Systemic Hypoxia Elevates Skeletal Muscle Interstitial Adenosine Levels in Humans

Dave A. MacLean, PhD; Lawrence I. Sinoway, MD; Urs Leuenberger, MD

**Background**—Adenosine is a potent vasodilator that has been shown to increase in cardiac tissue in response to hypoxia. However, peripheral vasodilatation also occurs during hypoxia, and the vasoactive substance(s) responsible for skeletal muscle vasodilatation have not yet been completely identified. Therefore, the purpose of this study was to measure and quantify skeletal muscle interstitial adenosine during acute systemic hypoxia.

**Methods and Results**—Skeletal muscle interstitial adenosine concentrations were determined by the microdialysis technique, in which 4 semipermeable microdialysis probes were inserted into the vastus lateralis muscle of 6 healthy male subjects and perfused at a rate of 5 μL/min with Ringer’s solution. Sixty minutes after the insertion of the microdialysis probes, systemic hypoxia was induced for 30 minutes by having the subjects breathe a mixture of 10.5% O₂ in N₂. Arterial oxygen saturation (fingertip oximeter) was lowered (P<0.05) from 96±0.7% to 74.9±1.4%, and forearm blood flow was increased 28%. During normoxia, the interstitial adenosine concentration was 0.44±0.08 μmol/L, and it was increased to 1.03±0.15 (P<0.05) and 0.85±0.09 (P<0.05) after 15 and 30 minutes of hypoxia, respectively.

**Conclusions**—These data are consistent with the concept that during acute systemic hypoxia, interstitial adenosine plays a key role in stimulating peripheral vasodilation. (Circulation. 1998;98:1990-1992.)

Key Words: dialysis ▪ blood flow ▪ oxygen

Systemic hypoxia is commonly seen in a variety of disease states, including heart failure, shock, pulmonary embolism, and lung disease. To compensate for the decreased O₂ delivery to tissues, a number of mechanisms are activated, resulting in an increase in ventilation, heart rate, cardiac output, and peripheral (muscle) blood flow. Although a number of different compounds have been suggested to be responsible for the hypoxia-induced increase in blood flow, the most widely recognized and investigated has been adenosine. Much of the previous work has used various heart models, and most of the evidence for a role of adenosine came from studies showing that hypoxia-induced hyperemia was attenuated by adenosine deaminase, adenosine receptor blockade, or the inhibition of adenosine production.

In contrast, the effects of hypoxia on skeletal muscle blood flow have been investigated to a much lesser extent. However, it has been shown that during systemic hypoxia, the release of adenosine from canine skeletal muscle was increased. Furthermore, it has been demonstrated in rodent muscle that adenosine largely mediates the vasodilation associated with systemic hypoxia, and that this vasodilation was dependent on nitric oxide synthesis. However, whether these same substances or mechanisms are responsible for the hypoxia-induced vasodilatation in human skeletal muscle is unclear. Furthermore, human skeletal muscle interstitial adenosine levels have never been measured during hypoxia, and these data would greatly advance our understanding of the regulation of peripheral blood flow. Therefore, the purpose of the present study was to measure and quantify interstitial adenosine levels during acute systemic hypoxia in humans.

**Methods**

**Subjects**
The experimental protocol was approved by the institutional clinical investigation committee, and written informed consent was obtained. Six healthy male subjects with a mean age of 32 years (range, 26 to 40 years) participated in this study. None of the subjects were taking any medication, and all refrained from the ingestion of caffeine-containing beverages for 24 hours before the studies.

**Microdialysis**
The fibers used to construct the microdialysis probes were obtained from an artificial dialysis kidney (GFE 18) that had a molecular weight cutoff of 3000 Da (ID, 0.20 mm; OD, 0.22 mm). Each end of a single fiber was inserted into a hollow nylon tube (ID, 0.50 mm; OD, 0.63 mm) and glued. The actual probe length (distance between the 2 nylon tubes) was 4 cm. Four microdialysis probes were inserted into the vastus lateralis muscle (~50% type I and 50% type II fibers) of 1 leg, with the skin and subcutaneous tissue at the microdialysis probe entrance and exit sites anesthetized with a local injection of lidocaine HCl. The probes were then inserted into the muscle via a 14-gauge cannula in a direction parallel to muscle fiber orientation. After insertion, the microdialysis probes were activated, resulting in an increase in ventilation, heart rate, cardiac output, and peripheral (muscle) blood flow.1–3
were attached to a perfusion pump (CMA model 102) and perfused at a rate of 5 μL/min with a Ringer’s solution containing 0.5 mmol/L lactate and 3.0 mmol/L glucose.

Materials
The subjects reported to the laboratory after an overnight fast, and after microdialysis probe insertion, they rested supine for 60 minutes before the experimental protocol was initiated. A sealed face mask with separate valves for inspired and expired gases was positioned over the subject’s mouth and nose for the delivery of a hypoxic gas mixture and the monitoring of minute ventilation (V̇E) and end-tidal CO₂. A 15-minute baseline collection period was conducted while the subjects breathed room air (normoxia), during which heart rate (ECG), blood pressure (Finapres), V̇E (pneumotachometer), end-tidal CO₂ (RGM, Ohmeda), and arterial oxygen saturation (O₂ Sat, pulse oximeter) were monitored every minute and collected on a Gould recorder. Meanwhile, microdialysis dialysate was collected over the entire 15-minute period. Systemic hypoxia was induced by having the subjects breathe a mixture of 10.5% O₂ in N₂ for 30 minutes. All cardiovascular and ventilatory variables were collected every minute, whereas dialysate was collected in two 15-minute blocks (0 to 15 and 16 to 30 minutes). In 1 subject, because of technical problems, hypoxia gas delivery was suspended after only 15 minutes. In 3 subjects, venous occlusion plethysmography was used to measure forearm blood flow (FBF). With this technique, forearm circumference is measured in a mercury-in-Silastic strain-gauge plethysmograph while a cuff is inflated to suprasystolic pressure to exclude blood flow to the hand, and blood flow is expressed as mL · min⁻¹ · 100 mL⁻¹. It should be noted that all cardiopulmonary data were averaged over the 15-minute collection periods to match the time frame used for dialysate collection.

Analyses
Dialysate adenosine (40 μL) concentrations were determined by the method of Tullson et al and high-performance liquid chromatography (HPLC). To determine the exchange fraction (called “probe recovery”) of adenosine over the microdialysis membrane, a small amount of [2-³H]adenosine (<0.1 μCi/mL) was included in the perfusate as the internal reference marker, as previously described. Five microliters of perfusate and collected dialysate was added to 3 mL of scintillation fluid and counted in a liquid scintillation counter for the determination of adenosine specific activity. Probe recovery was then calculated on the basis of the relative loss of [2-³H]adenosine (perfusate₀–dialysate₀/perfusate₀), and interstitial adenosine was calculated by dividing dialysate minus perfusate adenosine by probe recovery. It should be noted that despite the addition of a small amount of adenosine ([2-³H]adenosine, nmol/L range) to the perfusate, on analysis by HPLC, no adenosine was detected in any of the perfusate samples.

Statistics
The differences between normoxia and hypoxia were analyzed with the method of Tullson et al and high-performance liquid chromatography (HPLC). To determine the exchange fraction (called “probe recovery”) of adenosine over the microdialysis membrane, a small amount of [2-³H]adenosine (<0.1 μCi/mL) was included in the perfusate as the internal reference marker, as previously described. Five microliters of perfusate and collected dialysate was added to 3 mL of scintillation fluid and counted in a liquid scintillation counter for the determination of adenosine specific activity. Probe recovery was then calculated on the basis of the relative loss of [2-³H]adenosine (perfusate₀–dialysate₀/perfusate₀), and interstitial adenosine was calculated by dividing dialysate minus perfusate adenosine by probe recovery. It should be noted that despite the addition of a small amount of adenosine ([2-³H]adenosine, nmol/L range) to the perfusate, on analysis by HPLC, no adenosine was detected in any of the perfusate samples.

Results
The cardiovascular and ventilatory data are presented in the Table. There were ≈17% and 12% decreases (P<0.05) in O₂ Sat and end-tidal CO₂, respectively, during hypoxia compared with normoxia. In contrast, hypoxia resulted in ≈23% and 41% increases (P<0.05) in heart rate and V̇E, respectively, compared with normoxia. In the 3 subjects in whom FBF was measured, hypoxia resulted in a 1.3 mL · min⁻¹ · 100 mL⁻¹ increase (28%) in skeletal muscle blood flow. There were no significant shifts in mean arterial pressure throughout the experiment.

Microdialysis probe recovery remained unchanged from normoxia (51.7±2.2%) to hypoxia (0 to 15 minutes, 50.3±2.1%; 16 to 30 minutes, 49.3±2.2%). Interstitial adenosine levels more than doubled (P<0.05) during the first 15 minutes of hypoxia (Figure) and remained elevated (P<0.05) throughout the remainder of the experiment. It appears from the data that there was a small decrease in the interstitial adenosine levels during the final 15 minutes (16 to 30 minutes) of hypoxia. However, this decrease is primarily due to a reduction in sample size (n=6 to n=5) as a result of having to suspend hypoxia gas delivery in 1 subject during the final 15 minutes of the experiment.

Discussion
The major finding of this study was that during acute systemic hypoxia (≈75% O₂ Sat), interstitial adenosine levels were more than doubled, and these observations represent the first time that skeletal muscle interstitial adenosine has been measured under such conditions. It is well documented that during systemic hypoxia, peripheral blood flow is increased. In the present study, FBF was determined in 3 subjects to confirm this finding. It was observed that skeletal muscle blood flow was increased 1.3 mL · min⁻¹ · 100 mL⁻¹, whereas in a study by Leuenberger et al using exactly the same protocol, FBF was increased 1.2 mL · min⁻¹ · 100 mL⁻¹. These findings suggest that skeletal muscle blood flow was increased in the present study during hypoxia and that the increase was similar to that previous observed.

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxia (0–15 min)</th>
<th>Hypoxia (15–30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>89.2±3.6</td>
<td>88.4±4.5</td>
<td>88.1±7.0</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66.1±5.6</td>
<td>81.3±4.0*</td>
<td>79.9±4.4*</td>
</tr>
<tr>
<td>V̇E, L/min</td>
<td>8.1±0.9</td>
<td>11.4±1.0*</td>
<td>9.9±1.3*</td>
</tr>
<tr>
<td>Endtidal CO₂, %</td>
<td>39.6±1.4</td>
<td>35.0±1.5*</td>
<td>35.5±1.9*</td>
</tr>
<tr>
<td>O₂ Sat, %</td>
<td>96.4±0.7</td>
<td>80.3±1.7*</td>
<td>74.9±1.4*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure. All values are mean±SEM. *Significantly different from normoxia, P<0.05.
There are only a handful of studies in which interstitial adenosine levels have been measured, especially during hypoxia, and even these have yielded conflicting results. For example, both an increase and no change in interstitial adenosine levels have been observed in cardiac tissue in response to systemic hypoxia. Meanwhile, the only study in which skeletal muscle interstitial adenosine levels were determined was performed by Hellsten et al and illustrated that during 1-legged knee extensor exercise in humans, interstitial adenosine levels were increased as a function of exercise intensity and blood flow. These data provide new information regarding the direction and magnitude of change of interstitial adenosine in the face of increased metabolic demand and blood flow. In the present study, the resting interstitial adenosine concentration was 0.44 ± 0.08 μmol/L, which is slightly higher than that seen by Hellsten et al for human skeletal muscle but very close to those values observed by Wang et al for cardiac tissue.

In the present study, an acute bout of systemic hypoxia resulted in a doubling of the interstitial adenosine concentrations. These findings suggest that in human skeletal muscle, adenosine plays a very important role in the regulation of local blood flow. Similar findings have been reported for rodent muscle and canine muscle; it should be noted, however, that the level of hypoxia used in the present study is mild compared with those used in various animal models. It has been suggested, in animal preparations, that other compounds interact with adenosine to mediate the vasodilation associated with hypoxia. For example, it has been demonstrated that adenosine stimulates ATP-sensitive K⁺ channels on skeletal muscle to open, and the resulting release of K⁺ acts as a vasodilator. More recently, it has been shown that vasodilation in rats induced by adenosine during systemic hypoxia was dependent on nitric oxide synthesis. However, it is unclear whether a similar interaction between adenosine and nitric oxide exists in human skeletal muscle. These findings illustrate that the mechanisms associated with hypoxia-induced vasodilation are complex and that although adenosine is a key mediator, other compounds may interact with adenosine and also contribute to increased peripheral blood flow.

The measurement of interstitial compounds provides information not readily obtained from other conventional methods, such as arteriovenous differences and muscle biopsies. Detection of changes in interstitial concentrations is important because the interstitium is the site at which many of the metabolites are either produced or exert their effects. Therefore, interstitial determinations provide valuable information regarding not only the direction and magnitude of change in key metabolites but also their role in regulatory mechanisms. Furthermore, from the above discussion, it will be important in the future to measure all the putative regulators when investigating the mechanisms associated with hypoxia-induced vasodilation. In conclusion, the present study demonstrates that skeletal muscle interstitial adenosine is increased in response to acute systemic hypoxia. Furthermore, the microdialysis technique allows the measurement and quantification of compounds in the interstitial space and, when applied to future studies, will lead to valuable new insights into the mechanisms associated with hypoxia-induced vasodilation.

Acknowledgments
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References
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