Capillary blood flow and tissue metabolism in skeletal muscle during sympathetic trunk stimulation

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Pál, Miklós, András Tóth, Peipei Ping, and Paul C. Johnson. Capillary blood flow and tissue metabolism in skeletal muscle during sympathetic trunk stimulation. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H430–H440, 1998.—NADH fluorescence at tissue sites 15–20 µm in diameter and red blood cell velocity in adjacent capillaries were measured in resting sartorius muscle of the anesthetized cat during a 3-min period of sympathetic trunk stimulation. At stimulation frequencies of 2 and 4 Hz, red blood cell velocity fell briefly to 30–40% of control and then returned to ~75% of control values (vascular escape). No change in NADH fluorescence was observed. With stimulus frequencies of 6–12 Hz, flow reduction was greater and led to an increase in fluorescence when the flow reduction was >50% and was sustained for >30 s. NADH changes were more pronounced at tissue sites near venous capillaries than at sites near arterial capillaries. Hyperemia ensued after the end of sympathetic stimulation only when NADH fluorescence rose during stimulation. Resting blood flow in this muscle appears to be well above the minimum required to support oxidative metabolism. A shift to anaerobic metabolism does not appear to cause vascular escape during sympathetic stimulation but appears to be required for poststimulation hyperemia. These observations suggest that two separate oxygen-dependent mechanisms elicit vasodilation during and after sympathetic trunk stimulation.

Blood flow regulation; red blood cell velocity; reduced nicotinamide adenine dinucleotide; anaerobic metabolism; skeletal muscle metabolism

BLOOD FLOW TO STRIATED muscle appears to be regulated in accordance with the oxygen requirements of the tissue. To explain this relationship, it has been suggested that, under normal conditions, certain tissue areas may be anoxic or hypoxic and produce vasodilator metabolites that act on the arterioles, causing them to remain open sufficiently to support normal oxidative metabolism in most tissue areas (2, 3, 23). Consistent with this concept, there is substantial heterogeneity in oxygen delivery to skeletal muscle and a wide range of cellular oxygen levels among different tissue areas (8, 22, 23, 25, 28).

A linkage between metabolism and blood flow has also been invoked to explain the secondary dilation of the arterioles (vascular escape) that occurs after the initial constriction when skeletal muscle blood flow is reduced during sympathetic trunk stimulation (2, 3, 11). Tissue oxygen levels in cat sartorius muscle fell during sympathetic trunk stimulation (3), which in turn should increase the number of tissue areas that are oxygen-deficient and elevate the production of vasodilator metabolites. Support for this explanation of vascular escape is provided by the finding that escape is virtually abolished if tissue PO2 levels are maintained by elevating suffusate PO2 (2, 3) or by maintaining a constant rate of blood perfusion during sympathetic stimulation (10).

However, it has not been shown until now that a fall in tissue PO2 during sympathetic stimulation leads to a shift in metabolic state, and other mechanisms by which oxygen might alter vascular tone are possible. If the vascular escape during sympathetic stimulation is due to a shift to anaerobic metabolism in parenchymal cells, it should be possible to detect this shift by monitoring the metabolic state in localized tissue areas.

To test this prediction, we made use of the fact that, when oxygen supply is inadequate to fully support oxidative metabolism, the mitochondrial oxidative phosphorylation chain becomes more reduced, and the level of the mitochondrial NADH increases. Changes in NADH levels in vivo can be detected by monitoring its fluorescence at 450 nm (4, 27). Using an instrument recently developed in our laboratory, we have determined that changes in tissue fluorescence at 450 nm in skeletal muscle are quantitatively related to changes in NADH concentration (26, 27). In the present study, we monitored NADH fluorescence in very localized tissue areas (15–20 µm in diameter) in the exteriorized cat sartorius muscle while measuring red blood cell (RBC) velocity simultaneously in the capillaries surrounding these areas during sympathetic trunk stimulation. A rise in fluorescence was taken as indicative of a shift in metabolic state. A number of tissue sites in the arterial and venous regions of the capillary network were chosen for study and are considered as representative of these areas.

MATERIALS AND METHODS

Twenty-one cats of either sex weighing between 0.85 and 1.1 kg were given an intramuscular injection of ketamine hydrochloride (Ketaset; Bristol Laboratories), 15 mg/kg body weight, followed by cannulation of the jugular vein for administration of α-chloralose at 38 mg/kg. Supplemental doses of 10 mg/kg α-chloralose were given as needed to maintain a surgical level of anesthesia. Arterial pressure recordings were obtained via the left femoral artery using a Statham pressure transducer.

The right sympathetic chain was isolated as described by Boegehold and Johnson (2, 3). A bipolar silver wire electrode made of coated, 0.005-in.-diameter wire (A-M Systems) glued inside a cuff of Silastic tubing, 2.5 mm in diameter, was wrapped around the chain between L4 and L5. The ends of the cuff were sealed with petroleum jelly to electrically isolate the bared portion of wire. For reduction of blood flow to the muscle, a snare of Silastic tubing or an inflatable occluder (In Vivo Metric Systems) was placed around the sacral artery just above the iliac bifurcation, and the incision was sutured.

The right sartorius muscle was exteriorized as described previously (2) leaving vascular and neural supplies intact. An inflatable vascular occluder (In Vivo Metric Systems) was...
placed on the femoral artery feeding the muscle. During surgery, the muscle was held at its in situ length and kept moist with Plasmalyte solution (Travenol Laboratories). The solution was composed of (in meq/l) 140 sodium, 10 potassium, 5 calcium, 3 magnesium, 103 chloride, 47 acetate, and 9 lactate. The solution was adjusted to pH 7.4 before use and warmed to 37°C. After surgery, the animal was placed on a Plexiglas platform with the muscle fastened at its length, dorsal side up, on a pedestal that was electrically heated to maintain a 36°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 silicon grease to prevent exposure to the atmosphere and drying.

The animal was then mounted on a microscope system specially designed to observe the microcirculation and to monitor simultaneously NADH fluorescence in vivo (27). The system contains two light sources: a 100-W mercury arc lamp that provides ultraviolet light for NADH excitation at 366 nm and a 75-W xenon light source that is used for general field illumination at 405 nm. The transmitted and fluorescent light is collected by an objective and diverted by a beam splitter to a video camera (Cohu 2000) and to two photomultiplier tubes. With this arrangement, the microscope field can be viewed on a video monitor (Sony SL-300: Sony) for off-line measurement of RBC velocity while the photomultiplier tubes monitor NADH fluorescence at 450 nm and the transmitted excitation light at 366 nm. Letz H-20 (numerical aperture 0.40) and H-32 (numerical aperture 0.60) objectives were used, giving a final video magnification of ×1,000 and ×1,390, respectively. A Letz H-32 (numerical aperture 0.60) objective was used as the condenser.

A 1-h period was allowed for equilibration of the muscle after mounting the animal on the microscope stage. The sartorius muscle was then transilluminated with the 100-W mercury light source. The thin central region of the muscle was chosen for study. Capillary networks with clear visibility for the dual window velocity measurements were selected using 405 nm illumination, which provides a good contrast image of the RBC and of the vasculature. For fluorescence measurements, small avascular areas 20–30 μm wide were selected within the capillary network either in close proximity to the wall with an arteriole or near the bridge of a collecting venule. We did not attempt to obtain paired arterial and venous sites in a single capillary network. The tissue was illuminated at 366 nm for visualization of the microcirculation and for excitation of endogenous NADH. The tissue fluorescence at 450 nm, the transmittance of the excitation wavelength (366 nm), and arterial blood pressure were recorded during the experimental procedures on the Deskpro microcomputer and a Gould model 3400 strip chart recorder, while the video image of the microcirculatory field (~200-μm wide) was recorded on a Sony Super beta cassette video recorder. RBC velocity in capillaries adjacent to the site of fluorescence measurement during experimental procedures was measured off-line from the video recordings by the dual window method (15).

Experimental protocol. The protocol at each site consisted first of a control period of 1.5 min followed by a 5-min period of blood flow stoppage to the muscle induced by inflating cuffs on both the abdominal aorta and the feeding artery to the muscle. This period of total ischemia has been used previously to achieve maximal elevation of NADH (26). Flow was then restored, and data were recorded until flow and fluorescence stabilized, followed by an additional 5-min recovery period during which illumination of the tissue was blocked to minimize photobleaching. Next, a protocol of sympathetic trunk stimulation was recorded, consisting of a control period of 1.5 min, 3 min of stimulation, and a 3-min recovery period followed by an additional 5-min recovery period with illumination blocked. A Grass SD9D stimulator was used to stimulate the sympathetic trunk with 5-ms pulses of supramaximal stimulus intensity at frequencies of 2–14 Hz as described by Boegehold and Johnson (2, 3). At some sites, the stimulation was repeated using different frequencies. After the stimulation procedures were completed, a second 5-min period of ischemia and a recovery period were recorded.

Data analysis. The change in NADH fluorescence during the stop flow period was taken as an indication of the initial metabolic state. If the change was <25%, which occurred infrequently, the experiment was not included in the analysis. This condition, as noted previously (26), was most likely due to the presence of an air bubble under the muscle.

In some cases, there was an unstable recording of the 366-nm transmittance, which usually indicated the presence of a layer of blood vessels beneath the monitored tissue area. Such areas were not included in the study. Also, if a sustained change of >10% occurred in the transmittance signal during the protocol, the data were excluded from the study. In some instances, a gradual, modest decrease (5–15%) in the fluorescence signal occurred over the course of the experimental protocol, probably due to photobleaching. In such instances, the data obtained were corrected with the assumption that the change occurred in linear fashion over time.

The fluorescence and transmittance data files for each ischemic or sympathetic stimulation period were normalized to the mean value during the initial control period, and changes were expressed as percentage of control.

RBC velocity in the two to four capillaries surrounding the site of fluorescence measurement during each protocol was measured off-line from the video recordings with the dual window technique and averaged. In general, the changes in velocity and fluorescence were greater at higher frequencies of stimulation. However, the magnitude of the initial fall in RBC velocity and degree of escape as well as change in fluorescence during sympathetic stimulation varied considerably among tissue sites. To better assess the relationship between vascular escape and metabolic state, experimental results were consolidated into three arterial and three venous capillary groups based on frequency of stimulation and on patterns of RBC velocity and fluorescence change. Unless specified otherwise, all data are presented as means ± SE. Comparisons between groups were performed with the Student’s t-test with P < 0.05 required for statistical significance. In certain studies, the SigmaStat software statistical package (Jandel Scientific) was used for linear regression analysis.

RESULTS

Data collection for each muscle studied typically lasted 5–11 h. Systemic arterial pressure at the end of the experimental period (110 ± 5 mmHg, N = 21) was not significantly different from at the beginning of the experiment (111 ± 6 mmHg, N = 21). Control RBC velocity did not differ significantly between the arterial capillary regions (0.096 ± 0.025 mm/s, N = 33) and the venous regions (0.081 ± 0.015 mm/s, N = 32).

Ischemia. NADH fluorescence was stable during the control periods. Five minutes of complete ischemia caused an average 160 ± 9% increase in NADH fluorescence at all sites (N = 32). The magnitude of the increase in NADH fluorescence during ischemia is very similar to that described in detail in a previous study in this muscle (26). In Figs. 1–3, the average values were...
attained at the end of the period of ischemia are shown for comparison with the changes seen at the same sites during sympathetic stimulation.

Sympathetic trunk stimulation. Data are presented from 15 arterial and 17 venous sites in 21 preparations. One to four successful stimulation protocols were carried out at each site using frequencies between 2 and 14 Hz for a total of 33 stimulations at arterial sites and 32 at venous sites. Sympathetic stimulation caused an initial drop in capillary RBC velocity, with the fall dependent on the stimulus frequency. The minimum flow attained at any 12-s period during stimulation was significantly lower at 8–10 Hz (4.5 ± 4.3%) compared with 2–4 Hz (30.1 ± 10.3%). A subsequent increase in RBC velocity (sympathetic escape) was observed at both arterial and venous sites and was dependent on stimulus frequency, being present at arteriolar sites in 90% of stimulations at 2 and 4 Hz but only 35% at 8 and 10 Hz. In venous sites, escape was found in 100% of tests at 2 and 4 Hz and 36% at 8 and 10 Hz. There was
no significant difference in control RBC velocity between the groups that showed escape and those that did not.

Stimulation at 2 and 4 Hz typically led to vascular escape during sympathetic stimulation without a significant change in tissue NADH fluorescence. This pattern was seen during eight stimulations at four arterial sites, 4 at 2 Hz and 4 at 4 Hz as shown in Fig. 1. A similar pattern was seen during eight stimulations at five venous sites, 3 at 2 Hz and 5 at 4 Hz as also shown in Fig. 1. RBC velocity during sympathetic stimulation typically dropped briefly below 50% of control and then returned toward the control value. The rise in velocity during escape was significant in the arterial sites at 108 to 180 s after the stimulation began and in the venous sites at 156 s. No significant difference was
found in RBC velocity between the arterial and venous areas. At 4 Hz stimulation, the response at one arterial site and one venous site fell into patterns otherwise seen only at higher stimulus frequencies and are included in those groups.

A second pattern was characterized by a sustained reduction in velocity by 80–100% without escape and with an increase in NADH fluorescence (Fig. 2). This behavior occurred almost exclusively at higher frequencies of stimulation and was seen at six arterial sites during 14 3-min periods of sympathetic trunk stimulation, one stimulus period at 4 Hz, 2 at 6 Hz, 5 at 8 Hz, and 6 at 10 Hz. In these studies, NADH fluorescence began to rise 30–40 s after flow reduction, reaching a mean value of 141 ± 5.5% above the control level. As also shown in Fig. 2, a similar pattern was seen at six venous sites during 12 periods of stimulation, 4 at 6 Hz, 5 at 8 Hz, 2 at 10 Hz, and 1 at 12 Hz, with fluorescence beginning to rise 30–40 s after flow reduction and reaching a mean value of 145 ± 5.4% above the control.
level. At the end of the stimulation period, RBC velocity was $6.2 \pm 3.4\%$ of control at the arterial sites, which was not significantly different from the level at the venous sites ($21 \pm 14\%$). After the stimulation period was completed, RBC velocity returned to the control level in 36 s at the arterial area and in 120 s at the venous area and subsequently increased significantly above control at both sites. Flow returned subsequently to control levels 3 min poststimulation. NADH fluorescence returned to control within 20–60 s after stimulation ended.

During higher-frequency stimulation, some sites showed significant vascular escape, as shown in Fig. 3. At five arterial sites, vascular escape occurred during two periods of stimulation at 6 Hz, three at 8 Hz, four at 10 Hz, and two at 12 Hz. RBC velocity decreased to $31 \pm 16\%$ of control for 20 s and then gradually rose to a significantly higher level, reaching $84 \pm 13\%$ of control at the end of the stimulation period. Although a slight increase in fluorescence may have occurred, the change was not statistically significant. At six venous capillary sites, significant escape associated with increased fluorescence was found during 12 periods of stimulation, 1 at 4 Hz, 3 at 6 Hz, 3 at 8 Hz, 2 at 10 Hz, 1 at 12 Hz, and 2 at 14 Hz, as shown in Fig. 3. RBC velocity decreased to $13 \pm 7\%$ for a period of 50 s and then rose above control ($128 \pm 21\%$) as the stimulation continued. NADH fluorescence transiently increased to $130 \pm 5\%$ and then slowly returned to the control level by the end of the stimulation period. The return of fluorescence during stimulation appeared to lag behind the return of RBC velocity, perhaps reflecting significant oxygen loss to any adjacent tissue areas where flow remained low.

It appears from examination of Fig. 3 that flow reduction induced a greater rise in NADH at venous sites than at arterial sites. In fact, NADH rose significantly in 72% of all tests at venous sites and at only 42% of stimulations at arterial sites. In addition, the increase in fluorescence (expressed as the %rise in fluorescence times the duration of the increase) was more than two times greater in tests at venous sites ($49.7 \pm 8.5$) compared with arterial sites ($23.0 \pm 5.8$), as shown in Fig. 4. However, RBC velocity fell to the same average level during stimulation at venous sites ($46.3 \pm 4.3\%$ of control) as at arterial sites ($46.6 \pm 4.3\%$ of control).

From inspection of data at arterial and venous capillary sites in Fig. 1 and arterial capillary sites in Fig. 3, it appears that escape occurred in the absence of a significant rise in NADH. To evaluate this relationship further, we compared the escape index (the secondary return of flow as a fraction of the initial drop) with the increase in NADH expressed as the value above control in percent times the duration of the increase in minutes. As shown in Fig. 5, top, at arterial sites, escape and NADH increase appear to be almost completely dissociated; in only two instances did NADH rise substantially in the presence of vascular escape. The control RBC velocity at one of these sites ($0.046 \pm 0.006 \text{ mm/s}$) was significantly less than the mean for arterial sites ($0.096 \pm 0.025 \text{ mm/s}$). For the group overall there was a significant negative correlation between escape and the rise in NADH fluorescence, and the intercept was significantly different from zero. At venous sites as shown in Fig. 5, bottom, vascular escape was not dependent on a rise in NADH. The intercept was significantly different from zero, but the slope of the relationship between escape and NADH fluorescence was not.

It is of interest that poststimulation hyperemia was seen at arterial and venous sites in Fig. 2 and venous sites in Fig. 3 where NADH also rose during sympathetic stimulation but was not present at arterial and venous sites in Fig. 1 or at arterial sites in Fig. 3 where NADH fluorescence did not increase. To evaluate this relationship further, we plotted the magnitude of the poststimulation hyperemia versus the magnitude of the NADH fluorescence increase, both expressed as percentage rise above control multiplied by duration in minutes. As shown in Fig. 6, these two quantities were significantly related at both arterial and venous capillary sites; the slopes were significantly different from zero, whereas the intercepts were not significantly different from zero.

Relationship between reduction in RBC velocity and NADH fluorescence. Because the rise in NADH fluorescence appeared to depend on both the degree of flow reduction and duration, we analyzed the effect of both variables on the fluorescence signal. Figure 7 shows the NADH fluorescence change during the 3-min stimulation period with various degrees of flow reduction. It can be seen that a reduction of at least 50% in RBC velocity for at least 30 s was required to produce a rise in NADH fluorescence. A rise in fluorescence occurred more commonly at venous sites, as noted above.

An example of the time dependence of the increase in NADH during flow reduction at a venous capillary site is shown in Fig. 8. In this instance, periodic changes in RBC velocity due to vasomotion were observed, but these short-term changes in flow had no effect on the...
tends to restore blood flow and oxygen supply (1). A further elaboration of this theory by Schubert et al. (23) proposed that at least in cardiac muscle there are hypoxic regions normally present in the vicinity of the venous end of the capillary network that produce vasoactive metabolites that maintain the arterioles in a partially open state. Tissue sites with $P_O_2 < 5$ mmHg (which was considered to be the limit for oxidative phosphorylation) were found in the working heart (23).

In support of the concept proposed by Schubert et al. (23), Hanson and Johnson (12) found oxygen consumption in resting skeletal muscle to be highly dependent on blood flow, which would provide a feedback mechanism in which oxygen delivery is maintained according to tissue oxygen requirements. At the microcirculatory level, a microscale heterogeneity of blood flow distribution has been observed (7, 25) in skeletal muscle. Local regulatory mechanisms apparently modulate this heterogeneity of blood flow.
erogeneity to a degree (22), but at the level of the parenchymal cells a wide range of $P_O_2$ values is found (3, 6, 28). This heterogeneity of oxygen availability could limit the oxidative metabolism of a significant population of parenchymal cells at any particular time. However, evidence for flow dependence of oxygen consumption in whole organ studies is not universal. Stainsby and Otis (24) found that oxygen consumption in skeletal muscle of the dog hindlimb was independent of blood flow at rates $>1-2$ ml·min$^{-1}$·100 g$^{-1}$.

Durán and Renkin (8) observed both flow dependence and flow independence of oxygen consumption in different preparations of dog gracilis muscle and noted that one explanation for the latter behavior could be limitation of oxygen delivery by closure of capillaries or precapillary vessels with higher flow.

In our studies, a rise in NADH fluorescence was taken as an indication of a shift to anaerobic metabo-
lism due to lack of oxygen. The basis for this is considered in detail elsewhere (4, 26, 27). As shown in Fig. 7, a flow reduction of at least 50% for at least 30 s was required to increase the NADH level. Thus the tissue in this muscle is generally well oxygenated, and a considerable reduction in flow can occur before a shift to anaerobic metabolism takes place. This observation does not support the hypothesis that there are ischemic or hypoxic tissue areas in resting cat sartorius muscle. The finding is in agreement with our previous observation in arterial occlusion studies in this muscle that there is generally a considerable time lag averaging 48 ± 22 (SD) s after complete flow stoppage before NADH begins to rise (26).

It would be expected that tissue sites near the venous end of the capillary network where oxygen tension is lower would be more susceptible than arterial sites to oxygen deficiency and a shift to anaerobic metabolism. There is evidence that this is the case in the present study since, as shown in Fig. 4, the magnitude of increase in fluorescence was about two times greater at venous sites during sympathetic stimulation despite the fact that the fall in flow was the same as at arterial sites. In addition, as shown in Fig. 5, NADH fluorescence only rarely increased at arterial sites when vascular escape occurred, whereas it was much more common at venous sites during escape. This suggests that partial return of blood flow during escape is sufficient to maintain adequate oxygen delivery to arterial capillary sites but not to the downstream venous sites. In contrast, during total flow stoppage in this same muscle, the mean latent period before NADH fluorescence rose was virtually identical at venous and arterial capillary sites [47 ± 22 and 49 ± 22 s, respectively (26)]. As noted in that study, although oxygen tension would be lower on average at venous sites, the venous capillary network also has a greater blood volume and therefore a greater oxygen storage capacity during flow stoppage. When flow is reduced but not stopped, the greater storage capacity at venous sites would only confer a temporary benefit and would not permanently offset the deficit in oxygen delivery by the bloodstream.

Oxygen tension and vascular escape. Previous studies in the cat sartorius muscle by Boegehold and J ohnson (2) have shown that vascular escape from sympathetic stimulation is almost completely abolished by elevating the suffusate level of oxygen over the muscle. They concluded that a fall in oxygen levels in the muscle during sympathetic stimulation led to vascular escape. Subsequent studies revealed that elevation of suffusate level raised the parenchymal tissue Po2 levels during sympathetic trunk stimulation but not periarteriolar Po2 levels (3). This appears to eliminate the possibility that oxygen-sensing mechanisms within the arteriole itself such as autacoids released from the arterial endothelium (13, 18, 20) or shift to anaerobic metabolism of the arteriolar smooth muscle (16) are responsible for escape. They also found during sympathetic trunk stimulation under normoxic conditions that interstitial Po2 in several tissue areas in the vicinity of venous capillaries fell to 2 mmHg or less, which could induce a shift to anaerobic metabolism.

Anaerobic metabolism and vascular escape. Based on their observations cited above, Boegehold and J ohnson (3) proposed that vascular escape was due to a shift from aerobic to anaerobic metabolism in muscle fibers at sites of low tissue Po2. Their findings do not support this hypothesis. Significant escape was observed in this study with 2 and 4 Hz sympathetic chain stimulation without a change in NADH fluorescence at any of the arterial or venous tissue sites studied (Fig. 1). A similar finding was obtained at higher frequencies of stimulation in arterial areas (Fig. 3). Also, as shown in Fig. 5, there was not a positive correlation between the degree of escape and the rise in NADH at arterial and venous sites. With the assumption that the rise in NADH is coincident with a shift to anaerobic metabolism, these findings indicate that vascular escape is not dependent on a shift in the parenchymal cells to anaerobic metabolism. At high frequencies of stimulation, the situation may be more complex, as seen at venous capillary sites in Fig. 3. In these preparations, NADH rose concurrently with escape but then returned to control levels by the end of the stimulation period while escape persisted.

Possible mechanism of vascular escape. The vascular escape seen in sartorius muscle appears to be due to a linkage between tissue oxygen tension and vascular tone (2, 3) but, based on present findings, does not require a shift in NADH level of the parenchyma. Among the explanations that can be considered are a linkage through oxidases having a higher Michaelis constant for oxygen than oxidative phosphorylation as suggested by Duling (6) or through sites in the electron transport chain more sensitive to changes in oxygen levels than NADH, as suggested by Wilson et al. (29). Such mechanisms could be initiated at a higher oxygen tension than that required to alter the NAD/NADH redox couple. In addition, the possibility that other tissue sites such as capillary or venular endothelial cells release endothelin-derived relaxing factor or conduct vasodilator stimuli (30) when tissue or capillary blood oxygen levels fall cannot be excluded. These findings may also have implications for other local regulatory phenomena such as blood flow autoregulation in which vasodilator occurs well before the 50% reduction in blood flow required for a rise in NADH fluorescence occurs (19).

Role of anaerobic metabolism in poststimulation hyperemia and vascular escape. An unexpected finding in these studies was the relationship between a rise in NADH during stimulation and the hyperemia after stimulation, as shown in Fig. 6. On the basis of this observation, a shift to anaerobic metabolism during reduced flow appears to be a necessary precondition for poststimulation hyperemia. Such a shift was not always sufficient to cause hyperemia, perhaps because, in some instances, the sympathetic transmitter that accumulated during the stimulation period dissipated more slowly, allowing a more gradual return of flow. This may also be the cause of the delay in hyperemia at
venous sites shown in Fig. 3. In support of this explanation, Renkin (21) has shown that, when postocclusive hyperemia is prevented in skeletal muscle or intestine by perfusion at constant flow, the vasodilatation gradually disappears, although at a slower rate than in a free-flow preparation.

Several mechanisms have been proposed by which a change in parenchymal tissue metabolism could provide flow regulatory feedback to the supplying arterioles. Vasodilator metabolites released from the nearby parenchyma could readily diffuse to the arteriolar wall. Our data shown in Fig. 4 indicate that metabolic shifts can occur at low flow in parenchyma adjacent to arterial capillaries; however, such tissue areas are less susceptible to change than those near venous capillaries. Several recent studies suggest that vascular sites downstream from the arterioles may participate in flow regulation. The capillary network itself has been shown to sense and transmit vasoactive stimuli to the upstream arteriolar network (see Ref. 30 for references). ATP, released from RBC as they become deoxygenated in passage through the capillary network, may initiate such vasodilator stimuli (9). In addition, vasoactive substances present in venular blood can alter tone of nearby arterioles (14). Such mechanisms could together provide an integrated regulation of arteriolar tone in accordance with the metabolic needs of the tissue supplied.

A striking difference between the findings with escape and poststimulation hyperemia is the importance of the shift in NADH for hyperemia but not for escape. Based on this observation, we suggest that different mechanisms are responsible for escape and for poststimulation hyperemia. A point of similarity of the two mechanisms is that both appear to be oxygen dependent.

Vasomotion and anaerobic metabolism. Periodic changes in organ blood flow (flow motion or vasomotion) have been observed in the skeletal muscle during control and ischemic conditions (5). Studies on the brain revealed that ischemia-induced flow motion was correlated with similar fluctuations in tissue metabolism (17). It has been postulated that periodic shifts from aerobic to anaerobic metabolism cause the rhythmic contraction and dilation of the arterioles. In our study, we did not observe periodicity in NADH fluorescence during vasomotion. As shown in Fig. 8, a brief flow reduction as occurs spontaneously during vasomotion is not of sufficient duration to alter NADH fluorescence, although a sustained reduction in flow to the same level does produce a significant increase in fluorescence.

It appears therefore that the flow reduction during vasomotion may be too brief to initiate a shift to anaerobic metabolism.

Conclusions. In conclusion, these studies show that, in resting cat sartorius muscle, blood flow appears to be well above the minimum required to support oxidative metabolism; a flow reduction of at least 50% was required to induce a shift to anaerobic metabolism in the muscle, based on the criterion of a rise in NADH fluorescence. In addition, these studies reveal that vascular escape during sympathetic trunk stimulation is not due to a shift from aerobic to anaerobic metabolism. By contrast, poststimulation hyperemia was present only when a shift to anaerobic metabolism occurred. It appears therefore that two separate mechanisms, both triggered by a fall in tissue oxygen tension, are responsible for vascular escape from sympathetic stimulation and for poststimulation hyperemia. In addition, the correlation between a rise in NADH and poststimulation hyperemia provides further evidence for the metabolic hypothesis of blood flow regulation that a shift to anaerobic metabolism in the parenchyma leads to vasodilatation. In view of the evidence cited above that release of vasoactive factors from endothelium of arterial vessels may depend on local oxygen tension, the present observations point to a multiplicity of mechanisms that tend to maintain oxygen delivery when parenchymal or vascular oxygen tension falls.

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