Comparison of neural controls of resistance and capillary density in resting muscle

CARL R. HONIG, JAMES L. FRIERSON, AND JOHN L. PATTERTON

Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620

In each of six rats weighing 350–500 g, a gracilis muscle was disconnected from the remainder of the leg except for artery, vein, and obdurator nerve. Muscles were held at rest length with hemostats at origin and insertion, and all tributaries of the femoral vein except those draining the gracilis were tied. All leg arteries were left intact. After injecting 10,000 units of heparin intravenously, the femoral vein was ligated and an L-shaped plastic cannula 2 cm long was inserted into the distal end. Gracilis blood flow could then be measured by timed collection of effluent in tared capillary hematocrit tubes, of about 0.1 ml volume. From 0.5 to 3 min were required to fill the tubes, which were then rapidly weighed to the nearest 0.1 mg on a Mettler balance, stoppered with clay, and iced. To avoid congesting the muscle, the capillary tubes, which were about the same diameter as rat femoral vein, were held in a nearly vertical position below the gracilis. A tie was gently placed beneath the obdurator nerve proximal to its first branch to facilitate rapid denervation. To obtain reproducible responses to denervation, it was found essential that the gracilis be maintained at 37 C, and that the muscle and nerve be kept moist with a solution containing K+, Mg++, and Ca++. To accomplish this, a drip of modified Krebs solution, Tris buffered at pH 7.4, slowly flowed under the Parafilm with which the muscle and nerve were covered. The temperature of the drip at the outflow was measured with a thermistor. The temperature of the gracilis was continuously monitored with a thermistor, and heating lamps and rate of drip were adjusted to hold gracilis temperature at 37 C. The wet weight of each gracilis was determined at the end of the experiment.

Correlative measurements were obtained on six dog gracilis muscles, isolated as described above. Particular care was taken to preserve the two small arterics and veins to the distal portion of the gracilis. This provides more uniform perfusion than can be obtained in the conventional preparation in which only the main artery is used. Tributaries to all three gracilis veins were tied, 50,000 units of heparin were administered, and the femoral vein cannulated. A Y connector permitted gracilis effluent to return to the animal, or to be collected under oil in tared test tubes. Sufficient flow for accurate measurement could be obtained in a 10-sec collection period.

Measurement of Blood Flow

In each of six rats weighing 350–500 g, a gracilis muscle was disconnected from the remainder of the leg except for artery, vein, and obdurator nerve. Muscles were held at rest length with hemostats at origin and insertion, and all tributaries of the femoral vein except those draining the gracilis were tied. All leg arteries were left intact. After injecting 10,000 units of heparin intravenously, the femoral vein was ligated and an L-shaped plastic cannula 2 cm long was inserted into the distal end. Gracilis blood flow could then be measured by timed collection of effluent in tared capillary hematocrit tubes, of about 0.1 ml volume. From 0.5 to 3 min were required to fill the tubes, which were then rapidly weighed to the nearest 0.1 mg on a Mettler balance, stoppered with clay, and iced. To avoid congesting the muscle, the capillary tubes, which were about the same diameter as rat femoral vein, were held in a nearly vertical position below the gracilis. A tie was gently placed beneath the obdurator nerve proximal to its first branch to facilitate rapid denervation. To obtain reproducible responses to denervation, it was found essential that the gracilis be maintained at 37 C, and that the muscle and nerve be kept moist with a solution containing K+, Mg++, and Ca++. To accomplish this, a drip of modified Krebs solution, Tris buffered at pH 7.4, slowly flowed under the Parafilm with which the muscle and nerve were covered. The temperature of the drip at the outflow was measured with a thermistor. The temperature of the gracilis was continuously monitored with a thermistor, and heating lamps and rate of drip were adjusted to hold gracilis temperature at 37 C. The wet weight of each gracilis was determined at the end of the experiment.

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**Pao2, Pvo2, and O2 Consumption**

In each rat, the Pao2 of the venous blood used for flow measurement and the Pvo2 of a paired sample from the carotid artery were determined with an Instrumentation Laboratories 02 analyzer equipped with a microcuvette. In two animals, the Pao2 so obtained agreed within 5 mm Hg with values obtained with a macroelectrode on 3 ml of blood. Capillary hematocrits were obtained with each set of samples and were centrifuged together at the end of the experiment. Oxygen contents were estimated from the oxygen tensions and hematocrit ratios by use of oxyhemoglobin-dissociation curves for dog or rat blood similarly corrected for the Bohr shift. Oxygen consumption per 100 g gracilis was estimated from the Fick principle.

**Microscopy**

Flow and capillary density could not be measured conveniently in the same animals. For microscopy, smaller rats weighing 80-200 g were fixed to a plastic frame which was mounted on the stage of a Leitz microscope. The gracilis was separated from adjacent muscles, but its circulation was not isolated, and all leg vessels were left intact. All observations were made on the relatively flat distal end of the muscle. A cover glass was placed over this region to minimize glare, and a drip of modified Krebs solution was drawn under the glass by capillary action. The drip rate and radiant heat were adjusted to hold the surface of the muscle at 37 C.

The light source was a Chadwick-Helmuth point-source strobe light, model 135M-6. It provided 200 flashes/sec with the visual equivalent of 1,200 or 2,400 cd sec/flash. The lamp projected a beam which was collimated and brought to focus on the end of a bundle of optic fibers. The distal end of the bundle was joined to a single, tapered, polished glass fiber through an immersion-oil junction. The fiber measured 1 mm in diameter at its tip; a 45-deg polished beveled surface reflected the emergent light upward. The fiber was placed beneath the gracilis, and the muscle was viewed by transmitted light. Both the position of the light spot with respect to the muscle and the position of the muscle with respect to the objective could be varied with X-Y manipulators. Since each flash was only 30 µsec in duration, the temperature of the tip of the fiber remained the same as the temperature of the adjacent tissues. All measurements were made with a graduated eyepiece which was calibrated with a standard micron scale. The graduations were aligned normal to the long axis of the capillaries. The length of the scale, and hence of each measured field, was 720 µ. All observations were made with an 11 X objective and a 10 X eyepiece.

To operate the system, the fine vertical focus was adjusted to explore an optical section about 10 µ thick. This range of focus adjustment, which was kept constant in all experiments, was necessary in order to be certain of the identity of structures in the 4-µ depth of field of the microscope. The observer satisfied himself that no capillaries escaped attention, and that normal vasomotion (opening and closing of capillaries) was present. This done, capillaries that contained moving erythrocytes at that instant were counted, and the preparation was repositioned to a new field. In each experiment 6-11 fields were so examined over a 30-to 90-min period. These fields constituted the control observations. The obdurator nrvc was then cut while watching the last field to be counted. After observing this field for 10 min postdenervation, 3-11 additional fields were counted over a 20- to 90-min period. The mean capillarity for these fields was taken as the postdenervation value for that rat. A total of six rats were so studied. Two additional animals provided control information, but for various technical reasons no postdenervation data were obtained.

**Data Analysis and Statistics**

For each field the number of capillaries encountered along a line 1 mm long, oriented perpendicular to the long axis of the capillaries, was determined. The mean count for five to nine fields in a single muscle or for repeated countings of a single field was determined. To convert these means to capillaries per square millimeter we must assume some three-dimensional model. We selected the simplest, a square array, as did Krogh (12), and squared the mean number of capillaries per linear millimeter. If capillaries are packed in hexagonal rather than square arrays, i.e., each capillary is equidistant from its neighbor, the mean capillary density would be about 12% greater than the values shown in Table 1. Since neither model is precisely correct, Table 1 indicates values proportional to the true capillary density in gracilis muscle in vivo.

The above approach to estimation of capillary density is valid only for tissues in which mean intercapillary distance is several times as great as the optical section explored. In our case the mean intercapillary distance was 6 times the optical section, so that the latter, in effect, can be regarded as a surface. To compare capillary densities before and after denervation in a particular rat, each field before denervation was paired with a field after denervation, and the value of t was computed for the differences between the two counts. This procedure increased the likelihood that a small difference would be regarded as statistically significant. For comparisons among rats, the t test for independent sample means was employed (21).

**RESULTS**

**Blood Flow and Resistance**

Mean gracilis blood flow was 6.56 ml/100 g per min ± 0.77 SEM in six rats, and in five dog gracilis muscles it was 5.40 ml/100 g per min ± 0.23. The difference is not

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**TABLE 1. Effect of denervation on capillary density**

<table>
<thead>
<tr>
<th>No. of</th>
<th>No. of</th>
<th>Capillaries</th>
<th>Total Capillaries</th>
<th>Capillaries/mm²</th>
<th>Half Intercapillary Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate</td>
<td>Fields</td>
<td>Per Field*</td>
<td>Counted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>-------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>59</td>
<td>11.12 ± 0.55</td>
<td>645</td>
<td>240</td>
</tr>
<tr>
<td>Denervated</td>
<td>6</td>
<td>41</td>
<td>11.04 ± 0.23</td>
<td>443</td>
<td>234</td>
</tr>
</tbody>
</table>

Capillary density and intercapillary distance in rat gracilis before and after denervation. * Values are means ± SEM. † Values are means.
VASOMOTOR NERVES AND CAPILLARY DENSITY

and smaller more sustained increase to loss of vasoconstrictor tone.

obdurator nerve. Initial spike is probably due to active vasodilation,
which contains somatic and autonomic fibers to gracilis,

Capillary Density and Arrangement
did not produce detectable movement or fasciculation. The

significant at the 95% level. Cutting the obdurator nerve,
which contains somatic and autonomic fibers to gracilis,
did not produce detectable movement or fasciculation. The
time course of the flow increase postdenervation could not
be accurately measured in the rat gracilis, because the
volume of venous outflow was too small. The time course
was therefore studied in dogs. As shown in Fig. 1, the flow
response to denervation consisted of at least two phases.
During the first 2 min after cutting the nerve, flow in-
creased rapidly to peak values 2–5 times control. In each
dog this large initial increase was comparable to that pro-
duced in the same animal by a supramaximal dose of
nitroglycerine. Since nitroglycerine acts on all vascular
muscles, including those that are not driven by vasocon-
strictor nerves, the initial rise in flow cannot be fully ex-
plained by loss of vasoconstrictor tone. More direct evi-
dence that this transient is due, in part, to active vasodila-
tion has been provided by Jones and Berne (11). In every
dog the initial phase of active vasodilation was followed by
a smaller increase which lasted 30–90 min, after which

time the experiments were terminated (see Fig. 1). Since
we wished to relate release of vasoconstrictor tone to
microscopy, the initial transient was ignored in rats, and
flow was measured 5 and 10 min after cutting the obdura-
tor nerve. Mean flow in six rats was 160% control at 5 min
and 130% control after 10 min (P < .02 and < .05, respec-
tively). Mean arterial pressure was not affected by nerve
section (P > 0.5). Resistance therefore fell in every rat, as
shown in Fig. 2. The first experiments performed yielded
low initial resistances, and the last two the highest resist-
ances. It, therefore, seems likely that the rise in initial
resistance observed in successive experiments reflects de-
crease in trauma and preparation time. In agreement with
others (11) we regard the higher initial resistances as the
more normal ones. The usefulness of the plot in normalizing
differences in resistance among preparations is evident.
The regression lines obtained 5 and 10 min after denerva-
tion have the same intercept, but the slopes are significantly
different (P < .05), suggesting that myogenic tone had
begun to compensate for loss of extrinsic neural control
within 10 min.

Data obtained in dogs were similar except that the cor-
relation coefficients of the lines were lower, suggesting
greater heterogeneity in the larger muscles. The slope of
the regression line obtained in dogs for resistances 5 min
after denervation was 0.224, a value not significantly dif-
ferent from that observed in rats (P > .5).

Oxygen Consumption of Gracilis in Dog and Rat

Metabolic data will be presented in detail elsewhere and
are included here only as evidence of the functional integ-

ne synchrony with respect to a muscle fiber. In some fields, two
parallel capillaries were found separated by only about 5 µ.
Counter-current blood flow could frequently be observed
in such paired vessels. Closure of one or the other sphincter
of the capillary pair intermittently eliminated counter-
current flow, and of course changed the number of open

Capillaries in gracilis are up to 700 µ long, and, in the
case of intercapillary loops, as short as 100 µ. Most are
about 400–500 µ. They are by no means uniformly spaced
in the horizontal plane, though they tend to remain within
the vertical optical section that we employed. We do not
know whether capillaries have a three-dimensional sym-
metry with respect to a muscle fiber. In some fields, two

FIG. 2. Linear relation between resistances immediately prior to
denervation (initial resistance) and resistances 5 and 10 min after
denervation is interpreted in text. Each filled circle represents a
different rat. Regression equations and correlation coefficients are
indicated. Schematic denotes model of limb circulation where R =
component of resistance varied by a particular stimulus. Rp and Rs
represent parallel and series resistances which are not influenced by
stimulus (see ref 17).
capillaries in the field. We emphasize, therefore, that our figures for capillary density per millimeter cross section are mean values and that the variance of the means reflects fluctuations in capillary density with time and differences in capillary density at various points within the muscle.

In eight rats the mean capillary density was 240/mm² ± 10.7 SEM (Table 1). The range of the means obtained in individual rats was 220–294/mm². In every rat the precapillary sphincters exhibited contractile activity, measured by spontaneous, random changes in capillary density, whether or not the gracilis was innervated (see Fig. 3). When the number of capillaries in a particular field was measured repeatedly, the variance based on the F distribution for one field counted repeatedly (time mean) was the same as the variance among observations on many fields in the same muscle (space mean). Moreover, the variance of capillary density for all fields in eight rats was not significantly different from the variance among fields in an individual rat. We conclude that, under carefully controlled conditions, gracilis muscles are quite uniform and that variations in capillary density are principally due to the normal periodicity of precapillary sphincters.

To determine whether stimuli known to influence sphincters produce measurable changes in capillary density, the effects of epinephrine and asphyxia were observed. Injection of 1 µg of epinephrine intravenously lowered the capillary density in two rats to only 20% of the average control values for those animals, and in one rat asphyxia increased capillary density to 260% of control. Evidently the precapillary sphincters were capable of responding to gross stimuli. With respect to smaller changes, statistical analysis indicated that a 10% change in mean capillary density could have been detected as a response significant at the 95% level. Nevertheless, we observed no effect of denervation on capillary density, either during the first 2 min, when active vasodilation might be expected, or at subsequent times up to 1.5 hr. When a single field was observed for 10 min after denervation, the same fluctuations in capillarity, presumably myogenic in origin, occurred as in the control period. In those fields in which the fluctuations were rhythmic, denervation produced no change in frequency, or in the duration of the contraction phase. Thus, among the four examples shown in Fig. 3, capillarity initially increased, decreased, or remained the same. When additional fields were examined between 20 and 90 min after denervation and the counts for a particular animal were pooled, the mean values obtained in each rat were not significantly different from predenervation values for that animal. The pooled control and postdenervation means for all observations on all six rats are given in Table 1.

**DISCUSSION**

The principal finding we report is that acute denervation lowers resistance to blood flow in resting muscle but does not change the number of open capillaries. The following discussion considers the mechanism and significance of these results.

**Blood Flow and VO₂**

The mean blood flow and VO₂ that we observed in dogs fall within the range reported by others for dog gracilis muscle (20). No data are available in the literature for rat gracilis, but flow is not significantly higher in dogs similarly prepared. Because rat VO₂ is higher, O₂ extraction is much greater than in the dog despite comparable flow. The difference in VO₂ between dog and rat muscles is about that which might be predicted from the contribution of muscle to whole-body VO₂ (18). We conclude from this interspecies comparison, and from the resistance data discussed below, that the rat muscles were in an acceptable physiological state.

**Analysis of Resistance Changes**

To study the contribution of tonic vasoconstrictor nerve discharge to the control of gracilis circulation we simply cut the obturator nerve. A better experimental design might have been to produce reversible denervation with cold, or with local anesthesia, in order to observe responses to denervation repeatedly in the same animal. Unfortunately, this could not be done in rats for technical reasons. The muscles proved to be sufficiently uniform, however, to permit a quantitative analysis of responses among animals. The correlation coefficients in Fig. 2 provide a measure of this uniformity. Our analysis is based on the fact that the magnitude of vasodilator responses to neural and pharmacological stimuli of equal intensity is a precise linear function of the resistance from which response is initiated (17). As shown in Fig. 2, the extent of the vasodilations produced by acute denervation was also linearly related to initial resistances, probably because denervation elicits a maximal decrease in vasoconstrictor tone and hence represents a stimulus of comparable intensity in all animals.

The circulation in an isolated muscle can be represented by a variable resistance, R, to which other resistances are connected in series (Rs) or in parallel (Rp) (17); see Fig. 2. In the present experiments R might represent a population of arterioles supplied by tonically active vasoconstrictor nerves, Rp a population of arterioles not so innervated, and Rs precapillary sphincters, capillaries, and venules. In the case of vasodilation, Rp and Rs have relatively little effect.
and the slope and intercept depend primarily on the stimulus-related resistance R. The fact that initial and final resistances are linearly related suggests that denervation removes a constant fraction of total vasomotor tone. This neural component is estimated, from the slope of the 5-min line, to be about 75%, a figure in good agreement with that of Renkin and Rosell (19). Considered from the standpoint of the myogenic component, if the smooth muscles which generate R had been completely relaxed by denervation, and if basal, or myogenic tone were independent of initial resistance, the lines in Fig. 2 should have had 0 slope. Since the slopes are significantly different from 0, we conclude that differences in myogenic tone as well as in vasoconstrictor nerve discharge contribute to variations in resistance among different muscles. One interpretation of our analysis is that junction and pacemaker potentials interact in generating action potentials and tension at the same arteriolar smooth muscle cells, a possibility suggested by others on the basis of electrophysiological data (9). The significantly higher slope of the line obtained 10 min after denervation, for example, could be due to a rise in myogenic tone produced by release of smooth muscle pacemakers from inhibition by junction potentials. Alternatively, small intramuscular adrenergic ganglia (2) may compensate for loss of extrinsic neural drive. Regardless of the mechanism, the extent of compensation postdenervation varies with the initial state of the neuromuscular system as judged by the initial resistance.

**Capillary Density**

a) Arrangement of capillaries. The figures for capillary density which we and others have published are, in reality, means for both space and time. Nonuniformity of spatial distribution is much greater in the wide-mesh capillary net of skeletal muscle than in the closely spaced cardiac mesh (16). Perhaps the most extreme nonuniformity is that associated with counter-current blood flow. In the gracilis, capillary pairs separated by only about 5 μ can be found. In these paired vessels blood flows in opposite directions. Since the VO₂ of the tissue between the two capillaries is negligible, the pair represents, for practical purposes, a "compound capillary" of large radius, within which the longitudinal PO₂ gradient is minimized. The effect of such a counter-current compound capillary on O₂ transport is currently under study.

b) Number of open capillaries. The mean capillary density which we observed in rats is about 3 times the number Krogh counted in living guinea pigs (12). Better control of temperature and fluid environment, and our use of transmitted flash, rather than intense reflected light, may account for the difference. Our figure for rats is about 35% greater than that reported by Landis and Pappenheimer (13) for cat muscles on the basis of capillary counts in frozen sections. Since manipulation and exposure tend to increase capillary density as an artifact, we consider 250/mm² to be a high estimate of the number of open capillaries in resting rat gracilis in vivo. The total number of capillaries, both open and closed, demonstrable by anatomical methods, is different for red and white muscles, and varies with method of enumeration. A representative figure for gracilis is 2,500-3,000/mm² (15). Thus only about 10% of the available channels are open at rest at normal PaO₂. The principal objective of this study was to determine whether the stimulus to precapillary sphincters to so severely limit capillary density under normal resting conditions is, in part, dependent on vasomotor nerves.

c) Effect of denervation. Denervation produced no change whatever in the number of open capillaries, whether one field was observed continuously for 10 min after denervation (Fig. 3), or data for all fields were pooled for a single animal, or for all animals (Table 1). We interpret the effects of denervation on resistance to mean that autonomic nerves were operational and contributed significantly to control of arteriolar tone. The effects of epinephrine, asphyxia, and nitroglycerine on capillary density demonstrate that the precapillary sphincters were capable of response, and from statistical analysis we know that a 10% change in capillary density with denervation could have been detected. We conclude that precapillary sphincters are not driven by vasoconstrictor fiber discharge in resting muscle. Similarly, if the fall in resistance during the first 2 min after denervation is attributable to release of a vasodilator mediator (11), it is likely that such vasodilator fibers innervate arterioles but not precapillary sphincters. Evidence in support of this idea has been previously presented by Renkin and Rosell (19). There is no doubt that precapillary sphincters are innervated by autonomic nerves, for nerve-smooth muscle junctions have been visualized by electron microscopy (22). It would appear that such nerves are not tonically active, a conclusion consistent with the paucity of norepinephrine observed by fluorescence microscopy beyond the true arterioles (5) and E. Schenk, personal communication). When the sympathetic chain is stimulated with an electrode, contraction of precapillary sphincters, inferred from change in capillary filtration coefficient (1), accompanies the rise in resistance; but when sympathetic nerves are activated reflexly precapillary sphincters do not appear to participate in the vasoconstriction (10). Perhaps sphincter nerves can only be activated by intense preganglionic stimulation. Conversely, the capillary filtration coefficient does not indicate an increase in capillary density when vasoconstrictor discharge is reflexly inhibited (10). This strongly supports our conclusion that nerves to precapillary sphincters are not tonically active in skeletal muscle, and focuses attention on factors intrinsic to the gracilis, particularly the electrical properties of precapillary sphincter smooth muscles (4), local vascular reflexes (2), and the chemical mechanisms that couple circulation to metabolism (6, 7).

Though consistent with changes in capillary filtration coefficient, our findings appear to conflict with conclusions drawn from ⁸⁶Rb transport (8). The effect of denervation to increase the permeability PS was interpreted to mean that precapillary sphincters had relaxed and that capillary density had increased (8). It is possible that 50 hr after denervation, when the first postdenervation observations were made, there was indeed an increase in capillary density. Alternatively, it is possible that capillary density was unaltered and that the apparent change in transport was due to some other factor. Current models of transcapillary exchange involve the assumption that there is no interaction between diffusion fields around adjacent capillaries. Since we have
clearly identified counter-current blood flow in muscle capillaries, this assumption is no longer justified. We have no evidence that denervation influences the fraction of total blood flow that is counter current or any other aspect of capillary arrangement; but such effects are possible and could account for the discrepancy between anatomical measurements and filtration coefficient on the one hand and $^{86}$Rb transport on the other. Counter current capillary blood flow is but one aspect of the more general question of the degree of order which may exist in three-dimensional capillary arrays. Since it seems likely that drugs and physiological stimuli might influence transport by modifying the arrangement as well as the abundance of capillaries, we urge that caution be exercised in drawing anatomical conclusions from functional data, and that quantitative methods for determining the spatial distribution of capillaries be devised.

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REFERENCES


