Adenosine Concentrations in the Interstitium of Resting and Contracting Human Skeletal Muscle

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Background—Adenosine has been proposed to be a locally produced regulator of blood flow in skeletal muscle. However, the fundamental questions of to what extent adenosine is formed in skeletal muscle tissue of humans, whether it is present in the interstitium, and where it exerts its vasodilatory effect remain unanswered.

Methods and Results—The interstitial adenosine concentration was determined in the vastus lateralis muscle of healthy humans via dialysis probes inserted in the muscle. The probes were perfused with buffer, and the dialysate samples were collected at rest and during graded knee extensor exercise. At rest, the interstitial concentration of adenosine was 220±100 nmol/L and femoral arterial blood flow (FaBF) was 0.19±0.02 L/min. When the subjects exercised lightly, at a work rate of 10 W, there was a markedly higher (1140±540 nmol/L; P<0.05) interstitial adenosine concentration and a higher FaBF (2.22±0.18 L/min; P<0.05) compared with at rest. When exercise was performed at 20, 30, 40, or 50 W, the concentration of adenosine was moderately greater for each increment, as was the level of leg blood flow. The interstitial concentrations of ATP, ADP, and AMP increased from rest (0.13±0.03, 0.07±0.03, and 0.07±0.02 μmol/L, respectively) to exercise (10 W; 2.00±1.32, 2.08±1.23, and 1.65±0.50 μmol/L, respectively; P<0.05).

Conclusions—The present study provides, for the first time, interstitial adenosine concentrations in human skeletal muscle and demonstrates that adenosine and its precursors increase in the exercising muscle interstitium, at a rate associated with intensity of muscle contraction and the magnitude of muscle blood flow. (Circulation. 1998;98:6-8.)

Key Words: blood flow ▪ exercise ▪ adenosine ▪ muscles ▪ interstitium

In skeletal muscle, the match between oxygen demand and blood flow is closely regulated, with a linear relationship between work intensity and blood flow. This precise regulation of blood flow has been proposed to involve locally produced vasoactive metabolites, such as adenosine.

Traditionally, the metabolism of compounds in human skeletal muscle has been assessed by determinations of arteriovenous concentration differences over the muscle and measurements made on muscle tissue samples. However, largely because of a rapid uptake of adenosine by several cell types, such as erythrocytes and endothelial cells, measurements of adenosine in blood have provided little information regarding adenosine metabolism in human muscle. Moreover, determinations of purine compounds in human muscle biopsies have not demonstrated a significant elevation in adenosine during exercise. The muscle interstitium is a highly relevant compartment for indicating the concentrations of adenosine in the muscle, because this is the site at which it exerts several of its actions by binding to adenosine receptors on the surface of vascular and skeletal muscle cells. In the present study, the interstitial adenosine and adenine nucleotide concentrations were examined in human muscle with dialysis probes inserted into the vastus lateralis muscle. The adenosine levels were compared with the magnitude of leg blood flow at rest and during contraction.

Methods

Experimental Protocol

Seven healthy male subjects were informed of the experimental procedures before giving their informed consent. After local anesthesia, a 14-gauge cannula was inserted 2 to 3 cm into the distal part of the musculus vastus lateralis in a direction parallel to the muscle fiber orientation. The dialysis probes (described below) were introduced into the muscle via the cannula. After 1 hour of rest, the first dialysis perfusate samples were collected, and then the subjects performed 5 bouts of dynamic knee extensor contractions on a modified Krogh ergometer. The exercise bouts consisted of five 15-minute work periods, separated by 15 minutes of rest, performed in random order at work rates of 10, 20, 30, 40, and 50 W.

Microdialysis Technique

The dialysis probes consisted of a 4-cm-long semipermeable dialysis membrane (cutoff, 5000 to 6000 Da; ID, 0.20 mm; OD, 0.22 mm) attached at both ends to hollow nylon tubes (ID, 0.50 mm; OD, 0.63 mm). The probes were perfused at a rate of 5 μL/min with a Ringer’s acetate solution (pH 5.6) containing 0.5 mmol/L lactate and 0.63 mm). The probes were perfused at a rate of 5 μL/min with a Ringer’s acetate solution (pH 5.6) containing 0.5 mmol/L lactate and...
Leg Blood Flow

Leg blood flow was determined by ultrasound Doppler.9

Analysis

Adenosine, AMP, ADP, and ATP in perfusate and dialysate were quantitatively analyzed with reverse-phase high-performance liquid chromatography.10

Statistics

Differences between means were determined with a 1-way repeated-measures ANOVA. Significance was set at \( P<0.05 \).

Results

The interstitial concentration of adenosine in the muscle at rest was 220±100 nmol/L. During knee extensor exercise at 10 W, the interstitial adenosine concentration was higher \((P<0.05)\), and it was further elevated with increasing work rates (Figure, panel a).

The muscle interstitial concentrations of ATP, ADP, and AMP at rest were 126±30, 73±30, and 67±20 nmol/L, respectively. The concentrations of adenine nucleotides were higher \((P<0.05)\) at 10 W than at rest, and the nucleotide levels were moderately elevated with the increases in work rate (Figure, panel b).

Leg blood flow at rest was 0.19±0.02 L/min and was elevated \((P<0.05)\) for each increment in work rate (Figure, panel c). Vascular conductance was estimated on the basis of the measured leg flow values and values for blood pressure obtained in a similar study using the same exercise model.1

Estimated conductance was 2.0, 22.2, 28.3, 35.3, 38.8, and 40.4 mm Hg \( \cdot \) min\(^{-1}\) \cdot mL\(^{-1}\) at rest and during work rates of 10, 20, 30, 40, and 50 W, respectively. The correlation coefficients for the interstitial adenosine concentration and leg blood flow or estimated vascular conductance were \( r=0.98 \) \((P<0.001)\) and \( r=0.97 \) \((P<0.01)\), respectively.

Discussion

The present study shows, for the first time, interstitial concentrations of adenosine and its adenine nucleotide precursors in resting and contracting human skeletal muscle. The muscle interstitial adenosine concentration was measured with a microdialysis technique, and an average adenosine concentration of 220 nmol/L was found at rest. This value is of the same magnitude as that previously reported for interstitial canine cardiac muscle adenosine concentrations (100 to 220 nmol/L), values estimated from measurements of coronary arteriovenous adenosine concentration differences and blood flow.5

Exercise had a marked effect on the muscle interstitial adenosine concentrations with an \( \approx 5\)-fold increase from rest to a light work rate of 10 W. When exercise was performed

3 mmol/L glucose. Dialysate (outflow) was collected at rest and during the last 10 minutes of each work rate. To determine the relative exchange of adenosine over the dialysis membrane, \([2^\text{H}]\)adenosine \((<0.1 \mu\text{Ci/mL})\) was included in the perfusate. Scintillation fluid was added to the samples, which were counted in a liquid scintillation counter. The relative loss (recovery) of adenosine \((\text{RL}=\text{Out}_{\text{DPM}}/\text{In}_{\text{DPM}})\) at rest and at 10, 20, 30, 40, and 50 W was 60±8%, 65±5%, 64±5%, 61±4%, 60±4%, and 60±4%, respectively. The interstitial adenosine concentrations \((I)\) were calculated on the basis of the perfusate (inflow) and dialysate (outflow) concentrations, as well as recovery of each microdialysis probe, according to the following equation: 

\[
I = \frac{\text{Out} - \text{RL}}{\text{RL}} + \text{In}.\]

Concentration of (a) adenosine \((n=7)\) in human skeletal muscle interstitial fluid and (b) ATP \((\blacktriangle, n=6)\), ADP \((\square, n=5)\), and AMP \((\bullet, n=7)\) measured in dialysate. c, Femoral arterial blood flow. Measurements were made at rest and during graded dynamic knee extensor exercise. Data are mean±SEM. *Adenosine, ATP, and blood flow; #ADP; and §AMP denote significant difference \((P<0.05)\) between successive work rates.

at 20, 30, 40, or 50 W, the concentration was moderately greater for each increment. The pattern was similar to the response in blood flow as well as estimated vascular conductance. These findings provide support for the proposition that adenosine is of importance in muscle vasodilation. The nonlinear increase in the interstitial adenosine concentration could be explained by a multicomponent regulation of muscle blood flow, in which adenosine may act in synchrony with other vasoactive substances.

It should be mentioned that nucleotides could have been released from cells because of tissue damage induced by the insertion of the microdialysis probes into the muscle. Nevertheless, the 5 exercise intensities were performed in random order, and it appears unlikely that the change in intracellular adenine nucleotides and adenosine concentrations would be gradual if the cause of increase was merely due to damage.

The enzyme responsible for adenosine formation in skeletal muscle tissue is AMP 5’-nucleotidase. This enzyme exists both as a soluble cytosolic enzyme and as an ectoenzyme located in
the membrane of cells, with the active site probably facing out toward the extracellular space.\(^{11,12}\) Although the proportion of the enzyme existing in an ectoform has not been established in human skeletal muscle, studies on other animal cell types, such as cardiac myocytes, suggest that a majority of the enzyme is membrane-bound.\(^{13}\) With a large proportion of the enzyme located in the cell membranes of the muscle, the formation of adenosine would occur in the interstitial space, provided that the substrate AMP is present. In the present study, it was found that the concentration of AMP as well as ADP and ATP increased in the interstitium with exercise. As for adenosine, the interstitial concentrations of AMP, ADP, and ATP increased the most from rest to 10 W of exercise. These findings suggest that the interstitium is a potential site for adenosine formation. Furthermore, the \(K_m\) for AMP for 5'-nucleotidase in skeletal muscle of animals has been reported to be 19 \(\mu\text{mol/L}\) for the purified muscle enzyme,\(^{14}\) which is markedly higher than the observed dialysate concentration of AMP at rest and during exercise (from 0.05 to 3 \(\mu\text{mol/L}\)). Therefore, it is likely that the formation of adenosine is regulated, at least in part, by the availability of the substrate. The source of muscle interstitial adenine nucleotides remains to be elucidated, but nerve endings may be a likely origin.\(^{15}\)

Although adenosine has been proposed to be involved in the regulation of many processes of importance in human skeletal muscle during exercise, such as blood flow, sympathetic activity,\(^{16}\) and insulin-mediated glucose uptake,\(^{17}\) an increase in adenosine in contracting human skeletal muscle tissue has never before been demonstrated. The present study shows that adenosine is present in the human muscle interstitium and that the concentration of interstitial adenosine is associated with exercise intensity, muscle blood flow, and vascular conductance. Furthermore, the interstitial concentrations of the adenosine precursors AMP, ADP, and ATP are increased in parallel with adenosine, showing that the substrate for the ecto form of AMP 5'-nucleotidase is available to allow for an extracellular formation of adenosine.

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