Role of Adenine Nucleotides, Adenosine, and Inorganic Phosphate in the Regulation of Skeletal Muscle Blood Flow

By James G. Dobson, Jr., Rafael Rubio, and Robert M. Berne

ABSTRACT

Experiments were performed on isolated frog sartorius muscle and in situ dog skeletal muscle to determine whether adenine nucleotides and their degradation products are released during contraction in concentrations capable of producing arteriolar dilation. ATP was not detectable (<10^{-8}M) in the bathing solution of the resting or contracting frog sartorius muscle. Inorganic phosphate (P_i) in the muscle bath increased from 9 x 10^{-6}M to 28 x 10^{-5}M with 30 minutes of contraction (2 Hz) or with rest. With the dog hindlimb preparation, ATP, ADP, and AMP were not detectable (<5 x 10^{-8}M) in the venous blood collected after 5 minutes of ischemic contraction whereas P_i was present at a concentration of 3.7 x 10^{-4}M. Arterial blood levels required to elicit detectable vasodilation for ATP, ADP, AMP, and P_i were 28.7 x 10^{-4}M, 27.1 x 10^{-4}M, 31.4 x 10^{-4}M and 7.2 x 10^{-4}M, respectively. The adenosine concentration in dog muscle increased from 0.7 to 1.5 nmole/g with ischemic contraction, and hypoxanthine and inosine increased from 4.5 to 8.5 nmole/g and 2.0 to 5.5 nmole/g, respectively. The adenosine concentration in venous plasma collected from the hindlimb immediately after termination of the ischemic contraction period was 2.2 x 10^{-7}M as compared to 0.4 x 10^{-7}M in control venous and arterial blood samples. Hypoxanthine and inosine concentrations in venous blood increased 22- and 270-fold, respectively, following ischemic contraction. The calculated interstitial fluid adenosine concentration was twice the arterial concentration of adenosine required to elicit maximal arteriolar dilation. These findings suggest that adenosine may play a role in the metabolic regulation of skeletal muscle blood flow, whereas ATP, ADP, AMP, and P_i may not.

KEY WORDS autoregulation of blood flow inosine hypoxanthine skeletal muscle ischemia skeletal muscle contraction peripheral resistance reactive hyperemia functional hyperemia purine derivatives in muscle vascular smooth muscle tone dog frog

For a number of years, the adenine nucleotides have been periodically suggested as mediators of the vasodilation associated with an increase in metabolic activity or reduced oxygen supply to skeletal muscle, despite the lack of experimental verification for this hypothesis. The principal reasons for entertaining this idea have been the potent vasodilator property of this group of compounds, their presence in high concentration in muscle, and their key role in the regulation of energy metabolism.

Recently it has been reported that adenosine triphosphate (ATP) is released from isolated contracting frog sartorius muscle (1) and exercising human forearm muscle (2). In addition, inorganic phosphate (P_i), a vasoactive product of nucleotide hydrolysis, has also been postulated as a regulatory agent in blood...
flow to skeletal muscle, since levels of P1 have been shown to increase in venous plasma from contracting cat gastrocnemius muscle (3).

With respect to the heart, it has been proposed that adenosine functions as a mediator in the regulation of myocardial blood flow (4), and the possibility exists that adenosine may also serve a similar role in the metabolic regulation of skeletal muscle blood flow. Adenosine decreases skeletal muscle vascular resistance when administered intraarterially (5, 6), and accumulates in intact contracting, ischemic, contracting rat muscle (7). Furthermore, venous blood from heart and contracting skeletal muscle produces dilation of resistance vessels when infused into a forelimb artery but produces constriction when infused into a renal artery (5). The only endogenous compounds known to elicit these responses are adenosine and adenylic acid (AMP).

In view of the current interest in nucleotides and their derivatives as possible mediators of metabolic vasodilation and the availability of improved methodology for detection and quantification of adenine compounds, the present study was undertaken to determine the role of ATP, adenosine diphosphate (ADP), AMP, adenosine, and P1 in the regulation of skeletal muscle blood flow.

Methods

FROG SARTORIUS NERVE-MUSCLE PREPARATION

Sartorius nerve-muscle preparations were carefully dissected from pithed summer or winter frogs (Rana pipiens). Before dissection, the hindlimbs were perfused via the dorsal aorta with 10 ml of amphibian Ringer's solution to flush the blood from the muscle vasculature. The solution had a composition (in mm) of 111.60 NaCl, 1.88 KCl, 1.08 CaCl2 • 2H2O, 2.38 NaHCO3, 0.09 NaH2PO4 and 11.11 glucose. This solution was bubbled with air and had a pH of 7.4. The muscles were mounted at their resting length on glass rods and immersed in 300 ml of Ringer's solution for a 30-minute equilibration period at 25°C. After equilibration, the preparations were placed in a chamber containing 3.0 ml of fresh, aerated Ringer's solution. The sciatic nerve was suspended in air just above the solution on a platinum wire electrode, and the nerve was stimulated at 2–5 Hz for 30 minutes. Other preparations were allowed to rest for 30 minutes. The bathing solutions were then removed and immediately analyzed for ATP and P1.

DOG HINDLIMP PREPARATION

Twelve mongrel dogs weighing 18–22 kg were anesthetized with pentobarbital sodium (30 mg/kg, iv). The right leg was skinned to the ankle, and the circulation below this point was occluded by binding the ankle with a tight ligature. A stimulating electrode was placed on the peripheral end of the sciatic nerve and the leg was wrapped with a plastic sheet to prevent dehydration during the course of the experiment. The abdominal cavity was opened and the right common iliac vein and right external iliac artery were prepared for cannulation. After intravenous administration of 20,000 units of heparin, both vessels were severed and silicone rubber T-cannulas were inserted between the cut ends of each. The side branches of the T-cannulas were used for the collection of blood samples. Arterial blood flow was continuously recorded with a 3.5 mm cannulating electromagnetic flow probe (Biotronex). Adenosine, P1, and the sodium salts of ATP, ADP, and AMP were freshly prepared in 0.9% NaCl and were infused into the arterial cannula at a constant rate between 1.4 and 8.8 ml/min. Systemic arterial pressure was continually recorded from the left common carotid artery on a Honeywell Visicorder. Hindlimb blood flow achieved a steady, low, control value 30–40 minutes after insertion of the cannulas. Control arterial and venous blood samples were simultaneously collected after the initial equilibration period. The hindlimb muscle mass was then made ischemic for 5 minutes by occlusion of the arterial cannula and was induced to contract by electrical stimulation of the sciatic nerve with 5–10 V at 20–30 Hz during the ischemic period (Fig. 1). Experimental arterial and venous blood samples (40 ml) were simultaneously collected over a period of 8–40 seconds immediately upon termination of ischemic contraction. In some experiments, sartorius muscle samples were obtained by instantaneous freezing in situ by compression of the muscle into "wafers" (thickness 1–2 mm) with a pair of clamps precooled in liquid nitrogen. Six percent (w/v) dextran (mol wt = 60,000–90,000) in mammalian saline containing (in mm) 118.41 NaCl, 4.69 KCl, 2.52 CaCl2 • 2H2O, 25.00 NaHCO3, 1.18 MgSO4 • 7H2O and 1.18 KH2PO4 was infused intravenously over a 30-minute period to replace the blood withdrawn (approximately 160 ml). Following a second equilibration period, the control and experimental ischemic contractions were repeated and additional blood and muscle samples were obtained.
A typical pressure-flow recording from a dog hindlimb skeletal muscle preparation. Between 0 and 5 minutes the hindlimb muscle was stimulated to contract at 20-30 Hz while the arterial cannula was occluded. The brief decline in the flow tracing on release of the occlusion (5 minutes) was due to blood sampling procedures (see text). Note that arterial pressure (AP) was essentially constant during the course of the experiment.

Five minutes of ischemic muscle contraction constitutes a severe challenge. However, this procedure is not completely unphysiological, since 25-30 minutes after stimulation, hindlimb blood flow returned to control levels and the vascular bed showed normal reactive hyperemic responses to occlusions of the arterial blood supply for 5-10 seconds. This restoration of resistance vessel tone suggests that the experimental procedure had no lasting deleterious effect on the muscle vasculature, as illustrated in Figure 1.

ANALYTICAL PROCEDURES

Blood samples were collected in polyethylene centrifuge tubes immersed in ice and containing an equal volume of cold mammalian saline. Each sample was rapidly transferred to a refrigerated centrifuge and the bulk of the cellular elements was gently separated from the plasma by a force of 800-1,000 g for 10 minutes at 0°C. The dilute plasma was decanted and centrifuged a second time at 25,000 g for 10 minutes to remove the balance of the formed elements. It was then decanted and small aliquots (1-3 ml) were assayed immediately for ATP and Pi, while the remainder of the dilute plasma was subjected to ultrafiltration as previously described (8). Complete ultrafiltration required 20-24 hours at 0°C and removed proteins with molecular weight greater than approximately 30,000. The ultrafiltrates were stored at -20°C.

Tissue samples were stored (1-5 days) under liquid nitrogen prior to initiation of analytical procedures. The frozen muscle "wafers" were finely powdered in a hollow stainless steel cylinder fitted with a stainless steel piston, both precooled in liquid nitrogen. The pulverized muscles were then weighed and transferred to glass homogenizer tubes packed in dry ice. Based on tissue weight, 10 volumes of ice-cold 0.5N perchloric acid were rapidly added, and the mixture immediately homogenized for 90 seconds at 0°C with a blade homogenizer (Polytron) at a speed of 4,000-8,000 rpm. The homogenate was centrifuged at 7,000 g at 0°C for 10 minutes. The resulting supernatant fluid was filtered through fiber glass to remove insoluble lipids and then neutralized at 0°C to pH 7 with KOH. The neutralized extract was centrifuged, the precipitate (KClO₄) washed with 2-5 ml of distilled H₂O, and the supernatant fluids combined for storage at -20°C.

Activated charcoal was added to the ultrafiltrates of dilute plasma (2-5 mg/ml of original
blood sample) and to the neutralized acid extracts of muscle (50–100 mg/g wet weight) for the adsorption of purine derivatives. Each charcoal suspension was shaken for 3 hours at 25°C, separated by centrifugation (1,500 g for 10 minutes) and the supernatant fluid discarded. The charcoal was washed twice with 10 ml of water and centrifuged and the wash discarded. The purine derivatives were eluted from the charcoal by shaking vigorously for 90 minutes with 30 ml of 10% (v/v) pyridine in 50% (v/v) ethanol. The suspension was layered on an acid-washed celite column (3.0 g of celite in a tube 2.25 cm in diameter) and the eluate slowly filtered. Upon completion of filtration, an additional 15 ml of the elution fluid was placed on the column. Eluates were combined and dried with a stream of filtered air at 50°C, and the remaining solids were redissolved in 0.6 ml of H2O and either assayed for ADP and AMP or chromatographed for isolation of adenosine, inosine, and hypoxanthine.

The chromatographic procedures have been described previously (8). In brief, adenosine, inosine, and hypoxanthine were separated on thin-layer (0.5 mm) ion-exchange cellulose (Selectacel no. 69 phosphate) plates. The ascending chromatograms were developed with 15% (v/v) aqueous ethanol in closed tanks at 4°C for 4 hours. Ultraviolet fluorescent spots, which represented the isolated compounds, were eluted from the cellulose by washing four times with 5 ml of 50% (v/v) ethanol adjusted to pH 10 with NH4OH. Each cellulose suspension was stirred for 20 minutes and the supernatant fluid collected by centrifugation at 1,500 g for 10 minutes. Supernatant fluids were combined and air dried, and the residue redissolved in 1.5 ml H2O. The redissolved precipitate was centrifuged and used for enzymatic quantification of adenosine, inosine, and hypoxanthine. The percent recoveries for known quantities of adenosine, hypoxanthine, and inosine added to muscle extracts and plasma were 74.3 ± 1.5 and 78.4 ± 1.3, respectively. The percent recoveries of known amounts of ADP and AMP using this procedure were 74.3 ± 1.5 and 78.4 ± 1.3, respectively. Inorganic phosphate levels were determined on 0.5–1.5 aliquots of muscle bathing solutions and diluted plasma samples by selective and quantitative precipitation of Pi with a suspension of Ca(OH)2 in CaCl2 according to the method of Seraydarian et al. (13). The precipitates were dissolved in 2.0 ml of 0.25N HCl, and Pi determined colorimetrically with the acid molybdate method of Fiske and Subbarow (14).

Results

Frog Sartorius Nerve-Muscle Preparation

ATP was not detectable in the bathing solution after a 30-minute exposure to either resting or contracting muscle. If the nucleotide was present in the bathing solution, it was present in concentrations less than 1.0 × 10^-4M, the lower limit of sensitivity of our assay system. The concentrations of Pi in the bathing solutions from both resting and contracting muscle showed increases of ap-
ADENOSINE AND MUSCLE BLOOD FLOW

TABLE 1

<table>
<thead>
<tr>
<th>Period</th>
<th>Increase in P&lt;sub&gt;t&lt;/sub&gt; concentration above that of bathing solution* (10^{-4})M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>20</td>
</tr>
<tr>
<td>Contracting (2 Hz)</td>
<td>21</td>
</tr>
<tr>
<td>Resting</td>
<td>19</td>
</tr>
<tr>
<td>Contracting (2 Hz)</td>
<td>18</td>
</tr>
<tr>
<td>Resting</td>
<td>19</td>
</tr>
<tr>
<td>Contracting (2 Hz)</td>
<td>17</td>
</tr>
<tr>
<td>Contracting (2 Hz)</td>
<td>19</td>
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<tr>
<td>Contracting (2 Hz)</td>
<td>20</td>
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<tr>
<td>Contracting (5 Hz)</td>
<td>18</td>
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<tr>
<td>Contracting (5 Hz)</td>
<td>20</td>
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<tr>
<td>Contracting (5 Hz)</td>
<td>19</td>
</tr>
</tbody>
</table>

Freshly prepared ATP, ADP, and AMP \(10^{-6}\)M, and P<sub>t</sub> \(5 \times 10^{-8}\)M in normal mammalian saline were infused intra-arterially at different rates for a period of 1 minute. The resulting arterial plasma concentration was calculated for each experiment on the basis of the hindlimb blood flow at the onset of perfusion and the hematocrit ratio (range 0.43-0.45). The plasma levels of the adenine nucleotides and P<sub>t</sub> necessary to elicit a detectable vasodilation are shown in Table 2. A detectable vasodilation in the hindlimb preparation is defined in this study as a 10% increase in blood flow.

The concentrations of ATP, ADP, and AMP in arterial plasma required to elicit a detectable vasodilation ranged from 27.1 to \(31.4 \times 10^{-8}\)M. These concentrations are approximately sixfold greater than the lowest measurable plasma level for each nucleotide. The arterial plasma concentration of P<sub>t</sub> required to produce detectable vasodilation was \(7.2 \times 10^{-4}\)M, a level that was twice as great as that found in the plasma samples.

Adenosine in Muscle.—The adenosine concentration in control dog sartorius muscle was \(0.7\) nmole/g (wet weight) and increased to \(1.5\) nmole/g in ischemic contracting muscle (Fig. 2). The concentration of hypoxanthine and inosine in control sartorius muscle was 4.5 and \(2.0\) nmole/g, respectively. With ischemic muscle contraction the hypoxanthine and inosine levels increased to \(8.5\) and \(5.5\) nmole/g, respectively (Fig. 3).

Adenosine in Plasma.—Adenosine concentrations in control arterial and venous plasma, as well as in arterial plasma obtained after a

**TABLE 2**

Concentration of ATP, ADP, AMP and P<sub>t</sub> in Arterial and Venous Plasma of Skeletal Muscle of the Dog Hindlimb

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. Exp</th>
<th>Determined arterial and venous plasma levels at end of ischemic contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>7</td>
<td>(&lt;5.0 \times 10^{-8})M*</td>
</tr>
<tr>
<td>ADP</td>
<td>6</td>
<td>(&lt;4.8 \times 10^{-8})M*</td>
</tr>
<tr>
<td>AMP</td>
<td>6</td>
<td>(&lt;4.6 \times 10^{-8})M*</td>
</tr>
<tr>
<td>P&lt;sub&gt;t&lt;/sub&gt;</td>
<td>5</td>
<td>(3.7 \times 10^{-8})M</td>
</tr>
</tbody>
</table>

\*Lowest detectable plasma levels of these nucleotides. †Values represent mean ± 1 SE.
rates that produced an arterial plasma concentration of $2.8 \pm 0.6 \times 10^{-7} \text{M}$ (approximately the level found in venous blood after ischemic hindlimb contraction) elicited a $25 \pm 5\%$ increase in hindlimb blood flow. However, increasing the arterial plasma adenosine concentration to $33.2 \pm 2.6 \times 10^{-7} \text{M}$, a level equal to approximately one-half the sum of the concentrations of adenosine, hypoxanthine, and inosine found in venous blood after ischemic contraction, resulted in a $300 \pm 20\%$ increase in hindlimb blood flow. This degree of hyperemia is similar in magnitude to that observed following ischemic muscle contraction of the hindlimb in the present experiments.

**Discussion**

**Frog Sartorius Muscle**

The results of the experiments on the frog sartorius nerve-muscle preparations indicate that ATP is probably not released from contracting skeletal muscle in vitro in amounts required to significantly alter blood flow. These findings are not in agreement with those of Boyd and Forrester (1), who reported ATP concentrations of $1.97-19.7 \times 10^{-9} \text{M}$ in bathing solutions (2 ml) from contracting (2 Hz for 30 minutes) frog sartorius muscle. Although the sensitivity of the firefly assay system employed in the period of ischemic hindlimb contraction, ranged from 0.3 to $0.5 \times 10^{-7} \text{M}$ (Fig. 4). However, venous plasma levels of adenosine increased to $2.2 \times 10^{-7} \text{M}$ following ischemic contraction (Fig. 4). The control arterial and venous, as well as experimental arterial, plasma levels of hypoxanthine, ranged from 1.3 to $2.1 \times 10^{-7} \text{M}$, whereas the inosine levels were lower and ranged from trace amounts to $0.1 \times 10^{-7} \text{M}$ (Fig. 5). After ischemic contraction, venous plasma levels of hypoxanthine increased to $38.0 \times 10^{-7} \text{M}$ and inosine increased to $27.0 \times 10^{-7} \text{M}$ (Fig. 5). Neither of these degradation products of adenosine possess any significant vasoactivity.

**Adenosine Infusion**—Intra-arterial infusion of adenosine into the hindlimb preparation at
Plasma adenosine concentrations in blood collected from 12 dog hindlimb skeletal muscle preparations. Control arterial and venous levels represent plasma concentrations from well-perfused, resting hindlimb muscle; hyperemic levels represent arterial and venous plasma concentrations immediately on termination of 5 minutes of ischemic contraction. Vertical bars represent ± SEM for 24 determinations (2 per preparation).

Present studies allowed measurement of ATP concentrations as low as $1.0 \times 10^{-5}$ M. ATP was never detected in sartorius muscle bathing solutions from either resting or contracting muscle. Our results are also at variance with those of Abood et al. (15), who reported an increase in the release of ATP, ADP, AMP, creatine phosphate, and $P_i$ with in vitro contraction (0.33 Hz for 20 minutes) of frog sartorius muscle.

The reason for the differences between our results and those of Boyd and Forrester (1) and Abood et al. (15) is not readily apparent. It is possible that injury to frog sartorius muscle cells during dissection can release sufficient amounts of cell cytoplasm, which contains $2 \times 10^{-8}$ M ATP (16), to elevate the concentration of ATP in the extracellular fluid and bathing solution. Furthermore, a contracting, as opposed to a resting, muscle could liberate ATP and other phosphates from damaged cells by compression by adjacent, intact contracting cells. In our studies, the

Plasma hypoxanthine and inosine concentrations in blood collected from eight dog hindlimb skeletal muscle preparations. See legend of Figure 4.
muscles were repeatedly rinsed with frog Ringer's solution before the start of the experiments to wash all cytoplasm from cells that may have been damaged during dissection. In some experiments in which this precaution was not taken, small concentrations of ATP were detectable.

**DOG HINDLIMB PREPARATION**

Five minutes of ischemic muscle contraction in the dog hindlimb skeletal muscle preparation constitutes a strong stimulus but was used because such a maneuver should reveal whether any of the adenine nucleotides or Pi are released from the muscle into the venous blood. Since ATP, ADP, and AMP were not detected in venous plasma from either resting or ischemic contracting dog hindlimb skeletal muscle, and since plasma concentrations of these compounds necessary to elicit a detectable vasodilation were approximately six times greater than the lowest measurable plasma concentrations, we conclude that the adenine nucleotides are not directly involved in skeletal muscle blood flow regulation.

The plasma concentrations of the adenine nucleotides required to elicit a detectable vasodilation in our dog hindlimb preparation were similar to those necessary to induce a noticeable decrease in vascular resistance in the intact dog forelimb (17). Forrester and Lind (2) reported ATP concentrations in venous plasma from resting human forearm muscle 1.3—4.6 times greater than that required to elicit a detectable vasodilation in the hindlimb preparation. With sustained contraction of forearm muscle, the venous blood ATP concentrations were 300 times greater. These large plasma ATP concentrations from the human forearm are not reconcilable with our results or with the results of others (18, 19) and may reflect contamination from damaged cellular elements in the blood. Moreover, Forrester and Lind (2) suggested that platelets probably contributed to their plasma ATP concentrations. Although not reported in our studies, ATP was occasionally found in plasma after the first centrifugation (800-1,000 g) but not after the second centrifugation, suggesting that the plasma obtained after the initial low-speed centrifugation still contained formed elements (probably erythrocytes and platelets) that could have been responsible for the ATP observed. In two samples, in which hemolysis was apparent, ATP was also detected.

Control and experimental arterial and venous plasma levels of Pi of the dog hindlimb skeletal muscle preparation were less than $3.7 \times 10^{-4}$m. An arterial plasma concentration of twice this value was necessary to elicit a small arteriolar dilation. However, Pi was reported by Hilton and Chir (3) to be released from isolated, blood-perfused, contracting white-fibered skeletal muscle in the cat, in proportion to the frequency of contraction. Our hindlimb preparation contained both white- and red-fibered muscle, which may account for the difference between our results and those of Hilton and Chir (3). Barcroft et al. (20) reported that phosphate concentrations increased by 20% in blood from contracting human forearm muscle in which blood flow increased tenfold. However, when blood phosphate concentrations were increased as much as fourfold above control by infusion of a mixture of NaH$_2$PO$_4$ and Na$_2$HPO$_4$, flow did not change. Although Hilton and Chir (3) suggested that Pi may be involved in active hyperemia, organic phosphate in addition to Pi may have contributed to the response, since they used the Fiske and Subbarow (14) method for Pi determination. This method used without prior selective isolation of Pi, as performed in the present studies according to the methods of Seraydarian et al. (13), does not eliminate contamination with organic phosphate. Although our results indicate that the adenine nucleotides probably do not contribute to the increase in plasma phosphate levels reported by these investigators (3), it is possible that some unidentified organic phosphate(s) with unknown vasoactive property may be responsible for the apparent increase in plasma Pi levels.

Resting skeletal muscle of the dog contains measurable amounts of adenosine that increase when oxygen demand exceeds oxygen supply. These findings are in agreement with
previous studies on rat skeletal muscle (7). The adenosine present in the muscle is presumably restricted to the extracellular fluid, as has been postulated for the myocardium (4), due to the ubiquitous nature of adenosine deaminase. For example, at extracellular adenosine concentrations far in excess of those existing in anoxic muscle, adenosine was not detectable within red cell ghosts because of the high activity of adenosine deaminase (21). These ghost cells are capable of incorporating extracellular adenosine into intracellular adenine nucleotides or deaminating it to inosine, but free adenosine was found in the ghost cells only at an extracellular adenosine concentration of 1 mM. If indeed adenosine is confined to the extracellular space in skeletal muscle, and the extracellular fluid volume constitutes 20% of the skeletal muscle volume (22), the concentration of adenosine in the interstitial fluid after ischemic contraction would be 7.5 n mole/ml. This calculated interstitial fluid adenosine concentration (75 x 10^-9 M) is greater than twice the arterial plasma adenosine concentration (approximately 33 x 10^-9 M) required by intra-arterial infusion to produce a degree of hyperemia equal to that observed following ischemic muscle contraction. This suggestion is based on the assumption that since both interstitial fluid and plasma are in communication with the vascular smooth muscle of the resistance vessels, equal concentrations of adenosine in each of these compartments would have comparable effects in attenuating vascular smooth muscle tone.

Adenosine in venous plasma from the dog hindlimb increased about fivefold with ischemic contraction, whereas the levels of hypoxanthine and inosine increased 22- and 270-fold, respectively, above control levels. Studies on the rate of adenosine degradation in blood (8) indicate that about half of the adenosine is deaminated in the time required for the blood to pass from the capillaries to the collecting tube. Adenosine present in interstitial fluid is also converted to inosine and hypoxanthine in its passage through the vessel wall, since endothelial cells and pericytes contain adenosine deaminase and nucleoside phosphorylase (23) (enzymes that catalyze the conversion of adenosine to inosine and hypoxanthine, respectively). Hence, the concentration of adenosine at the level of the resistance vessels is undoubtedly much greater than that present in the blood collected from the iliac vein, a conclusion supported by the greater ratio of hypoxanthine and inosine to adenosine in venous plasma as compared to this ratio in the muscle.

Therefore, it appears that the concentration of adenosine reached in the interstitial fluid during ischemic contraction of the muscle is more than sufficient to account for the decrease in vascular resistance during the period of reactive hyperemia. These observations represent the first demonstration of adenosine in skeletal muscle effluents and suggest that adenosine may play a role in the local regulation of skeletal muscle blood flow in a manner similar to that proposed for cardiac muscle (4). Blood flow regulation by adenosine in skeletal muscle would predict that with an increase in oxygen consumption or a reduction in the arterial oxygen supply, cellular AMP levels would increase at the expense of ATP and the 5'-nucleotidase which in skeletal muscle is present in vessel walls and in muscle tissue adjacent to blood vessels (24), would dephosphorylate AMP to adenosine at a faster rate. Consequently, the interstitial fluid concentration of adenosine would increase, which in turn would cause skeletal muscle resistance vessels to dilate and muscle blood flow to increase to a level that would maintain muscle oxygen balance. Other vasoactive factors such as H^+, K^+ reduced PO_2, or increased osmolarity probably participate in the reactive hyperemia following ischemic contraction, but the extent of their participation, as well as that of adenosine remains to be elucidated. However, it appears unlikely that adenine nucleotides and inorganic phosphate are involved in the local regulation of skeletal muscle blood flow in a manner similar to that postulated for adenosine.
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