(s) released during exercise inhibit alpha-1 mediated contraction of vascular smooth muscle more effectively than that mediated by alpha-2 receptors. The potential for selective attenuation of vascular smooth muscle tone, which has also been reported by Faber and co-workers, opens a new area in the understanding of the regulation of arteriolar diameter. It may be significant in providing a mechanism for modulating the hyperemic response to exercise.
APPENDIX A

CALCULATION OF ARTERIOLAR NETWORK RESISTANCE AND THE CHANGE WITH MUSCLE CONTRACTION AND SYMPATHETIC STIMULATION

The pressure gradient in the arteriolar network

Our calculation of the initial or resting pressure drop across the vascular network is approximate. With arterial and venous pressures normalized to 100 mmHg and 4.9 mmHg respectively, House and Johnson (1986) found the pressure in the mid-region of second order arterioles was 76 mmHg. The 24 mmHg reduction in pressure from the femoral artery we assumed to be equally divided between the first and second order vessels. The 9 mmHg pressure reduction between mid-second order and mid-third order arterioles was similarly divided between the two orders. This allowed for the entrance effect between the large, second-order parent arterioles and the small third order daughter vessels (Zweifach, 1974; Zweifach and Lipowsky, 1984). House and Johnson (1986) also reported a pressure difference between mid-third order arterioles and mid-fourth order venules of 48 mmHg, with a further pressure reduction between fourth and first order venules of 9.5 mmHg. The pressure difference between first order venules and the femoral vein was 4.3 mmHg. These data are summarized in table 5.

Calculation of pressure drop in small vessels

The apportioning of pressure drop among the vessels located
between third order arterioles and fourth order venules (table 4) was estimated from the geometric data on lengths and branching ratios obtained in the cat sartorius by Koller et al. (1987) using the Poiseuille-Hagen formula:

$$R = \frac{(128LN)}{(\pi D^4 N)}$$

where $L =$ length, $\eta =$ viscosity, $D =$ diameter and $N =$ number of parallel segments for each arteriolar order (Lamport, 1955; Intaglialetta and Zweifach, 1971). The validity of the fourth power relationship of diameter to resistance has been demonstrated in the microcirculation (Lipowsky et al., 1978). Diameter values were taken from the current study. Koller used a centripetal ordering scheme instead of the centrifugal scheme used in this report. The first order arteriole is equivalent to the sixth order designation here, the second order is equivalent to the fifth and so on. One difference between the methods was that a small number of the vessels designated third order by Koller would also be designated third order in the present study, but most third order arterioles in Koller's study are fourth order in the present study. Viscosity varies little in these small arterioles and so was assumed to be constant (Papenfuss and Gross, 1986).

We do not have detailed quantitative data for the geometry of the capillary bed or for the fifth and sixth order venules. For the capillaries we assigned approximate values based on preliminary observations (Koller et al., 1987) of 12 capillaries per sixth order arteriole, with an average length of 650 microns. Capillary diameter
was assumed to be 5 microns throughout. We assigned values for resistance in the fifth and sixth order venules at 25 percent those of the fifth and sixth order arterioles, a proportion chosen arbitrarily as it approximated the ratios of pressure in consecutive orders of arterioles and venules from which pressure measurements had been taken.

The pressure drop between the third order arteriole and fourth order venule was apportioned among the vascular sections in proportion to the calculated resistance of each section (tables 4 and 5). These data were used to construct the pressure profile (figure 6).

Estimating the change in network resistance

The calculated distribution of resistance under control conditions was used as a reference to determine the new distribution of resistance during the experimental protocols. To make this calculation, a one mmHg drop in pressure for the control state was assigned a value of one arbitrary resistance unit (see table 6). The change was calculated from the ratio of control to experimental diameter, each taken to the fourth power. This was used to construct the experimental curves in figure 7. Changes in muscle blood flow with each protocol were estimated by taking the ratio of summed control to experimental resistance units, assuming for simplicity that capillary and venous resistance did not change.
Table 4. Estimate of small arteriole (third through sixth order, 3A-6A), capillary and small venule (fifth, 5V and sixth, 6V order) resistance using morphometric data; L, average micron length for the order and N, branching ratio are from Koller et al. (1987), D, micron diameter is from table 1. The change in pressure, $\Delta P$, was obtained by multiplying the fraction of $L/D^4N$ by the measured drop in pressure, 48 mmHg, across these vessels (House and Johnson, 1986).

<table>
<thead>
<tr>
<th>Vessel Orders</th>
<th>L (µm)</th>
<th>D (µm)</th>
<th>N</th>
<th>L/D^4N X 10^-2</th>
<th>Fraction L/D^4N</th>
<th>$\Delta P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A</td>
<td>556</td>
<td>11.1</td>
<td>1</td>
<td>3.66</td>
<td>.49</td>
<td>23.5</td>
</tr>
<tr>
<td>4A</td>
<td>423</td>
<td>8.1</td>
<td>3.42</td>
<td>2.87</td>
<td>.38</td>
<td>18.2</td>
</tr>
<tr>
<td>5A</td>
<td>163</td>
<td>7.7</td>
<td>11.83</td>
<td>.39</td>
<td>.05</td>
<td>2.4</td>
</tr>
<tr>
<td>6A</td>
<td>96</td>
<td>6.8</td>
<td>31.0</td>
<td>.14</td>
<td>.02</td>
<td>1.0</td>
</tr>
<tr>
<td>capillary</td>
<td>650</td>
<td>5.0</td>
<td>372</td>
<td>.28</td>
<td>.04</td>
<td>1.9</td>
</tr>
<tr>
<td>5V</td>
<td>-- .25 X 5A --</td>
<td></td>
<td></td>
<td>.10</td>
<td>.01</td>
<td>.5</td>
</tr>
<tr>
<td>6V</td>
<td>-- .25 X 6A --</td>
<td></td>
<td></td>
<td>.04</td>
<td>.01</td>
<td>.5</td>
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Table 5. Estimate of resistance across the vascular network combining pressure measurements (column A, from House and Johnson, 1986) with morphometric data (column B, calculated in table 4). The estimated pressure drop along each vessel order, (column A+B) are shown in figure 6.

<table>
<thead>
<tr>
<th>Vessel Orders</th>
<th>(A) Measured values (House &amp; Johnson)</th>
<th>(B) Calculated from vascular geometry</th>
<th>(A + B)</th>
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<tbody>
<tr>
<td>1A</td>
<td>12</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>2A</td>
<td>12, 4.5</td>
<td></td>
<td>16.5</td>
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<td>3A</td>
<td>4.5</td>
<td>23.5</td>
<td>28</td>
</tr>
<tr>
<td>4A</td>
<td></td>
<td>18.2</td>
<td>18.2</td>
</tr>
<tr>
<td>5A</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>6A</td>
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<td>1.0</td>
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<tr>
<td>capillary</td>
<td></td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>small venule</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3V - 6V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>large venule</td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>1V - 2V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vein</td>
<td>5</td>
<td></td>
<td>5</td>
</tr>
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Table 6. Estimate of the change in arteriolar network resistance during muscle contraction and sympathetic stimulation, first through sixth order arterioles (1A to 6A), capillaries, small venules and large venules. Control arbitrary resistance units (ARU) in column A (from column A+B in table 5) were multiplied by the ratio of diameters, D^4control/D^4experimental, to obtain the new ARU with each experimental procedure (column A x B). New ARU are shown in figure 7.

<table>
<thead>
<tr>
<th>Vessel Orders</th>
<th>(A) Control ARU</th>
<th>(B) control/experimental</th>
<th>(A x B) New ARU</th>
<th>(B) control/experimental</th>
<th>(A x B) New ARU</th>
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<tr>
<td></td>
<td></td>
<td>4 Hz MUSCLE CONTRACTION</td>
<td></td>
<td>30 Hz MUSCLE CONTRACTION</td>
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</tr>
<tr>
<td>1A</td>
<td>12</td>
<td>0.556</td>
<td>6.7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2A</td>
<td>16.5</td>
<td>0.303</td>
<td>5.0</td>
<td>0.078</td>
<td>1.3</td>
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<tr>
<td>3A</td>
<td>28</td>
<td>0.095</td>
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<td>0.122</td>
<td>2.2</td>
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<td>0.400</td>
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<tr>
<td>6A</td>
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<td>0.1</td>
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<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s. venule</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l. venule</td>
<td>8</td>
<td>8</td>
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8 Hz SYMPATHETIC STIMULATION

<table>
<thead>
<tr>
<th>Vessel Orders</th>
<th>(A) Control ARU</th>
<th>(B) control/experimental</th>
<th>(A x B) New ARU</th>
<th>(B) control/experimental</th>
<th>(A x B) New ARU</th>
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<tbody>
<tr>
<td>1A</td>
<td>3.1</td>
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<td>1.10</td>
<td>13.2</td>
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<td>2A</td>
<td>6.5</td>
<td>107.3</td>
<td>0.625</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>8.5</td>
<td>238.0</td>
<td>0.227</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>4A</td>
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<td>43.7</td>
<td>0.208</td>
<td>3.8</td>
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<td>7.0</td>
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<td>0.9</td>
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<td>1.4</td>
<td>0.667</td>
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<td></td>
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