Role of Potassium Ions in the Vascular Response to a Brief Tetanus

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ABSTRACT
Changes in the plasma potassium ion (K⁺) concentration in the venous effluent of isolated dog tibialis cranialis and extensor digitorum longus muscles were measured following 1 second of tetanic exercise (32 impulses/sec) during constant-flow perfusion. After a brief delay, venous plasma K⁺ concentration increased to a peak change of 0.23 ± 0.02 mEq/liter and then returned to the control level within 1.5 minutes after the beginning of stimulation, representing a net K⁺ loss of 1.7 μEq/100 g muscle. These experiments indicate a very short period of actual tissue-blood K⁺ transfer, since the time course of the venous efflux can be explained almost entirely by the distribution of vascular transit times. Our best estimate is that a 1-second tetanus produces an immediate 1.3-2.4-mEq/liter increase in interstitial K⁺ concentration which then returns to its control level with a time constant of 6-8 seconds due to rapid cellular reuptake. These K⁺ concentration changes have sufficient magnitude and the correct time course to play a significant role in the production of the vascular response to a brief tetanus.

KEY WORDS
- metabolic vasodilation
- skeletal muscle
- dog
- active hyperemia
- transcapillary flux
- potassium loss
- venous dispersion

The mechanisms involved in the local control of skeletal muscle blood flow are incompletely understood (1), but it appears that several factors act in concert to produce the increase in flow associated with exercise (2, 3). We have recently reported studies which support this idea and suggest that different factors dominate the control of vascular resistance in different experimental situations (4, 5). In particular, we have found that the vascular response to a brief tetanus is apparently too rapid to be caused by factors directly associated with muscle oxidative metabolism (4). A myogenic response to the extravascular compressive forces associated with a brief tetanus could be responsible for one-third to one-half of the response (6). Since the potassium ion (K⁺) has been strongly implicated as a metabolic vasodilator (2, 7, 8), we undertook the present study to determine the magnitude and the time course of K⁺ concentration changes associated with brief tetanic exercise and to evaluate the role of these changes in the vascular response to a brief tetanus.

Methods
We used seven mongrel dogs (20-25 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv, supplemented as required) and heparinized with sodium heparin (750 units/kg, iv, supplemented with 500 units/hour). We exposed the tibialis cranialis (TC) and extensor digitorum longus (EDL) muscles of the calf by making a longitudinal skin incision extending from the knee to the ankle on the anterior aspect of the left leg. After removing the fibularis longus muscle, with care not to disrupt the underlying fibular nerve, we severed the tendons of the TC and EDL muscles at the level of the distal transverse ligament. We cannulated the cranial tibial artery at the level of the proximal transverse ligament to obtain pressure measurements. We tied and cut the cranial tibial artery and vein just distal to this cannula. Working proximally, we freed the TC and EDL muscles and the accompanying vascular and nervous supply from other structures. The femoral insertion of the muscularis semimembranosus was severed, and the muscle was reflected to expose the underlying fibular nerve. The nerve was severed and freed from underlying structures to obtain a 5-cm length leading to the TC and EDL muscles. We exposed the cranial tibial artery and vein in the popliteal region after the femoral insertion of the gastrocnemius muscle had been severed. Removal of a 4-cm length of the head of the fibula completed the exposure of the cranial tibial artery and vein. We tied and cut all branches of these vessels to structures other than the TC and EDL. We then cannulated the cranial tibial artery and vein with PE 190 tubing; the flared tips of the cannulas were positioned near the tibia and tied in place. Blood was supplied from the contralateral femoral artery via a roller pump. Venous effluent returned to a funnel reservoir communicating with the contralateral femoral vein. Venous pressure was constant at about 5 mm H₂O as determined by the height of the free end of the outflow tubing. A portion of the venous effluent (2 ml/min) was drawn continuously through a cuvette densitometer to monitor venous O₂ saturation. Silastic tubing (Dow Corning) was used throughout the extracor-
poreal circuit except for short polyethylene cannulas and oximeter tubing. Paradise et al. (9) have used a similar preparation involving only the EDL muscle.

The free ends of the TC and EDL tendons were folded back on themselves and lashed to form a loop and then attached to transducers to obtain measurements of isometric force. We produced a brief tetanus with a 1-second burst of square current pulses with supramaximal parameters (1 msec, 1–5 mA) at a frequency of 32/sec delivered to the fibular nerve via platinum electrodes.

We collected venous blood samples in microhematocrit capillary tubes at timed intervals associated with muscular contraction. We immediately centrifuged the tubes and drew a 20-μl sample of plasma from each tube into calibrated disposable glass capillary pipettes. These samples were diluted 1:100 for determination of K⁺ concentration by flame photometry. The standard deviation of duplicate determinations on the same sample was ± 0.048 mEq/liter.

Throughout all experiments, flow was held constant with the roller pump. Venous K⁺ responses to a brief tetanus were observed with and without concurrent maximal dilation of the vascular bed with papaverine. A temporary maximal dilation lasting approximately 10 minutes was induced 4 minutes prior to a tetanus trial by a single close intra-arterial injection of 0.3–0.5 ml of papaverine HCl (30 mg/ml). A recovery period of 30 minutes was allowed following papaverine trials.

In each experiment, we measured the distribution of transit times through the vascular bed at the constant flow rate employed by observing the time course of the appearance of a dye, indigo carmine, at the venous blood sampling site following a close intra-arterial bolus injection. Dye concentration was measured with the oximeter positioned at the venous sampling site. This procedure measures transit delay and dispersion due to the vascular bed and the venous outflow tubing together.

Statistical treatment of the data followed standard statistical methods (10). Numerical analysis was performed on an IBM 360/67 digital computer.

**Results**

A representative response of our muscle preparation to 1 second of tetanus is shown in Figure 1. Since flow was constant, changes in perfusion pressure were proportional to changes in vascular resistance. Following the tetanus, resistance fell rapidly to a minimum within 6 seconds and then returned to the control level within 45 seconds. This vascular response to a brief tetanus is very consistent, and its time course has been examined in detail previously (4); the return to the control level is nearly monoexponential with a time constant of 10 seconds. The oxygen and K⁺ responses shown in Figure 1 are both venous measurements and thus do not represent tissue events directly, because they are influenced by delay and dispersion in venous vascular transit. Nevertheless, it is apparent that the period of elevated venous K⁺ concentration is shorter than the period of decreased venous O₂ saturation even though both O₂ and K⁺ were exposed to the same delay and dispersion in venous transit.

The average time course of alteration in venous plasma K⁺ concentration with and without concurrent papaverine-induced vasodilation is shown in Figure 2A. Trials with and without papaverine-induced dilations were alternated, and a total of six trials was carried out for each dog. After a brief delay, venous plasma K⁺ concentration rose sharply to a peak within approximately 20 seconds and then gradually returned to the control level by 1.5 minutes after tetanus in both situations. In these seven dogs, the average plasma flow rate was 14.5 ± 2.9 ml/100 g min⁻¹ and the average control plasma K⁺ level was 3.14 ± 0.15 mEq/liter. There was no statistically significant difference in the base-line venous K⁺ level with and without papaverine. The peak increase in K⁺ concentration observed following a 1-second tetanus was 0.207 ± 0.042 mEq/liter without papaverine and 0.230 ± 0.017 mEq/liter with papaverine-induced vasodilation. Although there was a slight tendency toward a higher peak change in venous plasma K⁺ concentration and a slower return to the control level during papaverine-induced dilation, at no sampling time did a statistically significant difference exist between the venous plasma K⁺ levels with and without the drug. This finding was the case regardless of whether the results from individual dogs were paired. Because of the small magnitude of these changes in relation to the base-line K⁺ level, statistical uncertainty in the data generally prevented detailed examination of individual experiments, even after the three trials of each type.
had been averaged for each dog. However, when we took the area under each average curve in Figure 2A and multiplied it by the average plasma flow rate for these experiments, we calculated an average net loss of 1.7 \( \mu \text{Eq} \) of K\(^+\) from each 100 g of muscle tissue following tetanus in the presence of papaverine and a net loss of 1.4 \( \mu \text{Eq} \)/100 g in the absence of the drug.

Figure 2B shows the average distribution of vascular transit times for the seven experiments in which papaverine was employed. This curve is the time course of venous concentration at the K\(^+\) sampling site of intravascular dye (indigo carmine) after a close intra-arterial bolus injection at time 0. The K\(^+\) efflux curve and the dye transit curve are similar both in delay and time course, which indicates that much of the shape of the K\(^+\) efflux curve is due to the delay and the dispersion of intravascular transit. Therefore, the K\(^+\) efflux curve spans a much wider period of time than does the actual tissue-blood K\(^+\) transfer. In fact, because the dye transit and K\(^+\) efflux curves nearly superimpose, we can conclude that the period of tissue-blood K\(^+\) transfer, like the bolus dye injection, must be very brief. This situation would mean that the peak concentration of K\(^+\) in the plasma as it leaves the capillaries must be significantly higher than the peak changes that we observed in the venous samples, since the same net mass of extra K\(^+\) must pass both sites. Estimates of the magnitude and the time course of these changes are presented in the Discussion.

Discussion

Our results clearly indicate that a transient increase in venous plasma K\(^+\) concentration follows a brief tetanus of skeletal muscle. As measured at a venous site, the time course of this increase closely approximates the time course of the appearance at the same site of an intra-arterial bolus of intravascular dye. This fact suggests that the time period of elevated end-capillary plasma K\(^+\) concentration, like a bolus injection of dye, is quite brief. From this simple qualitative analysis we conclude that the changes in interstitial K\(^+\) concentration associated with a brief tetanus are likely to be rapid enough to precede and therefore possibly be causally related to the decrease in vascular resistance which occurs over tens of seconds (Fig. 1). In the rest of this Discussion, we will present a more quantitative analysis of the data to get a better estimate of the magnitude and the time course of the interstitial K\(^+\) concentration changes associated with a brief tetanus.

The general steps involved in this analysis are: (1) assume a certain interstitial K\(^+\) concentration change, (2) relate this interstitial alteration to an end-capillary plasma K\(^+\) concentration change, (3) relate this end-capillary alteration to the corresponding venous plasma K\(^+\) concentration changes, and (4) compare these calculated venous alterations with the venous plasma K\(^+\) concentration measurements. The process is then repeated to determine the assumed interstitial K\(^+\) concentration change that gives the best match with our experimental venous plasma data.

Our first assumption was that the time course of the increase in interstitial K\(^+\) concentration associated with a brief tetanus can be represented as a sharp linear rise during the 1-second period of tetanus and a monoexponential return to the control level thereafter, as illustrated by the solid
curve in Figure 3A. This pattern of change was selected on the assumption that extra K⁺ appears in the interstitium as a result of transmembrane fluxes associated with skeletal muscle action potentials. Thus, interstitial K⁺ concentration would increase only during the 1-second period of stimulation when muscle action potentials occur. The monoexponential return of interstitial K⁺ concentration to the control level was meant to represent K⁺ removal from the interstitium by flow washout, reuptake into skeletal muscle cells, or other processes. In general, this assumed pattern of interstitial K⁺ concentration changes appears to conform to the results of Gebert (11), who found by direct measurement that interstitial K⁺ activity increases during a 15-second period of muscle activity at a rate proportional to the stimulation frequency and thereafter declines exponentially to the control value. In addition, calculations to be considered later show that the transmembrane fluxes of K⁺ necessary for the repolarization of the skeletal muscle cell membrane are sufficient to account for the interstitial K⁺ concentration changes postulated in the present paper.

In the second step of the analysis, we related a particular magnitude and time course of interstitial K⁺ concentration change to a corresponding alteration in end-capillary plasma K⁺ concentration, as illustrated by the top two curves in Figure 3A. For this purpose, we essentially took the Renkin model for transcapillary ion transport (12) and found a solution for end-capillary concentration in the case of time-varying interstitial concentration. The details of this solution are in the Appendix. We treated the interstitium as a well-mixed compartment. Sheehan and Renkin (13) have dealt extensively with this assumption and have concluded that radial gradients in the interstitium are negligible compared with transcapillary gradients, unless the diffusion coefficient for K⁺ in the interstitium is a small fraction of its free diffusion coefficient in water. We also believe that major radial diffusion gradients for K⁺ are unlikely based on the following simple considerations. If pores occupy less than 0.1% of the total capillary surface area and the average pore length is 0.5µ (14), then, even for the smallest lipid-insoluble molecules, the capillary wall presents a barrier equivalent to a water barrier 500µ thick. For the interstitium to present a comparable barrier, even in a 50µ path, the diffusion coefficient for K⁺ in the interstitium would have to be a tenth of that in water. As Sheehan and Renkin have pointed out (13), the available evidence suggests that the interstitial diffusion coefficient for K⁺ is not this low.

We also believe that major longitudinal K⁺ gradients are unlikely in our particular experimental situation. Since the extra interstitial K⁺ is derived from the skeletal muscle cells during the period of contraction and should therefore be delivered quite uniformly to the interstitial volume, it seems unlikely that interstitial gradients are present at the end of tetanus. Thereafter, however, longitudinal gradients could be established if flow removes relatively more K⁺ from the interstitium at the arterial ends of the capillaries than it does at the venous ends. Although such longitudinal gradients are probably a factor in these experiments, their importance appears to be minimal, since less than 10% of the extra K⁺ appears to leave the interstitium through flow washout.
To obtain the solution of the Renkin model outlined in the Appendix, we also assumed that capillary surface area and permeability to K⁺ are constant. We attempted to achieve this condition experimentally by maximally dilating the vascular bed with papaverine throughout the experimental period surrounding the brief tetanus. Presumably the product of capillary surface area and permeability remains constant at a maximal value with this procedure. Consequently, we performed our analysis only on the K⁺ data obtained in the presence of maximal papaverine-induced dilation.

In general, as shown in Figure 3A, our solution of the Renkin model indicates that changes in end-capillary K⁺ concentration follow much the same time course as do changes in interstitial K⁺ concentration. However, the magnitude of the changes might be considerably larger in the interstitium depending on the value of the product of capillary permeability and surface area (Renkin's PS).

Next we attempted to relate a specific time course of alteration in end-capillary plasma K⁺ concentration to the resulting venous alteration, as illustrated by the bottom two curves in Figure 3A. The venous curve results from the convolution of the end-capillary curve with an estimate of the distribution of the venous vascular transit times:

\[ K_v(t) = \int K_c(\gamma)h_v(t-\gamma)\,d\gamma, \]

where \( K_v(t) \) = venous plasma K⁺ concentration as a function of time, \( K_c(\gamma) \) = end-capillary K⁺ concentration as a function of time, \( h_v(t) \) = distribution of venous vascular transit times, and \( \gamma \) = variable of integration.

The distribution of venous vascular transit times, \( h_v(t) \), is estimated from the distribution of transit times across the entire bed, which was experimentally determined in each preparation with an intravascular dye. To obtain the venous estimate, we assumed that a certain percent of each particular transit time occurs on the venous side of the bed. This procedure is based on anatomical studies which indicate that connecting arterial and venous paths are nearly equal in length owing to the side-by-side course of supply and collection vessels (15-17). We tested a range of estimates of the venous vascular transit distribution based on from 50% to 100% of the transit time for each path spent in veins. However, since veins are reported to contain approximately 80% of the total bed volume in skeletal muscle (18, 19), we feel that 80% is the most reasonable estimate of the percent of the total transit time spent on the venous side.

In using the convolution equation, we also assumed that plasma K⁺ concentration changes occur simultaneously in all capillaries and that vascular transit has a time-invariant (stationary) effect on plasma K⁺ concentration in our constant-flow experiments. Moreover, we assumed that the mass of plasma K⁺ is conserved during venous transit, since the half-time for plasma-red cell K⁺ exchange is reported to be 36 hours for dog blood at 37°C (20). In addition, we assumed that plasma K⁺ concentration changes linearly when blood samples of unequal concentration are mixed.

The final step in our analysis was to test the venous plasma K⁺ concentration curve, predicted from a particular interstitial alteration through the steps listed in the preceding paragraphs, against the actual venous measurements and then through iteration to find the interstitial alteration which gives the best fit. This step was accomplished on the computer by minimizing the least-square error between venous predictions and measurements, weighted at each point according to the standard error of the experimental measurement. This entire procedure was repeated for several combinations of values for the product of capillary permeability and surface area and the percent of transit time spent in veins. For example, Figure 3B illustrates the minimal least-square error solution for 80% of the transit time spent in the veins and PS = 15 ml/100 g min⁻¹. The alterations in interstitial and end-capillary K⁺ concentrations which produced this venous curve are shown in Figure 3A.

When we examined the best solutions for different parameter values, it became apparent that our estimate of the rate of return of interstitial K⁺ concentration to the control level depended almost entirely on the percent of each transit time spent on the venous side of the bed, whereas our estimate of the magnitude of the interstitial changes depended heavily on the value of the product of capillary permeability and surface area. Figure 4 illustrates how both the magnitude and the speed of return of our best-fit estimates of alterations in interstitial K⁺ concentration in response to a brief tetanus vary with different assumed values of (1) the percent of transit time spent in the veins and (2) PS. The shaded area of the peak interstitial concentration curves and the heavy portion of the return time constant curve represent our opinion that the best estimate for the percent of transit time spent on the venous side is in the neighborhood of 80% and that capillary PS is between 10 and 15 ml/100 g min⁻¹ with maximal papaverine-induced dilation. We conclude that, in response to a 1-second tetanus, interstitial K⁺ concentration rises abruptly by 1.3-2.4 mEq/liter and then returns to its control level with a time constant of.
potassium ions and exercise hyperemia

Sensitivity of the values for peak change in interstitial K⁺ concentration and the corresponding time constant for return to its control level, which resulted in the best fit with measured alterations in venous plasma-K⁺ concentration, to variations in the assumed values of PS and the percent of transit time spent in the veins. Broken curves represent the best estimate of the peak interstitial K⁺ concentration change and are read from the right scale. The solid line is the corresponding recovery time constant and is read from the left scale. See text for further details.

between 6 and 8 seconds. Such alterations in interstitial K⁺ concentration would be rapid enough to control the vascular response to a brief tetanus (Fig. 1), which peaks within 5 seconds following tetanus and thereafter returns to the control level monoexponentially with a time constant of 10 seconds (4). Gebert (11), using direct microelectrode observation of interstitial K⁺ activity, has found sharp increases during a 15-second period of muscle activity with an exponential return to the control level thereafter. In his study, the time course of increased vascular conductance closely follows that of interstitial K⁺ activity, although the changes are not as rapid as those associated with the 1-second tetanus reported in the present paper. Our estimate of a 1.3-2.4 mEq/liter increase in interstitial K⁺ concentration during the tetanus from 10.8 to 19.0 pmoles of K⁺ must cross each square centimeter of surface membrane per impulse. This range agrees within reason with direct tracer flux measurements on single frog muscle fibers of 9.6 pmoles/cm² impulse⁻¹ (21) and on rat diaphragm of 10 pmoles/cm² impulse⁻¹ (22).

It is also interesting to note that, if we take 15 ml as the interstitial volume of 100 g of muscle, then a 1.3-2.4 mEq/liter increase in interstitial concentration requires the addition of 19-35 μEq of K⁺, whereas only 1.7 μEq or less than 10% of the initially added amount was actually lost to blood from 100 g of muscle tissue as a result of the tetanus. This fact, as well as the rapid return of interstitial K⁺ to control levels, suggests an effective cellular reuptake mechanism for K⁺. Regardless of whether it takes place passively or by active transport, this rapid uptake effectively prevents massive K⁺ loss from muscle during exercise.

Appendix

ANALYSIS OF TRANSCAPILLARY ION MOVEMENT

This analysis is similar to many treatments of transcapillary exchange (23) but differs from most in that we attempted to relate end-capillary concentration to a specified alteration in interstitial concentration rather
than to an alteration in arterial concentration as is the case of interest in indicator-diffusion techniques. Our assumptions are: (1) all capillaries are identical, (2) the interstitial concentration is uniform and a specified function of time, (3) capillary geometry and permeability are constant along its length and time invariant, and (4) capillary transit time is constant.

The response of end-capillary concentration to a unit impulse change in interstitial concentration at time zero is an exponential decay truncated at one transit time and given by:

$$h_e(t) = \begin{cases} ae^{-at}, & 0 \leq t < T \\ 0, & \text{otherwise} \end{cases}$$

where $$a = \frac{PS}{QT} \text{ (sec}^{-1})$$, $$PS = \text{Renkin's permeability-surface area product (ml/100 g min}^{-1})$$, $$Q = \text{plasma flow rate (ml/100 g min}^{-1})$$, and $$T = \text{capillary transit time} \text{ (assumed to equal 1 second).}$$

The response of end-capillary concentration, $$K_e(t),$$ to any given time course of interstitial concentration, $$K_i(t),$$ can be found from the impulse response, $$h_e(t),$$ and the convolution integral:

$$K_e(t) = \int_0^T h_e(\tau)K_i(t-\tau)d\tau.$$  \hspace{1cm} (2)

In our particular application, interstitial concentration was assumed to rise linearly for 1 second and then to return to the control level monoexponentially.

$$K_i(t) = \begin{cases} 0, & t < 0 \\ bt, & 0 \leq t < 1 \\ ce^{-ct}, & 1 \leq t \end{cases}$$

where $$a = \text{rate constant of return to control (sec}^{-1})$$, $$b = \text{rate of initial rise (mEq/liter sec}^{-1})$$, and $$c = \text{constant} = \frac{b}{e^{-c}} \text{ (mEq/liter).}$$

The corresponding time course of end-capillary concentration, $$K_e(t),$$ is obtained by direct application of Eq. 2 or by transform methods for generalized functions (24). For an interstitial alteration described by Eq. 3, the solution for end-capillary concentration is defined in four time intervals as follows:

$$K_e(t) = \begin{cases} \frac{b}{a}(e^{-at} + at - 1), & 0 \leq t < T \\ \frac{b}{a}(at - 1)(1 - e^{-aT}) + aTe^{-aT}, & T \leq t < 1 \\ \frac{b}{a}\left[e^{-at(1 - aT) + \frac{at}{a - a}e^{-at} - 1} - e^{-at} - (a - 1)e^{-at} - 1\right], & 1 \leq t < 1 + T \\ \frac{ba}{a - a}\left[e^{-at} - 1(e^{(T + 1) - 1})\right], & 1 + T \leq t \end{cases}$$

References

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