Acute systemic hypoxia elevates venous but not interstitial potassium of dog skeletal muscle

F. M. Mo and H. J. Ballard
Department of Physiology, The University of Hong Kong, Pokfulam, Hong Kong
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Mo, F. M., and H. J. Ballard. Acute systemic hypoxia elevates venous but not interstitial potassium of dog skeletal muscle. Am J Physiol Heart Circ Physiol 289: H1710-H1718, 2005. First published May 13, 2005; doi:10.1152/ajpheart.00614.2004.—Potassium release through ATP-sensitive potassium (K\textsubscript{ATP}) channels contributes to hypoxic vasodilation in the skeletal muscle vascular bed: It is uncertain whether K\textsubscript{ATP} channels on muscle cells contribute to the process. Potassium from muscle cells must cross the interstitial space to reach the vascular tissues, whereas that from vascular endothelium would have a higher concentration in venous blood than in interstitial fluid. We determined the effect of systemic hypoxia on arterial, venous, and interstitial potassium in the constant-flow-perfused gracilis muscles of anesthetized dogs. Hypoxia reduced arterial PO\textsubscript{2} from 138 to 25 and PCO\textsubscript{2} from 28 to 26 mmHg. Arterial pH and potassium were well correlated (r\textsuperscript{2} = 0.9); Both increased in early hypoxia and decreased during the postcontrol. In denervated muscles, perfusion pressure decreased from 95 to 76 mmHg by the end of the hypoxic period; neither venous nor interstitial potassium was elevated. In innervated muscles, perfusion pressure increased from 110 to 172 mmHg by the 11th min of hypoxia and then decreased to 146 mmHg by the end of the hypoxic period; venous potassium increased from 5.0 to 5.3 mM, but interstitial potassium remained unchanged. Glibenclamide abolished both the increase in venous potassium and the hypoxic vasodilation in the innervated muscle. Thus skeletal muscle cells were unlikely to have contributed to the release of potassium, which was suggested to originate from vascular endothelium. The sympathetic nerve supply may play a direct or indirect role in the opening of K\textsubscript{ATP} channels under hypoxic conditions.

ACUTE HYPOXIA PRODUCES systemic vasodilation in humans and other large mammals (4, 23), despite an increase in sympathetic vasoconstrictor nerve activity (27). The mechanisms accounting for this vasodilation have not been fully elucidated. However, the release of potassium through ATP-sensitive potassium (K\textsubscript{ATP}) channels has been reported to contribute to hypoxic vasodilation in a number of vascular beds, including that of skeletal muscle (21), which is important in determining peripheral resistance.

In the rat skeletal muscle circulation, hypoxia led to the formation and release of adenosine (18, 22), which, through an action on its receptors, opened K\textsubscript{ATP} channels (7) and released potassium; this was suggested to mediate the vasodilation (21), since glibenclamide abolished the increase in venous potassium and reduced the vasodilation that resulted from the systemic hypoxia. It was proposed that K\textsubscript{ATP} channels on the skeletal muscle cells were partially responsible for the potassium release (20). Hypoxia also increased the arterial potassium concentration ([K\textsuperscript{+}]; see Ref. 26); the source and mechanism of this potassium release remain uncertain. The effect of hypoxia on the venoarterial difference for potassium has been unpredictable (20), because of the high arterial level of potassium. Furthermore, the source of the potassium released in hypoxia could not be identified, since only the plasma potassium could be determined. Thus the involvement of K\textsubscript{ATP} channels in the vasodilator response to hypoxia is quite well established, but the contribution of K\textsubscript{ATP} channels on skeletal muscle cells remains a matter of conjecture.

In this study, we used interstitial microdialysis to investigate the contribution of skeletal muscle cells to the increase in venous potassium in hypoxia: Potassium released from skeletal muscle cells must necessarily pass through the interstitial space to reach the blood. For comparison, we also determined interstitial potassium during muscle contractions, a situation in which it is well established that potassium is released from the skeletal muscle cells. To further clarify the source of the potassium released in hypoxia, we also determined the venous [K\textsuperscript{+}]:Potassium that is released from the vascular endothelium must necessarily appear in the venous blood, but only a fraction of it (or none at all) would cross the diffusion barrier into the interstitial space.

Potassium release through K\textsubscript{ATP} channels was reported to be brought about by an action of adenosine on one of its receptors (7). Our studies have suggested that the mechanism and route of adenosine release during hypoxia may be influenced by the extent of sympathetic nerve tone on the muscle (unpublished observations). This raised the possibility that the nerves may also influence the release of potassium during hypoxia: We investigated this by comparing the potassium released from the innervated and denervated muscle during hypoxia. Finally, glibenclamide was administered to identify what portion of the venous potassium was attributable to release through K\textsubscript{ATP} channels and to estimate the contribution of K\textsubscript{ATP} channel opening to the hypoxic vasodilation.

METHODS

Surgical procedures. Experiments were performed in 25 male or female mongrel dogs weighing 8–23.5 kg (average wt 18.1 kg). All experimental protocols were approved by the University of Hong Kong Committee on the Use of Live Animals in Teaching and Research. Animals were anesthetized with ketamine and xylazine (5 and 2 mg/kg im) followed by pentobarbital sodium (6.9 ± 0.6 mg/kg iv, plus 0.08 mg·kg\textsuperscript{-1}·h\textsuperscript{-1}). Sodium bicarbonate (28 mM) in sodium chloride (150 mM) was infused at 0.05 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} to replace volume losses resulting from evaporation from the surgical areas.
A cannula was inserted in the trachea for positive pressure ventilation at a stroke rate of 17 min⁻¹. Arterial PCO₂ was determined using an Orion model 9502BN carbon dioxide electrode attached to an Orion model 710A meter (Orion Research, Beverly, MA), and the stroke volume was adjusted to keep PCO₂ at 36.4 ± 2.5 (SE) mmHg (n = 25). Arterial PO₂ was determined using a Strathkelvin Instruments (Glasgow, UK) model 781 oxygen meter, and the oxygen content of the inspired air was adjusted to keep PO₂ at 131 ± 3 mmHg. Arterial pH was measured using an AgCl pH electrode (model 5993–11; Cole-Parmer, Vernon Hills, IL) attached to an Orion model EA 920 ion analyzer and was maintained at 7.44 ± 0.02 by infusion of sodium bicarbonate (50–300 mM), if required. Esophageal temperature was kept at 36.2 ± 0.2°C by heating coils under the operating table. A pressure transducer (model P23XL; Gould) was connected to a carotid artery to monitor systemic arterial blood pressure; after connection of the perfusion circuits, systemic pressure was held constant at 74.1 ± 3.9 mmHg by a pressure reservoir connector to the brachial artery.

The gracilis muscle was vascularity isolated as previously described (1), and three microdialysis probes (LM10; Bioanalytical Systems, West Lafayette, IN) were implanted in the muscle using a needle. Probes were perfused at 2 μl/min with a solution of similar composition to the interstitial fluid, except that it was potassium free (in mM: 146 NaCl, 2.4 CaCl₂, 0.5 MgSO₄, 1.2 NaH₂PO₄, and 5.6 glucose, pH 7.3–7.4). The muscle was allowed to stabilize for 1 h before heparin sodium (500 IU/kg iv, followed by 100 IU·kg⁻¹·h⁻¹ iv) was given, and the perfusion circuit was connected as previously described (22). The gracilis muscle was perfused through isolated sections of the femoral and saphenous arteries with blood from the contralateral femoral artery; venous blood leaving the muscle was returned to the dog via a jugular vein. A pressure transducer (model P23XL; Gould) was connected to the arterial side of the perfusion circuit to measure the perfusion pressure. Signals were amplified using Gould 13–4615–50 transducer amplifiers and were recorded on a Gould RS 3400 recorder.

After 30 min of stabilization, the free flow was measured by timed collections of venous outflow, and the pump was set to deliver 150% of the free flow. In 12 dogs, the gracilis muscle was then denervated: in the 4 dogs that were to be used only for stimulation experiments, the obturator nerve was sectioned; in the 8 dogs that were to undergo “denervated muscle” hypoxia experiments (including the 2 that underwent stimulation followed by hypoxia), the femoral, sciatic, and obturator nerves were all sectioned. The remaining 13 dogs were used for “innervated muscle” hypoxia experiments, and all hindlimb nerves were left intact. Perfusion pressure decreased after nerve section but was allowed to recover to a stable value before the experimental procedures were begun. Thus microdialysis probes were perfused for >90 min before sampling.

Experimental protocol for muscle contractions. Six animals were used in this series. A 10-min control period was allowed and then muscle contractions were induced by stimulation of the peripheral end of the obturator nerve using 2-Hz, 6-volt, 1-ms pulses (model S48; Grass) for a further 10 min. Blood samples were collected from the femoral artery and vein at 0 and 10 min of control, at the end of 10 min of contractions, and at 10 and 20 min of postcontrol for pH, blood gas, and plasma potassium analysis. Microdialysis samples were collected for 10 min each for determination of interstitial potassium, except that it was potassium free (in mM: 0.25). Arterial PO₂ was determined using a Strathkelvin Instrument model 781 equipped with a thermistor and a barometric pressure sensor (model H11011; Strathkelvin Instruments, Glasgow, UK) at the arterial reservoir was adjusted to bring systemic arterial blood pressure back to its control value. In the denervated muscle experiments, microdialysate was not collected during the 5-min equilibration period, but in the innervated muscle experiments it was. In both cases, microdialysate was collected for two successive 7-min periods during the establishment hypoxia. After 20 min of hypoxia, the animal was returned to normoxia, and three postcontrol dialysate samples were taken. Blood samples were collected from the femoral artery and vein for pH, PO₂, PCO₂, and plasma potassium analysis at the end of each dialysate collection. The hypoxic protocol was performed one or two times in each dog: in experiments where the hypoxic treatment was performed two times in the same animal under identical conditions, the average result for that animal was used.

Effects of glibenclamide. Nine dogs were used to study the effects of glibenclamide on the responses of the innervated muscle to hypoxia; all of these dogs had previously been subjected to hypoxia in the absence of drugs, as described above. Control plasma and microdialysate samples were collected, and glibenclamide (1 mg in 1 ml) was then injected in the arterial supply of the muscle. After the muscles had been weighed at the end of the experiment, this dose was determined to be 19.1 ± 0.8 mg/kg muscle, which is sufficient to block the opening of K_ATP channels in skeletal muscle (21). One further set of control samples was collected in the presence of glibenclamide, and the hypoxia and recovery protocol was then repeated as above.

When all experiments had been completed, animals were euthanized with an intravenous infusion of saturated potassium chloride, and the gracilis muscle was removed and weighed.

Analytical methods. Microdialysate samples were collected in ice-cooled vials and stored at 0–4°C. Samples (40 μl) were diluted to 2.0 ml with deionized water just before analysis. Blood samples (1 ml) were rapidly withdrawn in precleaned syringes and centrifuged (15 × 15,000 revolutions/min). Plasma (500 μl) was transferred to a fresh vial and stored at 0–4°C. Duplicate plasma samples (60 μl) were diluted to 2.0 ml with deionized water for analysis. [K⁺] was determined by flame photometry. Microdialysis probe recovery was determined from the slope of a graph of dialysate [K⁺] vs. perfusate [K⁺]: At the end of five of the experiments, the probes were perfused with one potassium-free solution and two further concentrations of potassium (in the range 10–50 mM) for 10 min each. Dialysate was collected for 10 min at each concentration. Potassium recovery was 50% (r² = 0.84).

All concentrations have been corrected for dilution, and microdialysate concentrations have been further corrected for probe recovery.

Statistical methods. Values are means ± SE of the number of animals tested, unless otherwise stated. They were compared with their own precontrols using one-way repeated-measures ANOVA, followed by Fishers least significant difference test.

RESULTS

Twenty-five dogs were used; the gracilis muscles weighed 59 ± 4 g; resting blood flow was 5.3 ± 1.3 ml·min⁻¹·100 g⁻¹, and the constant flow was set to 7.3 ± 1.4 ml·min⁻¹·100 g⁻¹. Control values were monitored for up to 30 min before beginning each test; there was no significant variation in any of the measured parameters during the precontrol period.

Muscle contractions. Experiments were performed in six dogs. Control arterial PO₂ was 127 ± 6 mmHg, PCO₂ was 37 ± 4 mmHg, and pH was 7.39 ± 0.03; arterial blood gases and systemic arterial blood pressure remained constant through-

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out the experiment. Muscle contractions (2 Hz) produced a peak force of 4.5 ± 1.0 kg/100 g (n = 5) during the 1st min of contractions, which declined to 0.9 ± 0.2 kg/100 g by the 10th min.

Changes in the arterial perforation pressure and venous blood gases during and after muscle contractions are shown in Table 1. In some experiments, perfusion pressure recovery was monitored to completion, which took ∼2.5 h. Plasma and interstitial [K+] are shown in Fig. 1. Arterial [K+] remained unchanged throughout the experiment. Both venous and interstitial [K+] were increased during muscle contractions but had returned to control by 20 min of recovery.

Systemic hypoxia in denervated muscle. Eight dogs were tested. The arterial and venous blood gases and pH are shown in Table 2. Hypoxic treatment decreased arterial PO2 from 138 to 25 mmHg and arterial oxygen content from 22.7 ± 1.4 to 11.0 ± 1.3 ml/100 ml. Oxygen uptake by the muscle was 0.13 ± 0.02 ml·min⁻¹·100 g⁻¹ in the precontrol period. It decreased in the early part of the hypoxia [to 0.07 ± 0.03 (P < 0.005) at 12 min of hypoxia] but had returned to 0.13 ± 0.02 by the end of the hypoxic period, and increased above the precontrol, to 0.20 ± 0.03 ml·min⁻¹·100 g⁻¹ (P < 0.05), during the postcontrol. Arterial PCO2 decreased slightly during hypoxia, but venous PCO2 did not change. Arterial pH increased during the first 5 min of hypoxia, but returned to control at the 12th and 19th min of hypoxia, and dropped below control in the recovery period. Venous pH did not change during hypoxia, but it also decreased below precontrol during the postcontrol period.

The arterial perfusion pressure response to hypoxia is summarized in Fig. 2. After a delay of ∼5 min, arterial perfusion pressure began to decrease: it had dropped significantly below the control value (P < 0.05) by the 4th min of hypoxia, and remained at this level for the rest of the hypoxic period. Perfusion pressure began to increase as soon as the hypoxic treatment was stopped and had returned to control by the 5th min of recovery.

Systemic arterial blood pressure was controlled by a pressure reservoir connected to the carotid artery. In the first 5 min of hypoxia, systemic pressure increased more rapidly than the pressure control system could respond, and it increased from 74 ± 8 to 87 ± 8 mmHg. By about the 6th min of hypoxia, the systemic arterial pressure had been adjusted back to control, where it was maintained throughout the rest of the experiment.

The plasma and interstitial [K+] are shown in Fig. 3. Systemic hypoxia increased the arterial [K+] at the 5th min, but it had returned to control before the end of the hypoxic treatment. Neither the venous nor the interstitial [K+] were significantly changed by hypoxia.

Systemic hypoxia in the innervated muscle. Thirteen dogs were tested. The arterial and venous blood gases and pH are shown in Table 3: hypoxic treatment reduced the oxygen content of the arterial blood from 20.7 ± 1.3 to 11.7 ± 1.2 ml·min⁻¹·100 g⁻¹. The responses were similar to those in the denervated muscle in that both arterial and venous PO2 were decreased during the hypoxic treatment, and oxygen uptake by the muscle decreased from 0.13 to 0.03 ml·min⁻¹·100 g⁻¹ by the 12th min of hypoxia and increased above control to 0.21 ml·min⁻¹·100 g⁻¹ during the recovery period. Both arterial and venous PCO2 decreased slightly during hypoxia, whereas arterial pH increased above control after 5 min of hypoxia, but both arterial and venous pH decreased below control in the recovery period.

The systemic arterial blood pressure response to hypoxia was similar to that in the previous group of animals. The arterial perfusion pressure increased rapidly at the onset of hypoxia, reaching a peak after ∼10 min (Fig. 2). Perfusion

| Table 1. Arterial perfusion pressure and venous blood gases and pH in the denervated gracilis muscle before, during, and after muscle contractions |
|---------------------------------|-------------------|-------------------|
| **Arterial perfusion pressure, mmHg** | **Venous PO2, mmHg** | **Venous PCO2, mmHg** | **Venous pH** |
| Precontrol | Contractions | Recovery | Precontrol | Contractions | Recovery | Precontrol | Contractions | Recovery |
| 113±14 | 45±4* | 75±16| 64±5 | 19±2* | 64±4 | 41±3 | 76±5* | 61±8† |
| 3.73±0.02 | 7.12±0.04* | 7.24±0.05* |

Values are means ± SE of 6 tests, taken immediately before beginning the muscle contractions (precontrol), at the end of 10 min contractions (contractions) or after 20 min of recovery (recovery). Significantly different from precontrol: *P < 0.001 and †P < 0.01.

Fig. 1. Gracilis muscle arterial, venous, and interstitial potassium before, during, and after 2-Hz muscle contractions. Values are means ± SE of 6 estimations. [K⁺], potassium concentration. **P < 0.001 and *P < 0.01 vs. precontrol.
Pressure then decreased in all dogs: the perfusion pressure during the last 8 min of hypoxia was significantly lower than its peak value (P < 0.05), although it remained higher than control. When the hypoxia was withdrawn, perfusion pressure decreased rapidly back to control.

The plasma and interstitial [K+] are shown in Fig. 4. Arterial [K+] was significantly increased at 5 and 12 min of hypoxia, and it dropped below control in the early part of the recovery period. However, there was also an increase in venous [K+] at 12 and 19 min of hypoxia, and it dropped below control at 10 and 20 min postcontrol. Interstitial [K+] was not significantly changed at any time point in hypoxia or normoxia.

Effects of glibenclamide. Nine dogs were tested. Administration of glibenclamide did not alter arterial or venous blood gases or pH. The changes in the arterial and venous blood gases and pH in responses to hypoxia after glibenclamide Table 2. Arterial and venous blood gases and pH in the denervated gracilis muscle before, during, and after systemic hypoxia

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PO2, mmHg</td>
<td>PCO2, mmHg</td>
</tr>
<tr>
<td>Precontrol</td>
<td>10</td>
<td>137.4 ± 5.5</td>
</tr>
<tr>
<td>20</td>
<td>138.2 ± 4.3</td>
<td>28.7 ± 0.9</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5</td>
<td>24.5 ± 2.4*</td>
</tr>
<tr>
<td>12</td>
<td>25.8 ± 1.6*</td>
<td>25.9 ± 1.7†</td>
</tr>
<tr>
<td>19</td>
<td>23.7 ± 1.7*</td>
<td>26.9 ± 2.1†</td>
</tr>
<tr>
<td>Postcontrol</td>
<td>10</td>
<td>106.7 ± 2.6*</td>
</tr>
<tr>
<td>20</td>
<td>134.3 ± 3.5</td>
<td>29.1 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 tests. Significantly different from control: *P < 0.001 and †P < 0.05.
administration were similar to those in the innervated or denervated muscle in the absence of drugs: The hypoxic treatment significantly depressed both arterial and venous $P_{CO_2}$ ($P < 0.001$), whereas arterial pH increased at the onset of hypoxia ($P < 0.005$) and decreased below control in the recovery period ($P < 0.001$). Both arterial and venous $P_{CO_2}$ decreased significantly ($P < 0.05$) during hypoxia.

Resting femoral arterial perfusion pressure was increased by the administration of glibenclamide from $110 \pm 10$ to $130 \pm 8$ mmHg. Perfusion pressure increased in the early part of the hypoxia in a similar manner to that of the innervated muscles without glibenclamide (Fig. 2); perfusion pressure in the presence of glibenclamide did not differ significantly from that in its absence during the first 12 min of hypoxia. However, in the presence of glibenclamide, the perfusion pressure did not decrease in any animal during the second half of the hypoxic period. Thus, during the last 8 min of hypoxia, perfusion pressure in the presence of glibenclamide was significantly higher than that in its absence.

Changes in the arterial, venous, and interstitial $[K^+]$ during hypoxia in the presence of glibenclamide are shown in Fig. 5. The arterial $[K^+]$ increased in the early part of hypoxia in a similar fashion to that in the innervated muscle without glibenclamide. However, the increase in the venous $[K^+]$ toward the end of hypoxia was abolished by glibenclamide.

Because the effects of glibenclamide are not easily reversible, the hypoxic treatment with glibenclamide always followed hypoxic treatment in its absence. Therefore, control experiments were performed in four dogs in which the hypoxic treatment was performed two times in the absence of drugs. The two hypoxic treatments produced similar changes in venous potassium in both tests: venous potassium increased from $4.46 \pm 0.24$ to $4.81 \pm 0.36$ mM ($P < 0.01$; paired $t$-test) in the first test and from $4.22 \pm 0.24$ to $4.81 \pm 0.36$ mM ($P < 0.01$; paired $t$-test) in the second test.

Relationship between arterial pH and potassium. Figure 6 shows the mean values of the arterial pH and $[K^+]$ for all 21 dogs subjected to hypoxic treatment: These two parameters were well correlated ($r^2 = 0.90$).

**DISCUSSION**

This study showed that venous potassium increased during hypoxia in the innervated, but not in the denervated, muscles, suggesting that extrinsic nerves may have been involved in the potassium release under the conditions of this study. Elevation of venous potassium during hypoxia was abolished by glibenclamide, suggesting that the potassium was released through $K_{ATP}$ channels. Interstitial potassium was not elevated by hypoxia, although muscle contractions clearly increased both venous and interstitial potassium. Thus it is extremely unlikely that the potassium released in hypoxia could have originated from the skeletal muscle cells. We also confirmed earlier findings that arterial potassium was elevated in hypoxia and extended those findings to show that it dropped below control during the posthypoxic recovery period.
Hypoxic treatment. The hypoxic treatment decreased the oxygen supply to the muscle by 50%. We think that it took most of the first 5 min for the muscle oxygen to decrease to its steady-state hypoxic level, since the venous PO2, which lagged behind arterial PO2 by \( \approx \) 1 min due to the dead volume of the perfusion circuit, had not yet plateaued at 5 min. By the 12th min of hypoxia, oxygen uptake by the muscle had decreased from 0.13 to 0.07 ml \( \cdot \) min \( \cdot \) 100 g \( \cdot \) 1, although it had returned to its precontrol value by the 19th min. This does not necessarily indicate that the actual oxygen use by the muscle cells was limited during the middle part of the hypoxia, since the reduction in PO2 would cause the oxygen stores (e.g., those bound to myoglobin) to be released. Based on reported values for the myoglobin-bound oxygen content of dog muscle (8) and the correlation between venous PO2 and myoglobin-associated oxygen in muscle (3), we estimated that the myoglobin saturation decreased from \( \approx \) 85 to 40% between the 5th and 12th min of hypoxia, liberating an additional 0.07 ml \( \cdot \) min \( \cdot \) 100 g \( \cdot \) 1 of oxygen, which was similar to the deficit in oxygen uptake during that period. The oxygen uptake also rose slightly above control in the postcontrol period, which is consistent with “refilling” of the oxygen stores. Thus we feel that there is no clear evidence of oxygen insufficiency in the skeletal muscle cells during the hypoxic period and, hence, no reason to suppose that the intracellular ATP was altered.

Hemodynamic responses to hypoxia. The vasoconstrictor component of the hypoxic response predominated in the innervated muscle during the first 10 min of hypoxia. The rapid initial vasoconstriction was probably mediated by the sympathetic nerves, due to the rapidity of its onset in the innervated muscle and its absence in the denervated muscle (Fig. 2). The sympathetic tone appeared to have been maintained throughout the hypoxia: There was a rapid decrease in perfusion pressure in the innervated muscles upon the return to normoxia (Fig. 2), which was consistent with a reduction in vasoconstrictor tone. Vasodilation was superimposed on the initial vasoconstrictor response after \( \approx \) 10 min of hypoxia in the innervated muscle (Fig. 2). This was probably mediated by the opening of K\textsubscript{ATP} channels, since glibenclamide abolished the vasodilation (Fig. 2). K\textsubscript{ATP} channel opening also appeared to reduce the initial vasoconstriction in the innervated muscles during the first 1–2 min of hypoxia, since perfusion pressure was higher in the
presence of glibenclamide than its absence in this period (Fig. 2). Previously, glibenclamide had been reported to inhibit hypoxic vasodilation of isolated arterioles from rat skeletal muscle (30), but, in blood-perfused rat muscle, glibenclamide significantly reduced hypoxic dilation only during the 1st min of systemic hypoxia, and not during 2–5 min (7, 21), which is in agreement with the current findings in dog muscle. The present study is the first report that opening of K_ATP channels contributes to hypoxic vasodilation in skeletal muscle in the later part of sustained systemic hypoxia: We cannot tell from the present study whether the early and late opening of K_ATP channels involves the same opening mechanism, or even the same pool of channels. Interestingly, however, in the present study, the denervated muscles also exhibited hypoxic vasodilation during the latter part of the hypoxia, despite their failure to release potassium, which suggests that other mechanisms for hypoxic vasodilation (in addition to the opening of K_ATP channels) are present in the skeletal muscle vasculature; possibly, these alternative mechanisms were inhibited or overridden by the high level of sympathetic tone in the innervated muscles.

Venous potassium in hypoxia. Although there was net uptake of potassium from the blood under both normoxic and hypoxic conditions, hypoxia induced a small (~0.5 mM) increase in venous potassium in the innervated muscle. We may speculate that the [K+] within the vascular wall might have been higher, with only a portion of the potassium reaching the venous blood. We believe that this hypoxia-induced increase in venous potassium represents the release of potassium through K_ATP channels (which was superimposed on the potassium uptake) since it was blocked by glibenclamide. A glibenclamide-sensitive increase in skeletal muscle venous potassium during hypoxia has been reported previously (21), and the current findings are in good agreement with that report. The main purpose of this study was to investigate whether the venous potassium was released through K_ATP channels on skeletal muscle cells by taking parallel measurements of venous and interstitial potassium.

The hypoxic increase in venous potassium was abolished by denervation of the muscle, which suggests that extrinsic nerves may play a role in the potassium release. The sympathetic nerves supplying the skeletal muscle are strongly activated in hypoxia (19), as the reflex response to chemoreceptor activation; thus, these nerves might mediate the potassium release, either directly or indirectly. However, other types of nerves, such as cholinergic, purinergic, and nonadrenergic-noncholinergic, are also present in skeletal muscle, and the current study did not test their possible involvement.

Our findings do not show what cell type acted as the source of the increased venous potassium, but we can rule out skeletal muscle, since the interstitial potassium did not increase, and there was a net concentration gradient for potassium from the blood to the interstitial space. The blood also seems unlikely, because it is hard to see how activation of nerves could stimulate potassium release from blood cells. The nerve cells themselves are a possibility, but they are not the most likely source of the potassium, first because we are not aware of any reports of K_ATP channel opening on nerve cells during the depolarization and second because perivascular nerves terminate in the medial and adventitial layers of the blood vessels, so we would have expected similar increases in venous and interstitial potassium if it had come directly from the nerves.

K_ATP channels are expressed in both vascular smooth muscle and vascular endothelium (10, 28), and there are arguments for and against either of them acting as the main source of the released potassium; the apparent role for extrinsic nerves in the potassium release favors the smooth muscle as the source, since the nerves terminate close to smooth muscle cells. On the other hand, a study on isolated arterioles from rat skeletal muscle (30) suggested that α2D adrenergic receptors inhibited vascular smooth muscle K_ATP channels, whereas hypoxia opened the same pool of K_ATP channels; thus, hypoxia inhibited α2D constriction, and α2D receptor stimulation inhibited hypoxic vasodilation. Therefore, we would expect sympathetic nerve activation to reduce, not increase, potassium release from smooth muscle cells. Furthermore, we cannot see any reason why potassium release should be unidirectional, and we would have expected some elevation of the interstitial concentration to be detectable if potassium had come from the smooth muscle cells. The selective release of potassium in the blood and not the interstitial space tends to favor the vascular endothelium as the more likely source, although there is no obvious mechanism by which sympathetic nerves, which terminate in the adventitia, could directly influence the vascular endothelium. On the other hand, the glibenclamide-sensitive hypoxic vasodilation of isolated coronary vessels was completely abolished by endothelial denudation (17), which also favors an endothelial origin for the elevation in venous potassium. Thus, on balance, we believe that the vascular endothelium was the most likely source of the potassium released through K_ATP channels in the venous blood.

It is possible that the influence of the extrinsic nerves on potassium release may be an indirect, rather than a direct, phenomenon. For example, the highly constricted state of the vasculature in the innervated preparation may have resulted in some heterogeneity of flow, raising the possibility that there may have been small regions of more severe hypoxia in the innervated muscle, where potassium release was facilitated. It is also possible that the high perfusion pressure, resulting from the vasoconstriction of the innervated preparation, may have stimulated endothelial potassium release.

Opening of K_ATP channels has previously been reported to contribute to the hypoxic vasodilation of coronary (17) and skeletal muscle blood vessels (21). Opening of the K_ATP channels was suggested to be linked to the action of adenosine on one of its receptors, since glibenclamide could inhibit the vasodilator response to adenosine agonists (2, 7). Furthermore, in skeletal muscle, the adenosine-receptor antagonist 8-phenyl theophylline attenuated the rise in venous potassium induced by hypoxia or exogenous adenosine (21). Others have reported that the opening of K_ATP channels is a mechanism common to the whole group of physiological vasodilators that act through G protein activation of adenylyl cyclase and cAMP-dependent protein kinase, which includes agents acting on adenosine A1-, A2-, and A3-receptors, as well as β-adrenergic agonists (28). We have previously reported that adenosine was released in the venous blood in the denervated muscle during hypoxia (23), but we found that adenosine only appeared in the interstitial space of the innervated (but not the denervated) muscle in hypoxia (unpublished observation). This raises another possible mechanism by which the sympathetic nerves may indirectly
influence potassium release in the muscle: we may speculate that the activation of sympathetic nerves led to the increase in interstitial adenosine (for example, through the extracellular breakdown of ATP coreleased from the nerves themselves) and that this adenosine in turn diffused to the vascular endothelium where it acted on its receptors to cause the opening of $K_{\text{ATP}}$ channels.

**Arterial potassium in hypoxia.** The changes in the arterial [K$^+$] during and after hypoxia were similar in the innervated or denervated muscle, with or without glibenclamide. Thus it is unlikely that this increase in arterial potassium could account for the increase in venous potassium during hypoxia, which was seen only in the innervated muscle in the absence of glibenclamide. Elevation of arterial potassium in hypoxia has been reported previously (21, 24, 26). Here we extend those findings with the observation that arterial potassium falls below control in the posthypoxic period.

Arterial potassium and arterial pH both increased early in hypoxia, and dropped below control in the posthypoxic period (Tables 2 and 3 and Fig. 6), and the increase in arterial potassium at the onset of hypoxia could be abolished by preequilibrating the blood to a higher pH (24). We propose that the formed elements of blood are the most likely source(s) for the elevated arterial potassium, for, if vascular endothelium had been responsible, we would have expected a higher venous rather than arterial level. Similarly, it is unlikely that recirculation of the increased venous potassium in innervated muscles contributed significantly to the increased arterial concentration, for the venous concentration remained below arterial throughout hypoxia. We speculate that potassium transport out of the red blood cells might account for it. Two mechanisms exist, either or both of which might produce an efflux of potassium from the red blood cells in hypoxia. First, hypoxia caused red blood cell swelling (15) which, in turn, stimulated potassium efflux through chloride-dependent potassium transport (5). Second, pH elevation directly stimulated potassium efflux from red blood cells (25) through a volume-independent mechanism (6), which might involve the opening of Ca$^{2+}$-activated potassium channels, since hypoxia induced parallel increases in red blood cell mean cell volume, pH$\text{I}$, and intracellular Ca$^{2+}$ (15); Ca$^{2+}$-activated potassium channels in a number of other tissues are opened by elevation of pH$\text{I}$ (9). The good correlation between arterial pH and potassium (Fig. 6) tends to suggest that direct stimulation of potassium efflux from red blood cells by pH elevation is the more likely of the two possible mechanisms.

**Muscle contractions.** We measured interstitial potassium during muscle contractions for comparison with that during hypoxia, and to ensure that we could accurately determine interstitial concentrations using microdialysis, which was confirmed by the good agreement of our results with those of others.

Interstitial potassium increased from 3.4 to 5.7 mM during muscle contractions. It has long been known that venous potassium increased during muscle contractions (14). Previous studies have reported increases in interstitial potassium of up to 5 mM during muscle contractions (12); the magnitude of the increase was related to factors such as the force and duration of the muscle contraction (11, 13). Potassium was released from contracting muscle cells in the interstitial space; thus, extracellular potassium (16) and intracellular sodium (12) increased, whereas intracellular potassium decreased. Elevation of interstitial potassium produced local vasodilation (16), which helped to match oxygen supply to demand.

In conclusion, potassium was released in the skeletal muscle venous blood during hypoxia. Because the interstitial concentration was lower than the vascular concentration, the skeletal muscle cells could not have been responsible for the increase in venous potassium, which was suggested to originate from vascular endothelium. Glibenclamide abolished both the potassium release and the vasodilator component of the vascular response to hypoxia, suggesting that the opening of $K_{\text{ATP}}$ channels makes an important contribution to hypoxic vasodilation in the skeletal muscle circulation. Red blood cells are the most likely source for the increased arterial potassium in hypoxia.

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**REFERENCES**


