Microvascular pressure distribution in skeletal muscle and the effect of vasodilation

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The blood supply to skeletal muscle, the largest single structure of the whole body, has been studied from both the morphological and functional point of view using various methods of approach. Direct in vivo observation of the terminal vasculature with detailed photographic documentation of changes in vessel diameter was described by Zweifach and Metz (23), Hyman and Paldino (15), and Gray (13) for the spinal trapezius of the rat, by Barz (3) and Smaje et al. (21) for the cremaster muscle of the rat, and finally by Bränemark and Eriksson (4) for the tenuissimus muscle of the cat. With the recent development of quantitative micromethods for intravital microscopy (6, 22), it has become possible to undertake a detailed analysis of micropressure distribution in the successive segments of the arterioles and venules (11) in cat mesentery.

It is the purpose of this study to present the results of direct microvascular pressure measurements in a skeletal muscle of the cat and to map their distribution as related to vessel size. The first part of our study was devoted to an analysis of normal steady-state conditions. In the second part we studied the influence of vasodilator drugs on microvascular pressures.

The relationship between changes in capillary pressure and the decrease in systemic blood pressure triggered by vasodilation has not been as well established, by direct means, as the importance of this relationship in the microvascular system of skeletal muscle. Most of our current information has come from indirect studies of fluid exchange and pressure-flow relationships in whole limbs or in surgically isolated muscle preparations (14).

METHODS

Experiments were performed on 40 cats, each weighing about 2 kg, anesthetized with pentobarbital 30 mg/kg. No additional medication was used. The common carotid artery and jugular vein served as the site for systemic arterial and central venous pressure measurements. The m. tenuissimus was chosen because it is known to contain both fast and slow muscle fibers and can be taken to represent a typical skeletal muscle (8). Anatomically, it runs from the sacral and coccygeal bones down to fascia cruris of the hindlimb. It is about 0.5 cm wide, some three to six muscle fiber layers thick, and approximately 10–12 cm long. The central artery representing the main blood supply is accompanied by a vein and nerve. The basic anatomical layout is shown in Fig. 1. Details of the dissection have been described by Bränemark and Eriksson (4) and here we will describe only modifications of their original approach.

Close to the fascia cruris is the thinnest portion of the muscle, which contains only two to four layers of muscle fibers and, therefore, offers the most suitable site for transillumination for microscopy. Special care was taken to remove the connective tissue from the surface to demarcate the muscle fibers and the blood vessels between them. The cat was placed on its side in a plastic mold of the body leaving only the left hindlimb, bent at a 90° angle in the knee, fully exposed. A 12-cm-long light pipe with a prism fused to one end was used to direct the light and was slipped sidewise under the lateral edge of the muscle, so that the tissue rested loosely and flat on the surface prism, giving about a 5-mm² working area. A green filter was placed between the light source and light pipe for better optical resolution. Throughout the dissection, as well as during micropressure measurements, the muscle was kept moist with a continuous drip of isotonic Krebs solution containing 1% gelatin and maintained at 37°C.

The microscopic observations and micropressure measurements were made from the upper surface of the muscle, adjusted to the hydrostatic level of the heart. The light
source was mounted on the same holder as the plaster body mold for the cat, so that it moved with the cross stage of the microscope. Magnifications of X300–400 were obtained with UM Leitz objectives having a long working distance. By changing the focus, microvessels at different depths of the muscle were available for observation and micropuncture. The pressures in microvessels (7–200 μm) were measured by using glass micropipettes with sharpened tips to 2–4 μm (OD) filled with 2 M NaCl and inserted into selected microvessels with the help of a micromanipulator. The micropipettes were connected with the servo-nulling pressure-pump system developed by Wiederhielm et al. (22) and modified by Intaglietta et al. (16). The diameter of the examined vessel was determined in two ways. Vessel dimensions on polaroid prints were compared with a calibration grid. The second diameter-measurement technique is based on the image-splitting technique described by Baez (2), and modified by Intaglietta and Tompkins (17), in which the optical image splitting was replaced by an electronic shearing system with repeatability within 0.5%.

In the present experiments, microvascular pressures were evaluated in relation to the diameter of selected vessels and to the systemic arterial pressure. Infusion of papaverine (P) intravenously was administered in 54 runs in a dose from 100 μg/min per kg to 1 mg/min per kg body wt. Each infusion was maintained for several minutes to achieve a new steady state of the systemic arterial pressure. Isoproterenol (IPR) was administered intravenously in 48 check runs in a dose from 0.1 to 1.0 μg/min per kg body wt. Whenever a new steady-state level of systemic blood pressure was achieved, the infusion was discontinued. The effect of drugs was evaluated in relation to the dose, systemic blood pressure response, microvascular pressures, and to the size of given microvessels.

RESULTS

Steady-state conditions. Pressures in the central artery are within 95–90% of the systemic blood pressure. The diameter of this artery ranges from 70–100 μm, depending on
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this first generation as transverse arterioles (Fig. 2). The diameter of such arterioles varies from 70 to 20 μm. Direct measurements show the blood pressure in these vessels to average 70% of the systemic pressure (Fig. 4). The transverse arterioles subdivide into a number of lateral offshoots—short precapillaries or metarterioles (4) with diameters between 20 and 10 μm—or branch directly into five to eight true capillaries which then course between the muscle fibers (Fig. 3). Even when anatomically distinct precapillary sphincters cannot be determined, the significant site of precapillary resistance regulation in the skeletal muscle is located in vessels larger than 20 μm in diameter, as shown by the microvascular pressure drop. The true capillaries, i.e., smaller than 10 μm in diameter, are accessible for micropuncture only when they are located on the surface of the muscle; otherwise, direct intubation was difficult.

The pressure drop across the true capillaries proper is approximately 15% of the systemic pressure. Several venous capillaries form small venules. The blood pressure in these venules (<10–12 μm) is 24 mmHg and probably reflects closely (within 2–3 mmHg) the hydrostatic capillary pressure. Venular pressures then decrease sharply to 20 mmHg when confluent vessels 20 μm in diameter are formed. Further distally, the gradual decrease in venular pressure is proportional to the size of the vessel and in the central vein (average diameter: 140 ± 20 μm) ranges from 8 to 14 mmHg (Fig. 4).

The micropressure distribution in two different tissues (Fig. 4) indicates that in the mesentery, pressure falls within the dichotomization of the larger feeding vessels (>35–40 μm) on the arterial side by almost 50% before the blood enters the precapillaries and capillaries itself. On the other hand, in the skeletal muscle, the blood pressure remains above 60 mmHg in the terminal arterioles and precapillary vessels. The next abrupt fall in microvascular pressure in the muscle is the result of a spaying out of five to eight true capillaries from a single terminal arteriole. Capillary pressure in the cat mesentery is, on the average, less than 1 mmHg higher than the capillary pressure in skeletal muscle. But the pressure in collecting venules of the mesentery is higher (20.7 ± 1.5 vs. 11.5 ± 0.6 mmHg), presumably because they drain into the portal vein. In the skeletal muscle, the venular pressure falls gradually to the average value of 11.43 ± 0.65 mmHg in the central vein.

Precapillary and postcapillary resistance comprises the total resistance on the arteriolar and venular side, respectively. For this calculation, pressure closest to the capillary pressure were used, i.e., Pa and Pv. This approach is applicable, especially in view of the fact that the resistance of true capillaries is constant. In spite of the fact that flow was not measured, an attempt was made to express the pre-to-postcapillary resistance ratio in the following way:

\[ BP - Pa/Q = PrR \text{ or } BP - Pa = PrR \times Q \]

and

\[ Pv - VP/Q = PsR \text{ or } Pv - VP = PsR \times Q \]

where BP and VP are systemic arterial and central venous pressures, Q is the flow through a given vascular region, Pa = mean pressure in the smallest arterioles (10–12 μm), Pv = mean pressure in the postcapillaries and venules (10–15 μm), PrR = precapillary resistance and PsR = postcapillary resistance. If we assume that the inflow for the terminal part of the arterioles and the venular outflow are the same, dividing equation 1 by equation 2 results in the following relationship:

\[ (BP - Pa)/(Pv - VP) = PrR/PsR \]

Our findings suggest that the ratio of precapillary pressure drop to postcapillary pressure drop is the same as the precapillary-to-postcapillary resistance ratio (Table 1). The pre-to-postcapillary resistance ratio calculated in this way was, on the average, 3.37 ± 0.57, which is close to that derived from indirect measurement by Folkow and Melander (9, 20), or from direct microvascular pressure-flow determination established for the cat mesentery (12).

Effect of vasodilation. Two drugs were used to induce peripheral vasodilation, papaverine and isoproterenol. Papaverine was administered by intravenous infusion increasing the dosage in steps from 100 μg to 1 mg/min per kg body wt. Altogether 54 experimental runs were analyzed, and the micropressure changes in the skeletal muscles were compared with the systemic arterial pressure changes. As expected, the decrease in systemic arterial blood pressure due to vasodilatation was proportional to the administered dose. The pressure drop in the larger arterioles (>20–25 μm) is dose dependent, but as shown in Fig. 5, the reduction in pressure is proportionally smaller in the consecutively smaller arteriolar segments until reaching the terminal arterioles or precapillary vessels below 20 μm in diameter in which the micropressures actually showed a significant

![FIG. 4. Micropressure distribution in 2 different tissues in relation to average diameter of given vessels. Vertical bars express ± SEM and number of measurements is given in parentheses. Central BP—central arterial pressure—an average from all experiments.](http://ajplegacy.physiology.org/DownloadedFrom/bb9e-03532170)}
increase. This elevation of blood pressure in the precapillary vessels following systemic administration of papaverine is proportional to the dose and is transmitted to the venular side (Fig. 5). The effects of papaverine on blood pressure become less evident in the larger venules. In venules 80 μm in diameter and larger, no change in pressure was observed following papaverine.

The substantial difference in the effect of papaverine on arterioles smaller than 20 μm, as compared with that in larger vessels, suggests a considerable fall in the resistance in these large vessels since systemic arterial pressure dropped by approximately 12%, while the arteriolar micropressure in smaller vessels was increased by approximately 10%.

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![Diagram](http://ajplegacy.physiology.org/)

**FIG. 5** Changes in pressures during intravenous administration of papaverine. △ Syst. BP shows changes in systemic arterial pressure. Values in parentheses indicate range of true capillaries. Changes in microvascular pressures are plotted against control diameters (before dilatation).

**FIG. 6** Changes in pressures during intravenous administration of isoproterenol. Description is the same as in legend to Fig. 5.

**DISCUSSION**

The servo-nulling micropressure technique is well established as a reliable procedure with regard to accuracy and reproducibility (24). Special care was taken to include only the results of those experiments in which the blood flow was not impeded by the microneedle. Despite these precautions in very small capillaries (6–8 μm), it was sometimes difficult to achieve ideal conditions. As long as the micropipette was oriented along the midline of the vessel and the objectives were steady-state conditions or slow changes in blood pressure, the error of measurement was within 2% (16). On the basis of 188 micropressure measurements, conclusions can be drawn regarding the pressure distribution with the use of diameter to set up certain vessel categories in the skeletal muscle. Microvascular pressures were directly proportional to the systemic arterial pressure only in vessels with an average diameter of over 70 μm.

Since the major pressure drop in skeletal muscle is seen in arterioles with diameters ranging 35–20 μm, it can be concluded that vessels larger than 20 μm in diameter are the principal source of arteriolar resistance regulation in m. tenuissimus. Micropressures in vessels smaller than 20 μm (metarterioles) depend primarily on the precapillary resistance and to a lesser degree on the systemic arterial pressure. Bränemark and Eriksson (4) have shown that skeletal muscle arterioles down to a diameter of 20 μm continue to have smooth muscle cells in the vessel wall.

Our data on micropressure distribution in relation to vessel diameter, it can be concluded that the term precapillary resistance is related anatomically to the transverse arterioles and their branches which usually terminate in five to eight capillaries. This anatomical arrangement of the terminal vasculature in skeletal muscles is quite different from that in the mesentery and from that in other muscle beds, such as the spinotrapezius and cremaster muscles in the rat. The feeding artery for m. tenuissimus is a direct branch from the femoral artery and, therefore, the pressure in this central arteriole is close to the systemic arterial pressure. This is not the case in the cremaster and this may explain why the arteriolar and venular micropressures are lower in this muscle than in the m. tenuissimus (21). Furthermore, the average capillary length in the m. tenuissimus is 1,015 μm (8) creating a pressure drop of 15 mmHg, while in m. cremaster it is only 615 μm and the pressure drop is only 7 mmHg (21).

In the mesentery the feeding arterioles dichotomize into successively smaller terminal vessels (10) with relatively few abrupt side branchings as is the case in the m. tenuissimus. As a consequence, microvascular pressures are reduced in the mesentery more gradually with the subdivisions of the large arterioles.
The microvascular pressure in venules below 10 μm in diameter has been found to reflect closely (within 2-3 mmHg) the capillary pressure as determined directly in the skeletal muscle. The average of this pressure in venules smaller than 10 μm in our experiments was 24 ± 2.2 mmHg, which is in good agreement with the results obtained in cat mesentery experiments (12). In spite of the wide range of capillary pressures, various direct and indirect measurements of microvascular pressures performed recently on the dog hindlimb (11), or in the past on the wall of the cat intestine (18) or human skin around the nail fold (7), yielded values of capillary pressure essentially the same as in these experiments.

The vasodilatory effect of papaverine and isoproterenol on the peripheral circulation is well characterized. However, the effect of these substances on the terminal vascular bed is not well documented. A comparison of the mechanism of action of both drugs cannot be performed solely on the basis of systemic arterial blood pressure drop. Isoproterenol has, in addition to its dilatatory effect on the peripheral circulation, an inotropic and chronotropic effect causing an increase in stroke volume, heart rate, and thus in cardiac output, which compensates to some extent the effect of a drop in peripheral arterial resistance (1, 5). In the case of papaverine, however, a direct effect on the heart muscle is far less pronounced (5). It is possible, due to this difference in central influence, that the decrease of systemic arterial blood pressure after administration of IPR may be accompanied by a greater decrease in precapillary resistance than that induced by papaverine. The present experiments indicate a significant difference in the mode of action on the terminal vasculature. During the administration of papaverine there was a decrease in microvascular pressure in the larger arterioles (> 20 μm in diameter), while in smaller arterioles there was a consistent elevation in blood pressure, even when the administered dose was increased 10 times, which was transmitted through the capillary bed and was measurable on the venous side of the terminal vasculature. Thus, the pressure difference between the arteries and arterioles is, on the average, 22 mmHg after papaverine administration as compared with 35 mmHg during the control period. However, despite the reduced driving force, capillary pressure is elevated apparently because of the dilatation of the terminal arterioles or precapillary vessels, while the postcapillary vessels are probably less influenced. These conditions would give rise to filtration in the tissue space.

The smallest dose of isoproterenol used, 0.1 μg/min per kg, had an effect on both arteriolar and venular microvascular pressures and higher doses led to further arteriolar vasodilatation. Diana (6), in autoperfused-hindlimb experiments, using either constant flow or constant pressure during IPR administration, determined isogravimetrically a modest increase in capillary pressure. In our experiments with isoproterenol infusion, as the central arterial pressure decreases, both arteriolar and venular microvascular pressures drop, therefore, the mean capillary pressure can be assumed to be lower. This evidence would tend to support Mellander's (19) conclusion that IPR decreases precapillary as well as the postcapillary resistance. However, in contrast to Mellander's contention, according to which filtration increases, it can be assumed that the resulting decrease of capillary pressure in skeletal microvessels leads to absorption to fluid from tissue into the blood. Such an explanation may account for the observations that some vasodilator agents do not increase lymphatic flow while others double or even triple lymphatic flow (18). Our results indicating differences in effects of papaverine and IPR would appear to be germane to these findings.

In conclusion, in spite of the fact that papaverine and isoproterenol are both considered to be potent vasodilators, their different actions on precapillaries determine the final effect on the capillary pressure and, therefore, the conditions for fluid transfer in skeletal muscle.

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