Effect of occlusion duration on reactive hyperemia in sartorius muscle capillaries

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The phenomenon of reactive hyperemia has been demonstrated in the vascular beds of virtually all major organs of the body. The magnitude of the response varies from one tissue to another, being weak in liver (6) and kidney (8) but strong in myocardium (4, 5) and skeletal muscle (7, 11). The response also is dependent upon a variety of experimental factors including tissue temperature (20), intravascular pressure (14), and duration of occlusion (13).

Occlusion duration is an especially important factor in determining the magnitude of reactive hyperemia in skeletal muscle (13) and myocardium (4). Both peak flow and duration of hyperemia increase as the occlusion length increases. On this basis it is often supposed that reactive hyperemia in muscle is metabolically mediated, perhaps through accumulation of vasodilator metabolites or depletion of oxygen supply during the period of flow stasis.

However, there is also evidence that myogenic factors may be important in skeletal muscle during reactive hyperemia. Hyperemia can be induced in resting muscle by ischemic periods of a few seconds' duration, which is seemingly too short for significant metabolic changes to occur (11). Reactive hyperemia is considerably diminished when intravascular pressure is maintained during the occlusion (14).

According to the metabolic concept of reactive hyperemia, the degree of vasodilation should be dependent upon the duration of flow stoppage. According to the myogenic concept the vasodilation should be related to the intravascular pressure drop during the occlusion. The latter would be a function of occlusion duration only with very brief occlusions. Perhaps more significantly, the additional volume of blood which flows through the muscle in reactive hyperemia (excess flow) should be highly dependent upon occlusion duration according to the metabolic hypothesis, while according to the myogenic hypothesis it would not. In the present study we determined the effect of occlusion duration upon the magnitude of capillary reactive hyperemia as a means of assessing the relative contributions of metabolic and myogenic factors. The magnitude of capillary reactive hyperemia was also compared with the total flow response from the same muscle.

METHODS

Experiments were performed on 15 cats in a weight range of 1.4 - 2.6 kg. The animals were tranquilized with 1 mg/kg of propiopromazine hydrochloride and anesthetized with 75 mg/kg of alpha chloralose. The sartorius muscle was denervated and surgically isolated from the left hindlimb, arterial and venous cannulations were performed, and perfused with blood from the right femoral artery. The animals were heparinized (750 U/kg) shortly before cannulations, and booster doses of 375 U/kg heparin were given every 30 min during the experiment. The muscle-isolation technique has been described in detail in an earlier report (2). Arterial and venous pressures were monitored by Statham pressure transducers. Total venous outflow from the isolated muscle was measured in eight cats, using a recording drop counter. The venous blood was returned to the animal through the femoral vein. Venous outflow pressure was maintained at about 2 mm Hg for all experiments. The isolated, perfused muscle was mounted on a heated microscope stage and maintained at 37°C. The muscle was bathed with a physiological irrigating solution and covered with Saran Wrap. The muscles used in these experiments weighed an average of 5.4 g.

Red blood cell velocities in the capillaries were measured with use of the dual-slit photometric method of...
Wayland and Johnson (18). The muscle was transilluminated with a mercury-arc lamp, and the magnified image of the capillary under study was projected onto a screen containing dual parallel slits. The intensity of the light falling upon each slit was monitored with RCA 6199 photomultiplier tubes. When a red cell image crossed the slits, the light intensity on each slit was momentarily decreased causing pulses in the photomultiplier outputs. The time delay between the pulses of the two photomultiplier outputs was inversely proportional to the velocity of the red cell. The time delays were determined continuously with a Hewlett Packard 3721A correlator and the velocity was computed from the time delay with an analog divider circuit. The output of the divider circuit was continuously recorded with a Beckman type-R strip-chart recorder. Details of the velocity measurement and photomultiplier signal analysis have been described previously (18).

During observation of a single capillary, the arterial inflow to the muscle was occluded several times for intervals ranging from 5 s to 2 min. Following each occlusion, a period of 5 min or more was allowed for recovery from reactive hyperemia and the reestablishment of control conditions. From 3 to 23 occlusions were performed during the observation of each capillary, with 28 capillaries being studied in this manner. Capillaries were selected only on the basis of their suitability for visual observation and measurement.

**RESULTS**

A) Total blood flow. Control blood flow was $3.3 \pm 0.20$ (SE) ml/100 g·min, based on data from 83 measurement periods in eight cats. The effect of arterial occlusion on venous outflow from the sartorius muscle is shown in Fig. 1. This is a typical experiment and in it several things are apparent. First, the peak flow is highly dependent upon the duration of occlusion. Occlusions of 15 s duration produce a small, short-lived increase in flow after release. The peak flow gradually increases with longer occlusions while the duration of the hyperemic response is not particularly dependent on occlusion duration with occlusions of up to 60 s.

Shown in Fig. 2 is the effect of occlusion duration on peak flow and excess flow volume based on repeated measurements in eight preparations. The excess flow represents the integrated area under the curve of reactive hyperemia above control. Considering the peak flow first, it is apparent that this factor shows a gradual increase over the occlusion range tested. Following occlusions of 10-15 s duration, the peak flow is about 60% above control and rises to 250% above control after a 120-s occlusion. As shown in the lower panel, excess flow volume increases from about 0.27 ml/100 g for the 10- and 15-s occlusions to 1.90 ml for a 2-min occlusion.

Comparing the peak flow and excess flow volume responses following 10- to 15-s and 2-min occlusions, we note that the former increases by a factor of 4.5 and the latter by a factor of 7. It is apparent that the rise in peak flow is not sufficiently great to account entirely for the increase in excess flow volume. The greater rise in the latter is probably accounted for by the fact that the response duration increases after the 2-min occlusions.

B) Capillary flow. The control red cell velocity averaged $0.38 \pm 0.02$ (SE) mm/s, based on data from 213 control periods in 28 capillaries. An example of capillary reactive hyperemia is presented in Fig. 3. A 10-s occlusion produced a modest reactive hyperemia followed by a prolonged period of zero flow. This capillary was exhibiting periodic flow behavior during the control period. With slightly longer occlusions (15-20 s) the peak flow became higher and the period of hyperemia was somewhat extended. In these instances a period of zero flow
flow was also evident following recovery from hyperemia. With 60-s occlusions the peak flows were about 20% higher than with the 15- and 20-s occlusions and the responses were slightly longer. It is also worth noting that flow appeared to be sustained at near-maximal levels for a longer period with the longer occlusions. After 60-s occlusions a period of complete flow stoppage did not occur, in contrast to the shorter occlusions. Also, flow reached its peak value somewhat later following the longer occlusions.

The data on peak flow and excess flow for all capillaries are shown in Fig. 4. The peak-to-control ratios of the individual capillaries were approximately 3:1 following 10-s and 15-s occlusions and increased moderately to about 3.8:1 following occlusions of 60 and 120 s duration. Occlusions of 5 s duration produced only modest vasodilatation. Control flow values were taken as the average during the 60 s preceding occlusion.

The excess flow in capillaries is calculated from the area under the curve of reactive hyperemia above the control velocity. It is expressed in terms of the length of a column of blood of capillary dimensions. Excess capillary flow increased from about 2 mm after 10- and 15-s occlusions to approximately 14 mm following 120-s occlusions. It is evident that the increase in excess flow is due principally to a prolongation of the response since the peak flow did not increase greatly with the longer occlusions. Because of variability in capillary flow patterns, the duration could not be accurately determined in many of the capillaries.

The principal area of difference between capillary and volume flow reactive hyperemia was the peak flow response. Peak volume flow showed a graded increase as the occlusions became longer, while peak capillary flow reached near maximal values with short occlusions. Possible explanations for the difference in the two measurements are presented in the DISCUSSION.

**FIG. 3.** Capillary flow patterns from a single sartorius muscle preparation with varying occlusion duration.

**FIG. 4.** Effect of occlusion duration on capillary red cell velocity in 28 capillaries. Vertical bars represent SEM.

Comparison of capillary flow and total blood flow. There was an increase of about sevenfold in excess capillary flow as occlusion duration rose from 10 to 120 s. A similar increase in excess volume flow also occurred over this same span of occlusion times. Another area of considerable similarity between the two is in flow debt repayment, which is the ratio of excess flow to the flow deficit incurred during the arterial occlusion. The flow debt repayment (shown in Table 1) averaged 24.9% in volume flow and 29.7% in the capillary flow. These figures are the average values for all occlusion times of 10 s duration or more. No trend was discernible in the average values for flow debt repayment as the occlusion duration was varied in the range 10–120 s.

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tion, since the pressure fell to 40 mmHg in the first 5 s of occlusion from a control level of 105 mmHg and subsequently declined at a rate of about 1 mmHg/4 s. In earlier studies on cat mesentery we found a time lag of about 6 s between a step increase in venous pressure and the initiation of a constrictor response in the precapillary vessels (10). A similar period elapsed between reduction of arterial pressure and precapillary vasodilation, as manifest in capillary flow recordings (9, 10). On this basis it would appear that 10 s of occlusion are sufficient to initiate myogenic relaxation.

The finding that there was no increase in capillary excess flow when the occlusion duration was increased from 10 to 30 s suggests that there is probably no substantial accumulation of metabolites or lack of oxygen during the first 30 s of occlusion in this muscle. However, the sharp increase in excess flow when the period of ischemia is extended to 60 and 120 s is consistent with the notion that metabolic factors do become important with the longer occlusions.

Examination of the excess flow data in the volume flow studies leads to the same conclusions, since this factor was also substantially unchanged with 10- and 30-s occlusions. However, the peak volume flow showed a graded increase as occlusions became longer. There are two factors which might explain this discrepancy between capillary and volume flow. First, the volume flow recording technique we used was a drop counter, which integrates the flow over the interval between drops. This interval was approximately 2-3 s at the peak of reactive hyperemia. The red cell velocity system, on the other hand, has a time constant of approximately 1 s. Thus the red cell velocity recording would more nearly reflect instantaneous flow values at the peak of reactive hyperemia. However, since the peak flows persisted at near-maximal levels for more than 1-2 s, as seen in Fig. 3, the time constant of the drop counter is not a major reason for the discrepancy.

Probably more important is the fact that all capillaries do not reach their peak flow simultaneously. In an earlier study (3) we noted that the time-to-peak flow in capillaries following release of occlusion varied from 2 to 14 s. If the peak flow persists for a substantial period of time (i.e., 20-30 s), the variability in rise time might be relatively unimportant. This is probably true with the hyperemia which follows the longer occlusions. However, when the hyperemic phase is short, peak flows in the capillaries will not occur simultaneously and the volume flow will not accurately reflect the maximum vasodilation which occurs. The effect of occlusion duration on the pattern of hyperemia is evident in Fig. 3, in which the capillary red cell velocity maintains near-maximal levels for a longer time following the longer occlusions. This is probably the principal reason for the low peak-to-control ratio in volume flow following the short occlusions. It is perhaps significant that peak-to-control ratios from capillary and volume flow recordings are in good agreement following the longer occlusions, when hyperemia persists for a longer time. In addition, there may be a transient expansion of the veins and storage of blood in this compartment during reactive hyperemia. These observations lead us to suggest that, in general, volume flow recording can substantially underestimate the degree of vasodilation (or vasoconstriction) which may be taking place during rapid, transient phenomena. Capillary flow measurements would seem to provide a more accurate estimate of the magnitude of vasodilation which occurs in such instances.

It has been noted earlier that capillary and volume flow measurements are in good agreement as regards integrated values such as excess flow and flow debt repayment. The values for the excess flow rose about sevenfold in each case as occlusion duration increased from 10 to 120 s and flow debt repayment values were nearly identical, being 25% for volume flow and 30% for capillary flow. These values should be in close agreement if the capillaries chosen are a reasonable sample of the total population that contributes to the volume flow recording. The agreement suggests that the capillaries studied do not behave in a different manner from the population as a whole. Moreover, these studies provide no evidence that the muscle contains a substantial population of capillaries which flow during reactive hyperemia but are otherwise closed. If such a population existed in this muscle, we would expect to find a proportionately greater increase in excess flow and flow debt repayment in the volume flow than in the capillary red cell velocity. It is of course possible that such a population exists but is masked by another group in which flow is continuous during control and does not increase during reactive hyperemia. All capillaries studied by us flowed during the control period, although flow was intermittent in some. Similarly, these data do not support the notion that arteriovenous shunts open during reactive hyperemia in this muscle. Such a possibility has been raised by others in gross flow studies (5). If such shunts exist, they must behave in much the same manner as the capillaries.

In an earlier study of reactive hyperemia in calf muscles of the cat Konradi and Levmov (11) found a substantial hyperemia in volume flow following relatively short occlusions. In their studies, a 3-s occlusion produced a 43% increase in flow at the peak of reactive hyperemia and an excess flow of 2.7 ± 0.6 ml/100 g tissue. Prolongation of the occlusion to 30 s produced virtually the same peak flow (49% above control) and excess flow (3.0 ± 0.5 ml/100 g). The hyperemia persisted for 1.8 ± 0.2 min following the 3-s occlusion and 2.3 ± 0.2 min following the 30-s occlusion. Because the response was very nearly the same following 30 s of occlusion as after 3 s, they suggested that the response to short occlusions was
principally myogenic. When the occlusions were extended to 1, 2, and 4 min the peak flow increased in a graded fashion to 155% above control, excess flow rose to 17.5 ± 4.0 ml/100 g, and duration of hyperemia increased to 6.0 ± 0.9 min. These increases were taken as indicative of a metabolic factor becoming important with the longer occlusions. While our values for peak flow and excess flow are quantitatively quite different from those reported by Konradi and Levmov, both studies lead to the same conclusions regarding the operative mechanisms following short and long occlusions. Our comments above regarding deficiencies in the volume flow method for assessing the degree of vasodilation following brief occlusions would not apply to Konradi and Levmov's study since the hyperemic response in their preparation was quite prolonged even with short occlusions.

Our findings and those of Konradi and Levmov provide qualified support for the concept proposed by Barcroft (1) that a finite period of circulatory arrest may be required before metabolic factors become important in reactive hyperemia. Barcroft suggested that a period of 3 min is necessary for a fully developed metabolic effect on the basis that most muscles probably have sufficient stored oxygen to support metabolism during this period. 3 min is necessary for a fully developed metabolic effect on the basis that most muscles probably have sufficient stored oxygen to support metabolism during this period of time. Of course, such a latent period would vary considerably from muscle to muscle, depending on myoglobin content and $O_2$ consumption. Our data and those of Konradi and Levmov (11) suggest a time period greater than 30 s but less than 60 s for the metabolic effect to become apparent. It is possible that an increase in metabolite concentration is occurring in the first 30 s but has not reached threshold levels for vasodilator action on the vasculature. In addition, the possible contribution of aerobic metabolites which possess vasodilator properties cannot be ignored, as noted by Kontos (12).

Our findings support the hypothesis that brief interruptions of the blood supply to cat sartorius muscle (approximately 10-30 s in this study) cause a hyperemia which is probably largely of myogenic origin. Longer occlusions lead to a prolongation of the hyperemia, presumably due to metabolite accumulation. The time scale for metabolic effects probably varies substantially from tissue to tissue and even from one skeletal muscle to another. Thus the dissociation of myogenic and metabolic effects which is seen in cat sartorius would not necessarily obtain in other muscles.

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