The Role of Adenosine in Prolonged Vasodilation following Flow-Restricted Exercise of Canine Skeletal Muscle

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SUMMARY A period of prolonged vasodilation follows flow-restricted exercise of skeletal muscle. We tested the hypothesis that adenosine participates in mediating this vascular response. Vascularly isolated, anterior calf muscles of anesthetized dogs were stimulated to contract at a rate of 4 twitches/sec. Blood flow was held constant at 12.5 ± 1.3 ml/min per 100 g which was about 14% of the expected free flow for this exercise level. Skeletal muscle tissue adenosine was measured with an enzymatic, spectrophotometric assay of trichloroacetic acid extracts of 50 mg biopsy samples. Tissue adenosine rose from 2.30 ± 0.90 nmol/g in resting muscle to 22.5 ± 5.8 nmol/g by the end of the 22-minute exercise. Following exercise, tissue adenosine fell toward its baseline value with a time course very similar to the early portion of the return of skeletal muscle vascular resistance to its control level. Thus, skeletal muscle adenosine content (1) increases to a sufficient magnitude and (2) falls with an appropriate time course to be at least partly responsible for the early portion of prolonged vasodilation seen after flow-restricted exercise of skeletal muscle. Circ Res 44: 759-766, 1979

SEVERAL investigators have observed a prolonged period of vasodilation following flow-restricted exercise of skeletal muscle (Cerretelli et al., 1969; Morganroth et al., 1975; Owen et al., 1975; Morganroth et al., 1977). This prolonged vasodilation persists (~30 minutes) beyond the return of oxygen consumption to its control level (~3 minutes) (Morganroth et al., 1975). Several possible mediators of this response have been ruled out, including an impairment of vascular smooth muscle contractile ability, potassium release, elevated osmolality, lactate acid release, and factors closely linked to oxygen consumption (such as diminished vessel wall or tissue oxygen tension) (Morganroth et al., 1975). Prostaglandin and histamine release, perhaps as responses to ischemic tissue injury, have been implicated as mediators of prolonged vasodilation based on experiments with prostaglandin synthetase blockers and antihistaminergic agents (Morganroth et al., 1977). These blocking agents reduce the duration of prolonged vasodilation to approximately half of the initial value, but do not affect the early portion of this response. Furthermore, the time course of the release of prostaglandin E\(_2\), during and following flow-restricted exercise of skeletal muscle implies the participation of a mediator other than vasodilating prostaglandins in the first 15-20 minutes of the prolonged vasodilation response (Young and Sparks, in press).

Adenosine has been proposed as a local regulator of blood flow in skeletal muscle (Bern et al., 1971). Although tissue levels of adenosine do not change during twitch exercise of skeletal muscle under free flow conditions (Phair and Sparks, in press), during constant flow or ischemic exercise skeletal muscle adenosine levels rise (Bern et al., 1971; Bockman et al., 1976; Bockman et al., 1977; Phair, 1977). We performed the present experiments to determine whether adenosine might be responsible for the early portion of prolonged vasodilation observed following flow-restricted exercise.

Methods

Our experiments were performed using vasculurally isolated, in situ anterior calf muscles (tibialis cranialis and extensor digitorum longus) of 26- to 28-kg male mongrel dogs. These muscles have very similar fiber composition, both being made up predominantly of fast twitch, fatigue-resistant fibers and lesser numbers of slow twitch, fatigue-resistant fibers (Maxwell et al., 1977). Dogs were anesthetized with sodium pentobarbital (25 mg/kg, iv, or to effect) with supplemental doses given as required. The two muscles of interest were isolated as previously described by Mohrman and Sparks (1974). The fibular nerve innervating the two muscles was severed. Heparin was given intravenously (750 U.S.P. units/kg as a priming dose plus 110 U.S.P. units/kg per hour thereafter). Polyethylene cannulae were placed in the sole arterial supply and venous drainage of the muscles. The arterial can-
nula was connected to the contralateral femoral artery by an extracorporeal circuit of polyethylene and Silastic tubing. In four dogs this circuit contained a roller pump (Sage model 375) which allowed us to perfuse the muscles with arterial blood at a known, constant flow. In four other dogs no pump was in the circuit and free flow was allowed. The vascular resistance of the muscles was calculated by dividing the perfusion pressure by the flow rate per 100 g of muscle. Perfusion pressure was measured via a separate arterial cannula placed in the distal portion of the cranial tibial artery using a diaphragmatic pressure transducer (Statham P23DC). The venous outflow from the muscles was returned to the dog via a funnel-catheter placed in the inferior vena cava via the contralateral femoral vein. The venous outflow line was made as wide and as short as possible to minimize its resistance. The end of the outflow line was kept close to the level of the blood in the funnel (venous pressure). Systemic arterial blood pressure was measured via a side tube off the extracorporeal perfusion circuit proximal to the pump using another pressure transducer.

Arterial or muscle venous blood was passed through a cuvette optical densitometer (Gilford model 103-IR) whose output was proportional to the oxygen content of the blood. The densitometer was calibrated by independent analysis of blood oxygen content using an electrochemical oxygen analyzer (Lexington Instruments Lex-O_2-Con). Arterial blood gas and pH values were measured (Corning model 165/2) and kept within the physiological range for unanesthetized animals (Feigl and D’Alecy, 1972). Mechanical, positive pressure ventilation (Harvard Large Animal Respirator) was employed with adjustments of tidal volume and rate as required to maintain an acceptable level of arterial PCO_2. Inspired air was supplemented with oxygen if necessary to maintain an acceptable value of arterial Po_2, and intravenous sodium bicarbonate (15 g/liter) was given as necessary to combat metabolic acidosis.

The distal tendons of the muscles were connected to force transducers (Grass model FT.03). The knee was fixed in position and the resting length of the muscles was varied by moving the force transducers until an optimal length for tension development was obtained. The muscles were stimulated through needle electrodes placed in the muscle mass. Pulses of supramaximal (for tension development) constant current (70–100 mA) of 0.2 msec duration were provided by a stimulator (Grass model S48) through a stimulus isolation unit (Grass model SIU5A) and a constant current unit (Grass model CCU1A).

Muscle tension development, systemic arterial blood pressure, perfusion pressure, and venous blood oxygen content were recorded continuously on an ink-writing oscillograph (Grass model 7D).

Our experimental protocol was as follows. In four free-flow preparations the steady state flows and oxygen consumptions associated with a variety of twitch rates were observed. When venous O_2 saturation had reached a steady value after a stimulation period had been initiated at a particular twitch rate, venous outflow was measured by a timed collection. Flow measurements were repeated until flow was steady. The duration of the stimulation periods was approximately 5 minutes. Then the stimulator was turned off; the next stimulation period commenced as soon as flow and venous O_2 returned to control. Flow and oxygen consumption were observed at the following twitch rates: 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0/sec.

In four constant flow preparations, pump flow was set so that perfusion pressure was as close to systemic arterial pressure as possible without a flow (timed collection of venous outflow) exceeding 10 mL/min. Once perfusion pressure and venous blood oxygen content had achieved a steady state with acceptable arterial blood gas and pH values, two control muscle samples were obtained (see below). The muscles then were stimulated to contract at four twitches per second for 22 minutes. Two more muscle samples were obtained near the end of the exercise period (between 18.8 and 21.7 minutes). Then the stimulation was stopped. Four more muscle samples were obtained at approximately 5, 15, 25, and 35 minutes after the cessation of exercise. Control samples were taken most frequently from the tibialis cranialis and late samples most frequently from the extensor digitorum longus. Previous control studies showed that resting levels of adenosine are not different in the two muscles (Phair and Sparks, in press). The same conclusion can be inferred from the data presented by Bockman et al. (1976). Bockman’s data also suggest that the values in the two muscles are similar during exercise. Our data substantiate this. The samples taken just before and just after the end of exercise were distributed equally between the two muscles, and there was no trend in adenosine content which could be attributed to sample site.

After the last muscle sample had been obtained, the dog was killed with an intravenous injection of saturated potassium chloride. Both the experimental muscles and the corresponding muscles from the contralateral leg were excised and weighed. The experimental muscles weighed 10–21% more than the contralateral muscles [15 ± 5% (mean ± SD)] which is typical of past experience with this preparation. The contralateral muscle weight was used to normalize flow, vascular resistance, and muscle oxygen consumption to a per 100 g basis.

Muscle samples were obtained with a pneumatic tissue punch, as described by Phair and Sparks (in press). Essentially this was a sharpened die rotated via a flexible dental drill apparatus mounted on a pneumatic cylinder. Pressurized gas was used to propel the rotating die forward into and through the muscle. The operator stopped the rotation via...
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Figure 1  Relationship between skeletal muscle oxygen consumption (VO₂) and blood flow at a range of twitch rates from 0.25 to 6/sec.

A foot switch, withdrew the die with the sample from the muscle, and deposited the sample in a flask of liquid nitrogen (N₂) by opening a valve allowing pressurized gas to expel the sample. The time between first contact of the die with the muscle and deposition of the sample in liquid N₂ was 1-3 seconds. The sample sank below the surface of the liquid N₂, indicating freezing, in about 2 seconds. Sample size in these experiments was 52 ± 20 (SD) mg (n = 30; range, 20-102 mg).

Each muscle sample was pulverized under liquid N₂. The powder was placed in a vial containing 1 ml of shell-frozen 6% trichloroacetic acid and refrigerated overnight. The following day, the contents of the vial were transferred to a ground glass homogenizer and homogenized. The precipitated proteins and cell fragments were sedimented by centrifugation at 48,200 g (Sorvall model SS-3, 2 × 10⁶ rpm, 15 minutes). The supernatant extract was volumetrically transferred to a fresh tube and was neutralized to pH 6-8 using graded concentrations (10, 1, 0.1 M) of sodium hydroxide and hydrochloric acid. The hypoxanthine, inosine, and adenosine concentrations of these neutralized, protein-precipitated extracts were measured using an enzymatic, spectrophotometric assay based on that of Olsson (1970) and a highly sensitive, dual-beam, ultraviolet spectrophotometer (Aminco DW2). Since all the dilutonal factors related to the extraction procedure were known, the levels of these purines that existed in the muscle samples could be calculated and were expressed as nanomoles of purine base or nucleoside per gram net weight of tissue. Previous work in our laboratory has shown that at least eight muscle samples can be obtained from different regions of these resting muscles over a 1-hour period and analyzed using the procedures outlined here without any tendency for adenosine, inosine, or hypoxanthine to change outside the limits of assay reproducibility (Phair and Sparks, in press).

For purposes of data analysis, the purine concentrations of the two control muscle samples were averaged, as were the concentrations of the exercise samples. Thus, each dog in this series had one control, one exercise, and four post-exercise values for each purine measured (except one dog for which the 15-minute post-exercise sample was lost during extraction). An analysis of variance (Snedecor and Cochran, 1967) was performed for each set of purine values. Other values reported in the text are means ± one standard error of the mean unless otherwise noted. All statistical analyses were performed using the Michigan Interactive Data Analysis System (MIDAS).

Results

Free Flow Experiments

Figure 1 shows that oxygen consumption increased with twitch rate and that after an initial relatively flat response, flow followed oxygen consumption. At four twitches per second, flow was 88 ± 9 ml/min per 100 g. The resting resistance of the free flow preparation was 6.1 PRU₁₀₀ (range, 3.5-12.5) as compared to 9.2 (5.9-11.2) for the constant flow preparation. This difference between the two preparations may be a result of the lower perfusion pressures of the constant flow preparation and the resultant elastic recoil of resistance vessels.

Table 1 shows the values of arterial blood gases and pH, muscle flow, muscle vascular resistance, and muscle oxygen consumption (VO₂) for the constant flow dogs during the control state before exercise. Exercise at four twitches per second in-

<table>
<thead>
<tr>
<th>Table 1 Control Values for Four Constant Flow Dogs</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂ content [ml/dl (vol %)]</td>
<td>18.0 ± 1.4</td>
<td>15.4-21.9</td>
</tr>
<tr>
<td>Po₂ (mm Hg)</td>
<td>95 ± 6</td>
<td>82-109</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>36 ± 1</td>
<td>33-38</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.02</td>
<td>7.31-7.39</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48 ± 2</td>
<td>43-53</td>
</tr>
<tr>
<td>Muscle blood flow (ml/min per 100 g)</td>
<td>12.5 ± 1.3</td>
<td>10.5-16.8</td>
</tr>
<tr>
<td>Vascular resistance (PRU₁₀₀)</td>
<td>9.22 ± 1.25</td>
<td>5.90-11.2</td>
</tr>
<tr>
<td>Vascular conductance [(PRU₁₀₀)⁻¹]</td>
<td>0.12 ± 0.02</td>
<td>0.09-0.17</td>
</tr>
<tr>
<td>Resting VO₂ (ml/min per 100 g)</td>
<td>0.12 ± 0.02</td>
<td>0.07-0.16</td>
</tr>
<tr>
<td>Contractile muscle weight (g)</td>
<td>59 ± 2</td>
<td>56-63</td>
</tr>
<tr>
<td>Mean systemic arterial pressure (mm Hg)</td>
<td>133 ± 9</td>
<td>110-150</td>
</tr>
</tbody>
</table>
produced an increase in muscle oxygen consumption (VO₂) and a decrease in vascular resistance (Fig. 2, Table 2). The oxygen consumption (2.2 ± 0.1 as compared to 14 ± 3 ml/min per 100 g with free flow) was limited by the oxygen supply, as evidenced by the very low venous oxygen contents measured during exercise (Table 2). This level of oxygen consumption was insufficient to support tension development at the level obtained early in the exercise (Fig. 2), and tension development fell off by 77% from its peak by the end of the exercise period (Table 2). At the cessation of exercise, venous oxygen content quickly began to return to its control level. Vascular resistance, as judged by the perfusion pressure record, returned slowly. This is the prolonged vasodilation under study. In these experiments, the perfusion pressure never returned to its control value. This undoubtedly was because of the low resistance pathways (i.e., holes) in the muscle created by the punch biopsy protocol (see Discussion).

Table 3 and Figure 3 show the skeletal muscle tissue adenosine levels in our experiments. Control tissue adenosine level averaged 2.30 ± 0.90 nmol/g. Tissue adenosine concentration was dramatically elevated after 18 minutes of 4/sec exercise in each of the four dogs to a mean value of 22.5 ± 5.8 nmol/g. This was a statistically significant increase from control as judged by a one-way analysis of variance (P = 0.0004). Approximately 5 minutes after the cessation of exercise (actually 5.6 ± 0.4 min), the tissue adenosine level was still greater than the control value by at least a factor of 5-fold in every dog. This group of values was significantly greater than control (P = 0.0102). The difference between the exercise value and the 5-minute post-exercise value of tissue adenosine was not statistically significant. The values of tissue adenosine at 15, 25, and 35 minutes after exercise (15.4 ± 3, 26.4 ± 1.3, and 35.1 ± 0.2 minutes) showed a somewhat less clear trend. None of these last three values was significantly different from control, although only one dog had tissue adenosine levels during this period that fell to the pre-exercise control level (Table 3). Each of these last three points had a mean value for adenosine content which was significantly less than that found for the exercise group (P < 0.0025) and for the first post-exercise (5-minute) group (P < 0.05).

Figure 4 shows the levels of muscle tissue hypoxanthine and inosine before, during, and after flow-restricted exercise. Tissue inosine levels were clearly and statistically significantly elevated above control during flow-restricted exercise and minutes thereafter. Tissue inosine levels also were elevated over control in all our dogs for the remainder of the post-exercise period, but the large variability in the absolute values prevented statistical significance from being obtained. We speculate that the variability of the inosine content values may be explained by the non-steady state condition which adds variation in time course to variation in magnitude of the changes. Tissue hypoxanthine was elevated over control levels during the exercise and throughout the post-exercise period. Interestingly, hypoxanthine levels did not peak early and fall, as did adenosine and inosine levels. Rather, hypoxanthine levels tended to rise slightly throughout the post-exercise period. This probably reflects its slow buildup as the very high levels of inosine are dissipated via nucleoside phosphorylase.

**Discussion**

To date, a major portion of prolonged vasodilation following flow-restricted exercise of skeletal muscle lacks explanation. In the current study, we evaluated whether adenosine might mediate a portion of prolonged vasodilation; the results support this possibility.

Several criteria may be used to judge whether adenosine is a mediator of prolonged vasodilation of skeletal muscle. Adenosine is a vasodilator in...
skeletal muscle (Dobson et al., 1971). The enzymatic mechanism to produce adenosine is present in skeletal muscle (Rubio et al., 1973) and, under certain ischemic conditions, the adenosine level of skeletal muscle has been found to increase (Berne, 1971; Phair and Sparks, in press; Bockman et al., 1975; Bockman et al., 1976). The questions we set out to answer in the present study were (1) does skeletal muscle adenosine content increase under conditions that result in prolonged vasodilation and (2) if so, is the time course of changes in skeletal muscle adenosine content consistent with the proposed role of adenosine as a mediator of the early portion of prolonged vasodilation?

We exercised the isolated muscles of our four constant flow preparations at 4 twitches/sec. If flow had been allowed to increase freely, we would have expected the flow during exercise to have been 88 ± 9 ml/min per 100 g (Fig. 1). Instead we restricted the flow to these muscles to be only 12.5 ± 1.3 ml/min per 100 g. During this flow-restricted exercise, muscle adenosine rose in each preparation from a control value of 2.30 ± 0.90 nmol/g to 22.5 ± 5.8 nmol/g near the end of the 22-minute exercise period. Our value for resting muscle adenosine content agrees very well with values from other laboratories (Bockman et al., 1976). Our value for muscle adenosine content during flow-restricted exercise is higher than previously published values for working muscle (Berne et al., 1971; Bockman et al., 1976). Presumably this is due to the more vigorous exercise and the more restricted flow rates used in our study. The very low values of muscle venous oxygen content (Table 2) underscore how severe the exercise was in relation to the oxygen supply in the present experiments. Arterial hypoxemia, which might elevate skeletal muscle adenosine levels as it does cardiac muscle adenosine levels (Rubio et al., 1974), is not a likely explanation of our higher exercise value, since arterial blood PO₂ was controlled in our experiments (Table 1).

In addition to skeletal muscle adenosine content

\[ \text{[ADO]} \] is adenosine tissue concentration in nmol/g.

Table 3 shows the individual values of adenosine in each of the four dogs.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Dog 3</th>
<th>Dog 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest -5.0</td>
<td>5.6</td>
<td>-2.5</td>
<td>4.4</td>
<td>-4.5</td>
</tr>
<tr>
<td>Rest -2.5</td>
<td>3.4</td>
<td>-0.5</td>
<td>1.7</td>
<td>-1.5</td>
</tr>
<tr>
<td>Exercise 20</td>
<td>21</td>
<td>39</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Post-exercise 6.9</td>
<td>27</td>
<td>5.2</td>
<td>15</td>
<td>5.5</td>
</tr>
<tr>
<td>Post-exercise 15</td>
<td>3.6</td>
<td>16</td>
<td>—</td>
<td>15</td>
</tr>
<tr>
<td>Post-exercise 25</td>
<td>1.8</td>
<td>25</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>Post-exercise 35</td>
<td>3.7</td>
<td>36</td>
<td>9.2</td>
<td>35</td>
</tr>
</tbody>
</table>

Time is minutes before initiation of exercise for "Rest," minutes after initiation of exercise for "Exercise," and minutes after cessation of exercise for "Post-exercise" values.

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Figure 3 Tissue adenosine content. Last four bars represent contents at approximately 5, 15, 25, and 35 minutes after cessation of flow-restricted exercise. Numbers in parentheses denote number of dogs. Bars represent mean ± 1 SEM; * signifies P < 0.01; N.S. signifies P > 0.6. Each group is compared to control using analysis of variance.

Figure 4 Tissue inosine and hypoxanthine contents. Symbols are as in Figure 2 except, here, * signifies P < 0.05 and N.S. signifies P > 0.1. Open bars represent inosine; hatched bars hypoxanthine.
being increased at the end of the flow-restricted exercise, it is also elevated above control approximately 5 minutes (actually 5.6 ± 0.4 minutes) after cessation of exercise (15.7 ± 3.9 nmol/g). At this point, muscle oxygen consumption has returned to, or almost to, its control level (Fig. 2) while vascular resistance has returned only slightly toward its control level (Morganroth et al., 1975). Had the preceding exercise been performed under free flow circumstances, the vascular resistance would have been back to its control level at this time (Morganroth et al., 1975). The mechanism or mechanisms responsible for prolonged vasodilation, then, are surely operating at this 5-minute point, whereas they may or may not be operating at the end of exercise. Muscle adenosine content is, on the average, 920 ± 250% of control at this 5-minute point.

If adenosine does indeed play its proposed role in prolonged vasodilation, the magnitude of the increment in adenosine content must be sufficient to explain the early portion of this response. If all the adenosine measured at the end of the flow-restricted exercise were confined to the interstitial space (Dobson et al., 1971; Rubio et al., 1973) and if we assume 0.15 ml of interstitial fluid per gram of muscle, interstitial adenosine concentration would be 150 ± 39 μM at that time. Arterial plasma concentrations of this magnitude cause a 3- to 4-fold increase in blood flow to similarly prepared muscles under free flow conditions (Phair and Sparks, in press). Vascular conductance (reciprocal of resistance) rose by a factor of 2.7 ± 0.3 during the 4 Hz, constant-flow exercise used in the present study. Similarly, interstitial adenosine concentration is calculated to be 105 ± 26 μM at 5 minutes after exercise. Arterial plasma concentrations of this magnitude cause about a 3-fold increase in muscle blood flow (Phair and Sparks, in press). The skeletal muscle vascular conductance was estimated to be about two times control at this point (see discussion below). These conductance changes with adenosine infusion on the one hand and increments caused by flow-restricted exercise in muscle (and presumably interstitial) adenosine content on the other hand are in good agreement with each other. Of course, there are several assumptions made in this argument which might justifiably be challenged. The most important issue here is whether tissue adenosine in resting or contracting muscle is, in fact, confined to the interstitial space. The existence of more than one pool of adenosine in cardiac muscle has been suggested by tracer studies (Schrader and Gerlach, 1976) and the identification of adenosine-binding factors (Olsson et al., 1978). The issue of adenosine compartmentation in skeletal muscle has not yet been specifically examined. If there were compartmentation of adenosine within muscle away from arteriolar vascular smooth muscle, then some or all of the observed changes in the tissue adenosine content could, of course, exert no vasoactive influence.

Having demonstrated increases in skeletal muscle adenosine content with flow-restricted exercise, we next examined the time course of the tissue adenosine changes in relation to the time course of vascular resistance changes during prolonged vasodilation. In our experiments, the accurate measurement of vascular resistance (via measurement of perfusion pressure at constant flow) became impossible after several biopsies had been taken. Our biopsy procedure created low resistance pathways which greatly lowered the overall resistance to flow of the preparation. This prevented us from identifying just that portion of the total resistance that was ascribable to the intact vasculature. It is hard to say exactly when this problem became insurmountable. Resting perfusion pressure did not change as a result of the first two control muscle punches (see Fig. 2). Perfusion pressure fell to a very low level during the exercise, as expected, and suffered no net change as a result of the two exercise muscle punches. The problem became apparent only when it was observed that, by 35 minutes after exercise, the perfusion pressure trace had returned toward control by only 10, 57, 71, and 0% of the change caused by the exercise. Even with the existence of prolonged vasodilation, we would have expected the vascular resistance to be 90% or more recovered by this time (Morganroth et al. 1977; Fig. 4). We, therefore, discarded the post-exercise vascular resistance data from the present studies and estimated the time course of the prolonged vasodilation in these experiments based on previous work in our laboratory (Morganroth et al., 1977).

We were convinced that we had achieved circumstances that would ensure the presence of a prolonged vasodilation. Blood flow was restricted to approximately 14% of the expected free flow for 4 Hz exercise. Three of our preparations had blood flows that could be termed "low," and one preparation had a "medium" blood flow according to the classification of Morganroth et al. (1977). In that study, 20 minutes of 4 Hz exercise at low or medium flow rates always caused prolonged vasodilation with a 90% return of resistance to control (τ<sub>90</sub>) of at least 13 minutes. Furthermore, within each flow range, the τ<sub>90</sub> for resistance return was correlated (r ∼ 0.8) with the degree of fatigue seen in the exercising muscles during the flow-restricted exercise. In our experiments, the exercising muscles underwent severe fatigue, losing 60-90% of the peak developed tension by the last minute of exercise. We used the values for blood flow to, and degree of fatigue of, the muscles in our preparations to compute the expected τ<sub>90</sub>'s for resistance return to control during the prolonged vasodilation based on the empirical relations found in the previous study (Morganroth et al., 1977). We also assumed a monoexponential time course for resistance return. Whereas this is not strictly true (Morganroth et al., 1975, Morganroth et al., 1977), it is accurate enough for the point we wish to make. The range of the
predicted vascular resistance time courses for our four preparations as obtained by the calculations just described are shown as the shaded band in Figure 5.

Also shown in Figure 5 are the muscle adenosine contents obtained from control samples, at the end of flow-restricted exercise, and approximately 5, 15, 25, and 35 minutes following exercise. The scales for adenosine content and vascular resistance (normalized as fractional return to control) have been adjusted so that their relative time courses can be easily compared. The adenosine and resistance time courses appear to be similar; however, we do not have enough data on the time course of adenosine concentration between 5 and 15 minutes to know how well the time courses match during that time interval. The time course comparison does lend support to the hypothesis that adenosine is involved in the mediation of the early portion of prolonged vasodilation.

The time courses in Figure 5 suggest that elevated adenosine may not be responsible for the later portion of prolonged vasodilation, e.g., after 15 minutes. Perhaps prostaglandin and histamine release are responsible for the remaining vasodilation after tissue adenosine content has returned to near control values (Morganroth et al., 1977).

Temporal correlations must be interpreted cautiously in situations in which a nonlinear relation between the two correlated variables may exist. Insufficient data are available to characterize quantitatively the relation between tissue adenosine content and vascular resistance in skeletal muscle under the flow-restricted conditions of the present study. The large range of adenosine contents seen (a full order of magnitude between the extremes of control and exercise) almost ensures the presence of some nonlinearity. Furthermore, other factors associated with the ischemic exercise may modulate the vasoactivity of adenosine. For example, low O₂ (Gellai et al., 1973) and elevated H⁺ or carbon dioxide (Raberger et al., 1975; Merrill et al., 1978) levels seem to potentiate the vascular smooth muscle relaxing or vasodilator potency of adenosine in coronary vessels. Similar effects might be expected in skeletal muscle. Although these particular modulators essentially return to their control levels early in the course of prolonged vasodilation (Morganroth et al., 1975), the possibility of other as yet undetermined modulators participating in this response cannot be ruled out. Finally, the location of the measured adenosine within the tissue is again a critical issue.

We should consider the special circumstances of the vascular response under study. These skeletal muscles were subjected to a moderate workload which would normally require them to operate at about 70% of their maximal VO₂ (Maxwell et al., 1977; Fig. 1). However, blood flow to the muscles was severely restricted to only about 14% of the expected free flow rate. This flow restriction limited oxygen delivery to the tissue. Despite nearly complete extraction of oxygen from incoming blood (Table 2), muscle oxygen consumption was limited to 2.2 ± 0.1 ml/min per 100 g, which is only about 16% of the free flow oxygen consumption for this exercise load (Fig. 1). This level of oxygen consumption was insufficient to support tension development at the usual level, presumably due to an inadequacy of high energy phosphate generation. As a result, tension development fell by the end of our 22-minute exercise period to only 10–40% of the tension developed early in the exercise. Under those conditions, tissue adenosine rose by approximately one order of magnitude on the average (Fig. 2).

Vascular resistance fell to near minimal levels. The correlation of tissue adenosine content and vasodilation suggests that adenosine mediates the vascular response to exercise under these conditions. Yet equally great vasodilation can be achieved by skeletal muscles exercised under free flow conditions without any significant change in tissue adenosine (Phair and Sparks, in press). If there is another, non-adenosine, as yet unidentified, steady state regulator of skeletal muscle vascular resistance, as is implied by this free flow result, it may also be participating in the vasodilation of flow-restricted exercise, invoked by the combination of relative ischemia and metabolic demand. The addition of the adenosine mechanism in the present situation would explain a portion of the prolonged vasodilation seen. Whereas the unidentified mechanism usually responsible for exercise dilation quickly recedes following exercise (Morganroth et al., 1975), muscle adenosine levels, raised by the combination of exercise and ischemia, fall more slowly and prolong the vascular recovery. After some minutes of recovery, muscle prostaglandin release will have risen from the low rate seen during ischemic exer-

**Figure 5** Comparison of time courses of tissue adenosine content (closed circles, dashed line) and estimated vascular resistance (shaded band). Adenosine points are those shown in Figure 2. Vertical bars represent 1 SEM for adenosine content. Horizontal bars represent 1 SEM for time of biopsy. Note that adenosine scale has been shifted (non-zero at origin). See text for explanation.
ercise (Young and Sparks, in press) prolonging the vasodilation even further.

The severity of the stress placed on these muscles by the flow-restricted exercise is underscored by the large increases in tissue inosine and hypoxanthine, as well as tissue adenosine (Fig. 4). Inosine may be formed in skeletal muscle by the action of 5' nucleotidase on IMP which has been formed by deamination of AMP. This pathway may even quantitatively dominate the AMP-to-adenosine-to-inosine pathway (Rubio et al., 1973). Therefore, tissue inosine and hypoxanthine levels are inappropriate variables for evaluating the participation of adenosine in vascular control of skeletal muscle. The level of inosine reached during ischemic exercise, however, may represent the breakdown of a significant fraction of muscle adenine nucleotides. Inosine and hypoxanthine show only very modest increases in skeletal muscle during exercise with free flow perfusion (Phair and Sparks, in press).

Although it is a severe stress, ischemic or flow-restricted exercise may have clinical relevance. Atherosclerotic disease may provide an impediment to flow through a large artery in a limb (for example) that is so severe that exercise of that limb may be performed under pathologically flow-restricted conditions. Prolonged vasodilation follows exercise of calf muscles of patients with severe arteriosclerotic disease (Lassen and Kampp, 1965).

In summary, we have confirmed that skeletal muscle tissue adenosine levels rise during flow-restricted exercise. Tissue adenosine levels fall with a time course similar to the early portion of the recovery of vascular resistance following this type of exercise. These results support the hypothesis that adenosine mediates the early portion of the prolonged vasodilation seen following flow-restricted exercise of skeletal muscle.

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