Adenosine contributes to hypoxia-induced forearm vasodilation in humans

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Adenosine contributes to hypoxia-induced forearm vasodilation in humans. J. Appl. Physiol. 87(6): 2218–2224, 1999.—In humans, hypoxia leads to increased sympathetic neural outflow to skeletal muscle. However, blood flow increases in the forearm. The mechanism of hypoxia-induced vasodilation is unknown. To test whether hypoxia-induced vasodilation is cholinergically mediated or is due to local release of adenosine, normal subjects were studied before and during acute hypoxia (inspired O2 10.5% ~20 min). In experiment I, aminophylline (50–200 µg·min−1·100 ml forearm tissue−1) was infused into the brachial artery to block adenosine receptors (n = 9). In experiment II, cholinergic vasodilation was blocked by atropine (0.4 mg over 4 min) infused into the brachial artery (n = 8). The responses of forearm blood flow (plethysmography) and forearm vascular resistance to hypoxia in the infused and opposite (control) forearms were compared. During hypoxia (arterial O2 saturation 77 ± 2%), minute ventilation and heart rate increased while arterial pressure remained unchanged; forearm blood flow rose by 35 ± 6% in the control forearm but only by 5 ± 8% in the aminophylline-treated forearm (P < 0.02). Accordingly, forearm vascular resistance decreased by 29 ± 5% in the control forearm but only by 9 ± 6% in the aminophylline-treated forearm (P < 0.02). Atropine did not attenuate forearm vasodilation during hypoxia. These data suggest that adenosine contributes to hypoxia-induced vasodilation, whereas cholinergic vasodilation does not play a role.

cholinergic vasodilation; aminophylline

In humans, systemic hypoxia leads to an increase of sympathetic nerve traffic directed to skeletal muscle (23, 24, 27). Activation of the sympathetic nervous system through hypoxia has been attributed to hypoxic stimulation of peripheral arterial chemoreceptors (10, 21), but central effects of hypoxia may also play a role (10). In classic human studies, brief periods of moderate-to-severe hypoxia (PO2 ~35 Torr) have been shown to increase cardiac output and forearm blood flow (FFB) (11, 18). Because arterial pressure did not change, the increase in FFB was due to vasodilation (18). A more recent study (13), where muscle sympathetic nerve activity (MSNA), FBF, and skin blood flow were measured simultaneously, suggested skeletal muscle vasodilation during hypoxia despite an increase in sympathetic activity directed to this vascular territory. Thus, in skeletal muscle, the neural and vascular responses to hypoxia appear to be dissociated.

One potential mechanism that may explain simultaneous sympathoexcitation and vasodilation during hypoxia may be the local release of vasodilator metabolites (21). In this theory, similar to vasodilation in exercising muscle, metabolites or other vasoactive factors may oppose sympathetic vasoconstrictor influences, thereby leading to vasodilation in skeletal muscle (12). Candidate substances include nitric oxide, adenosine, and a series of other factors (21).

In recent investigations in rats, hypoxia-induced vasodilation in the hindlimb was attenuated by systemic administration of the adenosine-receptor antagonists aminophylline and 8-phenyltheophylline (16). These observations support the concept that release of adenosine in skeletal muscle may contribute to the vasodilation seen during systemic hypoxia (16). Adenosine, a metabolic by-product of ATP utilization, has long been recognized to play a pivotal role in the regulation of coronary vascular tone during myocardial ischemia (3, 7, 12) and may also be an important regulator of skeletal muscle blood flow (12). Alternatively, it is conceivable that during hypoxia increased sympathetic nerve traffic directed to skeletal muscle may represent activity in vasodilator nerves, i.e., hypoxia-induced vasodilation could be cholinergic in nature. Evidence of cholinergic vasodilation has been found in skin of the human forearm (1, 4) but also in forearm muscle (25).

Therefore, the aim of this study was to determine whether the local release of adenosine or cholinergic vasodilation was responsible for hypoxia-induced vasodilation in humans. To this end, we examined the effects of hypoxia with and without regional pharmacological blockade of adenosine receptors in the forearm as well as with and without blockade of cholinergic vasodilator pathways.

METHODS

Subjects. We studied 22 healthy men (age 27 ± 1 yr) who were not taking any medications. The studies were performed after an overnight fasting period. Caffeine products were held for at least 12 h. The study protocol was approved by the Institutional Clinical Investigations Committee, and each study participant signed written informed consent.

Instrumentation. All subjects were studied in the supine position. Electrocardiographic electrodes were connected to determine heart rate and to monitor the heart rhythm. Before cannulation of the brachial artery, Allen's test was performed to confirm the presence of a functional ulnar artery (9). After local anesthesia of the antecubital fossa with 2% lidocaine hydrochloride (Abbott Laboratories, North Chicago, IL), a 20-gauge 10-cm catheter was positioned retrogradely in the brachial artery of the nondominant arm and was connected to a pressure transducer (Abbott Critical Care Systems, Abbott Laboratories). The arterial catheter was flushed intermit-
tently with heparinized saline and was used for measurement of arterial pressure and selective forearm infusion of drugs. Mean arterial pressure was calculated as diastolic pressure plus one-third pulse pressure.

Respiratory measurements. Minute ventilation (l/min), end-tidal CO₂ (%) and arterial oxygen saturation (%) were determined via a respiratory gas monitor and an ear-lobe oximeter (Ohmeda RGM 5200, Ohmeda, CO). Respirations were monitored via a pneumobelt strain-gauge device.

Plethysmography. Blood flow in the cannulized (experimental) and opposite (control) forearms was determined by venous occlusion plethysmography (13, 18, 20, 30). In this method, venous return from the forearm is intermittently interrupted with an inflatable upper arm cuff, and the rate of increase of the forearm volume, an index of arterial inflow, is determined with a mercury-in-Silastic strain gauge. To this end, both forearms were supported and slightly elevated. Venous occlusion cuffs were placed on the upper arms. An inflation pressure of 50 mmHg was chosen as it prevents venous outflow without impeding arterial inflow into the limb (20). The strain gauges were placed ~10 cm distal to the antecubital fossa. To ensure that the forearm veins were "empty" at the beginning of each flow measurement, the forearms were elevated to at least ~10 cm above the level of the right atrium. One minute before each series of forearm flow measurements, wrist cuffs were inflated (inflation pressure 250 mmHg) to exclude blood flow through the hands (13, 20). At least six to eight simultaneous flow curves (over ~5 min) were obtained in each condition and averaged. After each flow measurement, enough time was allowed for the plethysmographic signal to return to baseline, thus ensuring that all changes of forearm volume induced by venous occlusion were due to transient changes of intravascular volume rather than extravasation of fluid. It is important to point out that these flow measurements reflect total forearm flow, which predominately represents flow through skeletal muscle and, to a lesser extent, flow through skin and bone (20). Therefore, plethysmographic flow measurements do not permit the detection of differential effects of hypoxia or drug infusions on these different vascular territories.

The strain gauges were calibrated before each study by determining the deflection of the signal from baseline corresponding to a 1% increase in their length. FBF was expressed as milliliters per minute per 100 milliliters of forearm tissue. Forearm vascular resistance (FVR) was calculated as mean arterial pressure divided by FBF and was expressed as millimeters Hg per minute per 100 milliliters of forearm tissue.

Adenosine-receptor blockade. To block adenosine receptors in the human forearm, we infused aminophylline into the brachial artery as described by Taddei et al. (28). In preliminary experiments, we observed mild vasodilation with intraarterial (ia) infusions of aminophylline, even at rates that were not expected to vasodilate. Aminophylline (Abbott Laboratories) infused at 25, 50, 100, and 200 µg·min⁻¹·100 ml forearm tissue⁻¹ ia for 10 min increased FBF by 22 ± 10% (n = 7), 32 ± 7% (n = 5), 59 ± 14% (n = 6), and 48 ± 18% (n = 5) respectively. Therefore, to determine an infusion rate of aminophylline that inhibited adenosine-induced vasodilation, we performed individual blocking experiments. Aminophylline (Adenocard, Fujisawa USA, Deerfield, IL) was begun at 5 µg·min⁻¹·100 ml forearm tissue⁻¹, and was increased if necessary to 10 µg·min⁻¹·100 ml⁻¹ ia to achieve an approximate doubling of FBF after 5 min of infusion (aminophylline only). Aminophylline was then added at 50 µg·min⁻¹·100 ml⁻¹ ia, and FBF measurements were repeated after 10 min of simultaneous infusion of both drugs. If no attenuation of FBF was seen, the aminophylline infusion was increased at 10-min intervals to 100 and 200 µg·min⁻¹·100 ml⁻¹, respectively, until FBF was attenuated (adenosine plus aminophylline). Once attenuation of FBF was demonstrated, the adenosine infusion was stopped while aminophylline was continued, and FBF measurements were repeated after 10 min (aminophylline only).

Cholinergic blockade. Cholinergic blockade in the forearm was achieved by ia infusion of atropine sulfate (Fujisawa USA), as described by Blair et al. (4). This infusion regimen has been shown to abolish vasodilation induced by ia infusion of acetylcholine at 20 µg/min while not affecting systemic hemodynamics (4). To confirm the adequacy of cholinergic blockade, we validated this technique in three subjects. After measurements of baseline FBF, acetylcholine was infused at 40 µg/min for 5 min, and FBF measurements were repeated. While the acetylcholine infusion was continued, atropine sulfate (0.4 mg ia) was infused over 4 min, and FBF measurements were repeated. Acetylcholine for ia infusion was prepared from sterile 1% ophthalmic solution of acetylcholine chloride (MiOchol, Ciba Vision Ophthalmics, Duluth, GA).

Experiment I: Effects of aminophylline on the forearm vascular responses to hypoxia. In one group of subjects, after instrumentation and acclimatization to the face mask for ~15 min, FBF was determined in both forearms (baseline). The aminophylline infusion was then begun at the minimum dose that produced an attenuation of adenosine-induced vasodilation in the prior drug titration protocol (see above). After 10 min of the aminophylline infusion, FBF measurements were repeated in both forearms (aminophylline vs. no drug). The subjects then breathed 10.5% O₂ in N₂ for ~20 min. Hemodynamic, ventilatory, and FBF measurements in both forearms were repeated during the last 5 min of hypoxia (hypoxia plus aminophylline vs. hypoxia alone). The subjects were then returned to room-air breathing.

Experiment II: Effects of atropine on the forearm vascular responses to hypoxia. In a separate group of subjects, after instrumentation and after acclimatization to the face mask for ~15 min, FBF was determined in both forearms (baseline). The subjects then breathed 10.5% O₂ in N₂ for ~20 min. After 5 min of hypoxia, FBF was determined in both forearms (hypoxia). After 10 min of hypoxia, atropine sulfate (0.4 mg ia) was infused over 4 min. Hemodynamic, ventilatory, and FBF measurements in both forearms were repeated over 5 min immediately after the atropine infusion (hypoxia plus atropine vs. hypoxia alone). The face mask was then disconnected, and the subjects returned to room-air breathing.

Drugs. All drug solutions were prepared in half-normal saline and were infused with Harvard precision-infusion pumps. The ia infusion rates were adjusted for forearm volume, which was determined by the volume-displacement technique.

Statistics. Comparisons of baseline and hypoxia measurements and comparisons between the control and the infused forearms were performed with Wilcoxon’s signed rank test. Where multiple comparisons were performed, the probability value was adjusted with the Bonferroni method. A P value of <0.05 was considered statistically significant. The data are presented as means ± SE.

RESULTS

Effects of moderate-to-severe hypoxia on hemodynamics, on ventilatory parameters, and on FBF and FVR

(n = 17). The effects of hypoxia on hemodynamics, on ventilatory parameters, and on the FBF and FVR are shown in Table 1. Moderate-to-severe hypoxia was
Table 1. Effects of systemic hypoxia on hemodynamic parameters, on ventilatory parameters, and on forearm blood flow and forearm vascular resistance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hypoxia</th>
</tr>
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<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>94 ± 2</td>
<td>91 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>56 ± 2</td>
<td>73 ± 3†</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>7.7 ± 0.4</td>
<td>12.2 ± 1.0†</td>
</tr>
<tr>
<td>End-tidal CO₂, %</td>
<td>5.6 ± 0.1</td>
<td>5.0 ± 0.1†</td>
</tr>
<tr>
<td>Oxygen saturation, %</td>
<td>97 ± 0.4</td>
<td>77 ± 1†</td>
</tr>
<tr>
<td>Forearm blood flow, ml·min⁻¹</td>
<td>2.7 ± 0.2</td>
<td>3.6 ± 0.5*</td>
</tr>
<tr>
<td>Forearm vascular resistance, mmHg·ml⁻¹·min⁻¹·100 ml⁻¹</td>
<td>42 ± 4</td>
<td>31 ± 3*</td>
</tr>
</tbody>
</table>

Values are means ± SE from 17 subjects. *P < 0.01 and †P < 0.001 compared with baseline.

associated with an increase of minute ventilation and heart rate, whereas no significant change of arterial pressure was noted. During hypoxia, FBF increased by 38 ± 11%, and FVR decreased by −25 ± 6%. In four subjects, arterial blood-gas determination at the end of the hypoxic protocol demonstrated a PO₂ of 37 ± 2 Torr, a PCO₂ of 28 ± 3 Torr, and a pH of 7.45 ± 0.02.

Effects of aminophylline on adenosine-induced forearm vasodilation (n = 8). The effects of adenosine and aminophylline ia infusion on FBF are shown in Fig. 1. Because ia administration of these drugs did not affect systemic pressure, only the FBF data are presented. Adenosine at 5–10 µg·min⁻¹·100 ml forearm tissue⁻¹ increased FBF by 148 ± 37%. When 50–200 µg·min⁻¹·100 ml⁻¹ of aminophylline were added, FBF was reduced by 40 ± 7% (P < 0.01; single-sample sign test). The infusion rate of aminophylline that achieved attenuation of adenosine-induced increases in FBF was 50 µg·min⁻¹·100 ml⁻¹ in one subject, 100 µg·min⁻¹·100 ml⁻¹ in three subjects, and 200 µg·min⁻¹·100 ml⁻¹ in four subjects.

Experiment 1: Effects of aminophylline on hypoxia-induced forearm vasodilation (n = 9). The infusion rate of aminophylline in these studies was 139 ± 21 µg·min⁻¹·100 ml⁻¹ and was established by the individual blocking studies described above, except in one subject in whom an empiric infusion rate of 100 µg·min⁻¹·100 ml⁻¹ was used.

In the experimental forearm, FBF was 2.9 ± 0.2 ml·min⁻¹·100 ml⁻¹ at baseline, increased significantly during the aminophylline infusion to 4.1 ± 0.3 ml·min⁻¹·100 ml⁻¹ (P < 0.03), but was unchanged at 4.4 ± 0.5 ml·min⁻¹·100 ml⁻¹ during hypoxia plus aminophylline (P = not significant [NS]). Accordingly, in the experimental forearm, FVR was 32 ± 1 mmHg·ml⁻¹·min⁻¹·100 ml at baseline, exhibited a downward trend to 26 ± 2 mmHg·ml⁻¹·min⁻¹·100 ml (P = 0.1) during the aminophylline infusion, and remained unchanged at 24 ± 3 mmHg·ml⁻¹·min⁻¹·100 ml during hypoxia plus aminophylline (P = NS). In the control forearm (no drug), FBF was 2.4 ± 0.3 ml·min⁻¹·100 ml⁻¹ at baseline, was unchanged at 2.5 ± 0.4 ml·min⁻¹·100 ml⁻¹ (P = NS) while aminophylline was infused in the opposite forearm, and increased significantly to 3.3 ± 0.6 ml·min⁻¹·100 ml⁻¹ during hypoxia (P < 0.02). Accordingly, in the control forearm, FVR was 41 ± 4 mmHg·ml⁻¹·min⁻¹·100 ml at baseline, was unchanged at 48 ± 7 mmHg·ml⁻¹·min⁻¹·100 ml (P = NS) while aminophylline was infused in the opposite forearm, and decreased significantly to 34 ± 4 mmHg·ml⁻¹·min⁻¹·100 ml during hypoxia (P < 0.02). The effects of hypoxia on FBF and FVR in the experimental (hypoxia plus aminophylline) and control forearms (hypoxia alone) are shown in Fig. 2.

Because in the experimental forearm ia aminophylline infusion resulted in vasodilation, in a subgroup of subjects, we compared the effects of the nonspecific vasodilator nitroprusside to those of aminophylline on the vasodilator response to adenosine (n = 5). During
infusion of sodium nitroprusside (Elkins-Sinn, Cherry Hill, NJ; 0.25–1.0 µg·min⁻¹·100 ml⁻¹ ia), FBF rose by 45 ± 11% and rose further by 229 ± 76% above baseline when 5 µg·min⁻¹·100 ml⁻¹ ia adenosine were added (P < 0.05). In contrast, in these subjects during aminophylline infusion at 100–200 µg·min⁻¹·100 ml⁻¹ ia, FBF rose by 52 ± 12% while the addition of 5 µg·min⁻¹·100 ml⁻¹ ia adenosine only increased FBF by 114 ± 33% above baseline (P = NS).

Effects of atropine on acetylcholine-mediated forearm vasodilation (n = 3). After 5 min of infusion of 40 µg/min ia acetylcholine, FBF rose from 2.8 ± 0.5 to 5.9 ± 1.8 ml·min⁻¹·100 ml⁻¹. After 0.4 mg ia atropine was infused over 4 min while the acetylcholine infusion was continued, FBF decreased in all three subjects to 3.3 ± 0.6 ml·min⁻¹·100 ml⁻¹. In the control forearm, FBF did not change. Thus atropine attenuated the acetylcholine-induced vasodilation. At the dose employed, atropine did not result in detectable systemic effects.

Experiment II: Effects of atropine on hypoxia-mediated forearm vasodilation (n = 8). In the experimental forearm, FBF was 3.1 ± 0.2 ml·min⁻¹·100 ml⁻¹ at baseline, increased during hypoxia (before atropine) to 4.0 ± 0.4 ml·min⁻¹·100 ml⁻¹ (P < 0.05), but remained unchanged at 4.4 ± 0.6 ml·min⁻¹·100 ml⁻¹ after the atropine infusion (P = NS). Accordingly, in the experimental forearm, FVR was 32 ± 3 mmHg·ml⁻¹·min·100 ml at baseline, revealed a downward trend to 25 ± 3 mmHg·ml⁻¹·min·100 ml during hypoxia before atropine (P = 0.07), and remained unchanged at 23 ± 3 mmHg·ml⁻¹·min·100 ml after the atropine infusion (P = NS). In the control forearm (no drug), FBF was 2.9 ± 0.3 ml·min⁻¹·100 ml⁻¹ at baseline, revealed an upward trend to 3.7 ± 0.5 ml·min⁻¹·100 ml⁻¹ during hypoxia before atropine (P = 0.14), but remained unchanged at 4.0 ± 0.8 ml·min⁻¹·100 ml⁻¹ after atropine infusion in the opposite forearm (P = NS). Accordingly, in the control forearm, FVR was 34 ± 3 mmHg·ml⁻¹·min·100 ml at baseline, did not change significantly at 28 ± 3 mmHg·ml⁻¹·min·100 ml during hypoxia before atropine (P = NS), and remained unchanged at 27 ± 4 mmHg·ml⁻¹·min·100 ml after atropine infusion in the opposite forearm (P = NS). The effects of hypoxia on FBF and FVR in the experimental (hypoxia plus atropine) and control forearms (hypoxia alone) are shown in Fig. 3.

DISCUSSION

The principal findings of this study are that hypoxia-induced forearm vasodilation in humans is attenuated by intrabrachial artery infusion of the adenosine-receptor antagonist aminophylline but not by cholinergic blockade with atropine. These findings support the concept that the skeletal muscle vasodilation, previously observed during hypoxia, is in part mediated by the local action of adenosine, whereas cholinergic vasodilation does not play a role.

Circulatory and ventilatory effects of systemic hypoxia. In agreement with prior studies, we found that moderate-to-severe acute systemic hypoxia produced an increase in ventilation, a rise of heart rate, and a substantial vasodilation in the human forearm (13, 18, 21). Based on simultaneous measurements of total and skin blood flows in the forearm, this rise of FBF is largely due to an increase of flow in skeletal muscle (13). Teleologically, the increase in blood flow is aimed at maintaining O₂ delivery to metabolically active tissue despite decreased arterial O₂ saturation (21). However, studies from several laboratories have demonstrated that sympathetic nerve traffic directed to skeletal muscle (MSNA) increases during brief exposure to hypoxic gas (13, 23, 24, 27). In general, MSNA measured in the peroneal nerve reflects sympathetic vasoconstrictor nerve traffic directed to the skeletal musculature as a whole (29). Norepinephrine spillover into the circulation, a measure of neuronal norepinephrine release, rises also during hypoxia (13). Thus, during systemic hypoxia, the sympathetic and vascular responses in skeletal muscle appear to be dissociated. Similar to the sympathetic response to exercise, during hypoxia, increased sympathetic outflow to skeletal muscle may serve to maintain blood pressure in the face of hypoxia-induced vasodilation (22).

Potential mechanisms of hypoxia-induced skeletal muscle vasodilation. Because sympathetic withdrawal cannot account for vasodilation observed during hypoxia, a number of alternative mechanisms should be considered. For example, in light of evidence that hypoxia may lead to adrenal release of epinephrine (23), hypoxia-induced vasodilation could be mediated by activation of vascular β-adrenergic receptors. However, the forearm vasodilator response to hypoxia is not altered by β-receptor-blocking agents infused into the forearm, suggesting that epinephrine is not responsible (18). In addition, hypocapnia, which occurs as a result of hypoxia-induced hyperventilation, is not a likely contributor to vasodilation associated with hypoxia (19). Because peroneal sympathetic nerve recordings

Fig. 3. Percent change of FBF and forearm vascular resistance during hypoxia in experimental forearm treated with atropine and in (untreated) opposite forearm. Hypoxia caused FBF to increase and forearm vascular resistance to fall. There was no difference in responses of untreated forearm (hypoxia, hatched bars) and atropine-treated forearm (hypoxia plus atropine, open bars). NS, not significantly different hypoxia plus atropine vs. hypoxia.
demonstrate an increase in nerve traffic during hypoxia and because a cholinergic vasodilator system may exist in humans (1, 4, 25), cholinergic vasodilation should be considered to play a role during hypoxia. However, we found that ia infusion of atropine at a dose that markedly inhibited acetylcholine-mediated vasodilation did not prevent forearm vasodilation during hypoxia. This finding strongly argues against an important role for cholinergic vasodilation in the skeletal muscle vascular response to hypoxia and, instead, suggests that local nonneural mechanisms of vasodilation are operative (21). It seems plausible that these mechanisms may be fundamentally similar to the mechanism(s) responsible for vasodilation in exercising muscle. Exercise-induced vasodilation has been linked to nitric oxide, prostaglandins, but also to adenosine (12).

Adenosine release as a potential mechanism of hypoxia-induced skeletal muscle vasodilation. Adenosine, a metabolic by-product of ATP utilization, has potent vasodilator effects in cardiac and skeletal muscle and has been shown to play an important role in the regulation of coronary vasomotor tone (3, 7). The potential role of adenosine in regulating skeletal muscle blood flow is less clear (2, 3, 7, 12), probably in part because of the difficulty in measuring adenosine in tissue or in venous effluent. In recent investigations in lightly anesthetized rats, the hypoxia-induced hindlimb vasodilation was markedly attenuated by systemic administration of the adenosine-receptor antagonists aminophylline or 8-phenyltheophylline (16). Moreover, topical application of 8-phenyltheophylline on rat hindlimb muscle reduced the increase in arterial diameter induced by hypoxia or by topical adenosine (15). Based on these findings, these investigators postulated that local release of adenosine is an important contributor to hypoxia-induced vasodilation in skeletal muscle. This hypothesis had not been tested in humans.

Our data from the human forearm are remarkably similar to those of Neylon and Marshall (16) where the hypoxia-induced increase in femoral vascular conductance was markedly attenuated by aminophylline. During hypoxia of comparable degree (P O2 ~ 37 Torr), we found that, compared with the control forearm, the increase in blood flow and the corresponding decrease in vascular resistance were markedly reduced in the forearm infused with aminophylline. It should be pointed out that in the animal studies cited above aminophylline was given intravenously, and this was predictably associated with systemic effects, including stimulation of ventilation, an increased heart rate, but also an increase of baseline femoral blood flow of ~22% (16). In contrast, we infused aminophylline into the arterial supply of the forearm to modulate locally the effects of adenosine that might be released during hypoxia. With this experimental approach, systemic effects of aminophylline that confound the interpretation of the data are avoided, and the opposite (uninfused) forearm can be used as a control.

A series of published reports suggest that aminophylline (a combination of theophylline and the pharmacologically inactive solvent ethylenediamine) attenuates the cardiovascular and respiratory responses to adenosine in animals and in humans (5, 16, 28), presumably via competitive inhibition of adenosine A1 and/or A2 receptors. For example, aminophylline attenuates the increase in arterial pressure and ventilation elicited by large intravenous doses of adenosine (5). Furthermore, the forearm vasodilation induced by ia adenosine is markedly attenuated by concomitant infusion of aminophylline (28). One important confounding issue in our study and in that by Neylon and Marshall (16) is that aminophylline, at the dose levels used, induced direct vasodilation. This effect of aminophylline may be related to the inhibitory properties of aminophylline on phosphodiesterase (7, 28). It has been reported that inhibition of adenosine-induced vasodilation can be achieved at infusion rates of aminophylline that are not associated with detectable vasodilation (28). Unfortunately, in our studies, the infusion rates necessary to attenuate adenosine-induced vasodilation were higher and increased baseline flow. The reason for this discrepancy is not clear but may relate to large interindividual differences in the vascular responses to this drug we observed.

Our interpretation of the data rests on the assumption that vasodilation should have occurred in the experimental forearm as it did in the untreated control forearm during hypoxia, unless aminophylline interfered with the mechanism of hypoxia-induced vasodilation. For several reasons, we believe that despite an increase in baseline flow induced by aminophylline, our data support this assumption. First, in two of our nine subjects, aminophylline alone did not result in direct vasodilation, yet it markedly attenuated vasodilation in response to hypoxia. Second, in our blocking studies, despite direct aminophylline-induced vasodilation in six of eight subjects, we demonstrated that aminophylline opposed the vasodilation resulting from simultaneously infused adenosine. Third, in the studies with nitroprusside, treatment of the forearm with this non-specific vasodilator did not attenuate additional vasodilation induced by adenosine while similar vasodilator doses of aminophylline markedly attenuated further vasodilation in response to adenosine. Finally, it should be pointed out that, in classic studies cited earlier (18), ia infusion of α- and/or β-receptor-blocking agents produced greater than twofold increases of baseline blood flow, yet did not prevent further increases in flow resulting from hypoxia. Taken together, these considerations strongly suggest that aminophylline inhibited hypoxia-induced vasodilation not because of its non-specific vasodilator effect but rather through specific interference with the action of adenosine at the level of adenosine receptors.

One important limitation of our studies is that we cannot exclude the possibility that differential effects of hypoxia or of the infused drugs on muscles vs. skin blood flow affected our results. However, at a dose that caused forearm vasodilation, aminophylline blunted a
further increase in total FBF during hypoxia. Thus, unless this was due to an attenuation of hypoxia-mediated vasodilation in skeletal muscle, substantial vasoconstriction in the skin should have occurred. Studies in animals and humans suggest that this is not the case (13, 23). It also appears unlikely that atropine blocked cholinergic vasodilation during hypoxia in one tissue while vasodilation was accentuated in another vascular territory, therefore resulting in no net effect of atropine on FBF during hypoxia.

The concept that adenosine may be an important contributor to hypoxia-induced skeletal muscle vasodilation would be strengthened further by the demonstration of increased adenosine concentrations in skeletal muscle. In a dog model based on arterial and venous blood measurements, adenosine output did not change during hypoxia (2). However, because of rapid uptake and removal of adenosine by red blood cells and endothelial cells (7), these data are difficult to interpret. More recently, we have conducted microdialysis experiments in humans to measure adenosine in the skeletal muscle interstitium. Indeed, in these studies, an approximate doubling of adenosine during acute hypoxia was found (14). The present data extend this observation and suggest that the increase of skeletal muscle interstitial adenosine during acute hypoxia in humans may be functionally important. During hypoxia, adenosine could contribute to vasodilation via an effect on vascular smooth muscle, via prejunctional inhibition of norepinephrine release, or perhaps via an interaction with other vasodilator systems (7). Whether the local release of adenosine could also contribute to hypoxia-induced sympathetic activation via stimulation of metabolically sensitive muscle afferents will require further investigation (8).

Our studies do not permit us to comment definitively on other potential vasodilator mechanisms evoked by hypoxia. For example, nitric oxide has been demonstrated to contribute to hypoxic vasodilation in animal models (17), and recent investigations in humans have demonstrated attenuation of forearm vasodilation due to hypoxia with inhibitors of nitric oxide (6). In this regard, it is of note that an interaction between nitric oxide and adenosine during hypoxia has been postulated recently (26). According to this concept, the adenosine-mediated hypoxic vasodilation is dependent on nitric oxide synthesis.

In conclusion, our studies suggest that hypoxia-induced vasodilation in the human forearm is in part mediated by the local action of adenosine released under hypoxic conditions. In contrast, sympathetically mediated cholinergic vasodilation does not appear to play a role. The origin of adenosine in skeletal muscle and the link of adenosine formation and/or release to cellular metabolism during hypoxia remain unclear.

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