Energy state, pH, and vasomotor tone during hypoxia in preconstricted pulmonary and femoral arteries

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Leach, R. M., D. W. Sheehan, V. P. Chacko, and J. T. Sylvester. Energy state, pH, and vasomotor tone during hypoxia in preconstricted pulmonary and femoral arteries. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L294–L304, 2000.—To assess effects of smooth muscle energy state and intracellular pH (pHi) on pulmonary arterial tone during hypoxia, we measured ATP, phosphocreatine, Pi, and pH, by 31P-NMR spectroscopy and isometric tension in phenylephrine-contracted rings of porcine proximal intrapulmonary arteries. Hypoxia caused early transient contraction followed by relaxation and late sustained contraction. Energy state and pH decreased during relaxation and recovered toward control values during late contraction. Femoral arterial rings had higher energy state and lower pH, under baseline conditions and did not exhibit late contraction or recovery of energy state and pH, during hypoxia. In pulmonary arteries, glucose-free conditions abolished late hypoxic contraction and recovery of energy state and pH, but endothelial denudation abolished only late hypoxic contraction. NaCN had little effect at 0.1 and 1.0 mM but caused marked vasorelaxation and decreases in energy state and pH, at 10 mM. These results suggest that 1) regulation of tone, energy state, and pH, differed markedly in pulmonary and femoral arterial smooth muscle, 2) hypoxic relaxation was mediated by decreased energy state or pH, due to hypoxic inhibition of oxidative phosphorylation, 3) recovery of energy state and pH, in hypoxic pulmonary arteries was due to accelerated glycolysis mediated by mechanisms intrinsic to smooth muscle, and 4) late hypoxic contraction in pulmonary arteries was mediated by endothelial factors that required hypoxic recovery of energy state and pH, for transduction in smooth muscle or extracellular glucose for production and release by endothelium.

Vasomotor responses to hypoxia constitute fundamental adaptations to a commonly encountered stress. In systemic arteries, hypoxia typically causes vasodilation, which increases blood flow and oxygen delivery to hypoxic tissue. In pulmonary arteries, hypoxia causes vasoconstriction, which diverts blood flow from hypoxic to normoxic lung regions, thereby preserving ventilation-perfusion matching and maximizing systemic arterial oxygen content. Despite the importance of these responses and many years of investigation, the precise mechanisms by which hypoxia changes vasomotor tone remain unclear.

Much of the work performed to investigate vasomotor responses to hypoxia has been carried out in isolated arteries. In rabbit aortas precontracted with norepinephrine, hypoxia caused sustained vasorelaxation, which was endothelium independent and associated with decreased intracellular phosphocreatine ([PCr]) and P (Pi) concentrations in vascular smooth muscle (13, 50, 51). The consistency of the relationships between these indexes of energy state and vasomotor tone during a variety of interventions, such as cyanide exposure and manipulation of tissue phosphate concentration and creatine pool size (25, 50), suggested that hypoxia-induced changes in vascular tone were mediated by changes in energy state. Because hypoxia caused greater vasorelaxation in arteries contracted with norepinephrine, which acts via receptor-linked transduction pathways, than in arteries contracted with KCl, which acts via nonspecific smooth muscle depolarization, hypoxia-induced decreases in energy state were thought to inhibit energy-dependent steps in smooth muscle transduction pathways upstream (e.g., the phosphatidylinositol cycle) rather than downstream (e.g., actin-myosin interaction) from myosin light chain kinase (14).

The hypoxic vasomotor response was quite different in preconstricted pulmonary arteries: early transient contraction was followed by relaxation and late sustained contraction (6, 27, 30). Early hypoxic contraction was partially or completely endothelium dependent and blocked by inhibitors of nitric oxide synthase, suggesting that it was caused by hypoxic inhibition of nitric oxide-mediated vasorelaxation (27). The relaxation phase of the response was endothelium independent, but late hypoxic contraction was found to be endothelium dependent and independent (6, 27, 30). It is not known whether pulmonary arterial smooth muscle energy state changes during hypoxic relaxation and late hypoxic contraction or what role such changes might play in these vasomotor responses.

In previous studies of resting arterial tissue (31), we demonstrated that baseline energy state was lower and intracellular pH (pHi) was higher in pulmonary than in femoral arterial smooth muscle. Furthermore, during

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hypoxia, energy state deteriorated in femoral arteries but did not change in pulmonary arteries. These results suggested that production and/or utilization of ATP differed in resting pulmonary and systemic arterial smooth muscle under baseline and hypoxic conditions; however, these experiments provided no information about how these differences might affect vasomotor tone. Because changes in energy state can affect a wide variety of cellular processes, including signal transduction, contractile protein interaction, and activities of ion pumps and channels, we hypothesized that changes in energy state contributed to hypoxic vasomotor responses in precontracted pulmonary arteries.

To test this hypothesis, we determined the relationship between isometric tension and energy state during hypoxia in isolated pulmonary and femoral arteries precontracted with phenylephrine. As noted previously (6, 27, 30), precontraction was necessary for hypoxia to elicit changes in vasomotor tone in these vessels. In precontracted pulmonary arteries, we also determined the effects of glucose-free conditions, cyanide, and endothelial denudation. We assessed smooth muscle energy state by 31P-NMR spectroscopy, which allowed continuous measurement of ATP concentration ([ATP]), [PCr], and [Pi], as well as of pH, which typically changes with energy state (31) and can also have profound effects on vasomotor tone (4, 28, 39). Our results indicate that regulation of vasomotor tone, energy state, and pH was markedly different in contracted pulmonary and femoral arterial smooth muscle and suggest that 1) hypoxic relaxation in these arteries was mediated by decreased smooth muscle energy state and/or pH, resulting from hypoxic inhibition of oxidative phosphorylation, 2) energy state and pH recovered during hypoxia in pulmonary but not in femoral arteries due to acceleration of glycolysis signaled by mechanisms intrinsic to smooth muscle, and 3) late hypoxic contraction in pulmonary arteries was mediated by factors derived from endothelium that required hypoxic recovery of smooth muscle energy state and pH, for transduction or extracellular glucose for production and release.

METHODS

Tissue preparation. All protocols and procedures were approved by the Animal Care and Use Committee in accordance with National Institutes of Health guidelines. Precontracted porcine proximal intrapulmonary and femoral arteries were used because they exhibited different vasomotor responses to hypoxia and provided sufficient tissue mass for 31P-NMR measurements (31). Pigs weighing 25–35 kg were anesthetized with ketamine (20 mg/kg im) and pentobarbital sodium (12.5 mg/kg iv) and killed by exsanguination. Proximal intrapulmonary arteries (5–7 mm ID, and superficial femoral arteries (1–2 mm ID) were isolated, placed in oxygenated Ringer solution at 25°C, cleaned of connective tissue, and cut into 5-mm-long paired rings. The rings were suspended between two stainless steel stirrups in organ chambers filled with phosphate-free Krebs bicarbonate (PFKB) solution gassed with 95% O2-5% CO2 at 37°C. This solution contained (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 2.5 CaCl2, 25.0 NaHCO3, and 10.0 glucose. One stirrup was anchored in the chamber, and the other was connected to a strain gauge (model FT03, Grass Instrument, Quincy, MA) for continuous measurement of isometric tension (model 7E polygraph, Grass Instrument). If necessary, endothelium was removed from the rings after they were mounted by gentle rubbing of the intimal surface with a cotton swab. The rings were stretched at 10-min intervals in increments of 0.5 g to resting tensions of 4 g in pulmonary arteries and 5 g in femoral arteries. Preliminary experiments revealed that contractile responses to KCl (80 mM) were maximal at these resting tensions. The rings were then stimulated with 3 × 10−7 M phenylephrine, which produced contractions that were ~50% of maximum. Endothelial function was tested by exposing the precontracted vessels to ACh (10−7 M) and bradykinin (10−8 M) and was assumed to be intact when rings relaxed >50% to these agents and absent when relaxation did not occur. To confirm that endothelial denudation did not seriously damage smooth muscle, contractile responses to phenylephrine in rings subjected to denudation were required to be ≥80% of responses measured in control rings with endothelium.

One ring of each pair was used for continued measurement of isometric tension and remained in the organ chamber at rest in fresh PFKB solution until experiments began (1 h). The other ring was used for measurement of energy state and pH by 31P-NMR spectroscopy. These vessels were mounted on supports at their resting, stretched diameter. In pulmonary arteries, these supports were Z-shaped pieces of plastic. In femoral arteries, which were too small (1–2 mm ID) for these supports, two or three blunted solid glass rods (0.75–1.5 mm OD) were gently inserted into the lumen to achieve the required degree of stretch. Endothelial integrity of the stretched, mounted vessels was confirmed by removing some of the rings from supports, remounting them in organ chambers for measurement of tension, and testing responses to ACh and bradykinin after precontraction with phenylephrine as described above.

For each NMR experiment, 6–8 pulmonary arterial rings (0.12 ± 0.01 g total dry wt, 0.62 ± 0.03 g total wet wt) or 10–15 femoral arterial rings (0.11 ± 0.01 g total dry wt, 0.50 ± 0.04 g total wet wt) mounted at their resting, stretched diameters were carefully arranged in a 20-cm glass NMR sample tube (10 mm OD) and superfused through a multipor polycarbonate catheter with PFKB solution. The superfusion system has been described previously (31). A peristaltic pump delivered perfusate at 20 ml/min through insulated plastic tubing to the NMR sample tube, which was positioned in the bore of the NMR magnet. At this flow rate, the temperature in the sample tube was 37°C when the temperature in the perfusate reservoir was 50°C. The PFKB solution in the reservoir and the fluid surface in the sample tube were gassed with 93% O2-7% CO2. We chose this concentration of oxygen to ensure adequate tissue oxygenation and to allow comparison with our previous studies in resting arterial tissue (31). As found previously (31), 7% CO2 was necessary to achieve the desired level of CO2 in the sample tube perfusate. Once perfusion was initiated, rings used for 31P-NMR spectroscopy and measurement of tension were stimulated continuously with phenylephrine (3 × 10−7 M). One hour was allowed for stabilization of vascular energy state before experiments began.

31P-NMR spectroscopy. NMR measurements were performed with a Bruker MSL-500 NMR spectrometer and a vertical 11.8-T superconducting magnet with an 89-mm bore as previously described (31). A commercial 31P probe enclosing the NMR sample tube was tuned to 202 MHz. After the sample tube was placed in the probe, field homogeneity was optimized by shimming the first induction decay (FID) of protons at 500 MHz. The proton spectral line width was 25.9 ± 10 Hz. The NMR magnet was gassed with 7% CO2-93% O2, and the sample tube was adjusted to 37°C. As found previously (31), 7% CO2 was necessary to achieve the desired level of CO2 in the sample tube perfusate, and peristaltic flow was used to superfuse the sample tube.
2.0 Hz (range 14–37 Hz) in pulmonary arteries and 8.9 ± 0.8 Hz (range 5–14 Hz) in femoral arteries. $^{31}$P spectra were obtained using a 12-s pulse width (60° flip angle) from FID acquired over 100 ms. For each spectrum, 904 scans were collected over 15 min, with a 1-s interpulse interval. Interventions described below were started or stopped by switching perfusates in synchrony with onsets of these 15-min scan collections. FID was subjected to Fourier transformation after application of 20-Hz line broadening. Spectral resonance positions are given in parts per million (ppm) relative to PCr. In experiments where metabolic inhibition caused loss of PCr, the position of the PCr peak determined during the baseline period was used as the reference.

$[PCr], [ATP], [P_i],$ phosphomonoesters (PME), and phosphodiesters (PDE) were assessed by integrating areas under the appropriate peaks of spectra summed over 30 min. As in our previous study (31), this collection time provided adequate signal-to-noise ratios while allowing us to follow changes occurring slowly over time. The baseline for integration was determined by Fourier transformation of the summed FID from the total 6-h experiment to produce a single spectrum with high signal-to-noise ratios. Concentrations are expressed as percentages of values measured during the second hour of the baseline period. Absolute concentrations were not determined because tissue mass varied from day to day and external standards could not be introduced into the NMR tube in a reproducible manner. $[PCr]/[ATP]$ and $[P_i]/[ATP]$ were calculated from relative concentrations after correction by appropriate saturation factors (31).

The $pH_i$ was determined from the chemical shift difference (Δ$\sigma$) between the PCr and P$_i$ peaks according to the following equation: $pH_i = pK_a + log(\Delta\sigma/\Delta\sigma_0)$, where $pK_a$, $\Delta\sigma$, and $\Delta\sigma_0$ are constants equal to 6.70, 5.64, and 3.18, respectively (53). The Δ$\sigma$ was measured as the distance in parts per million between the resonance positions of the highest points on the P$_i$ and PCr spectral peaks. The P$_i$ peak was well differentiated from the baseline noise and easily separated from the PME and PDE peaks; therefore, any influence of the PME or PDE peak on the P$_i$ peak should be negligible, constant, and unlikely to affect $pH_i$ determination.

Effects of hypoxia in pulmonary and femoral arteries. The effects of hypoxia were determined in pulmonary arteries from six animals and femoral arteries from five animals. Experiments consisted of baseline, exposure, and recovery periods, each 2 h long, during which the vessels were stimulated continuously with phenylephrine (3 x 10$^{-7}$ M) during a 2-h baseline period followed by 3-h exposure and 1-h recovery periods. During the exposure period, perfusate NaCN concentration was increased progressively at hourly intervals to 0.1, 1.0, and 10 mM. Cyanide-containing perfusates were prepared by addition of appropriate amounts of 10 M NaCN to stock PFKB solution gassed with 95% O$_2$-5% CO$_2$ (isometric tension measurements) or 95% O$_2$-5% CO$_2$ (isometric tension measurements). The resulting alkaline solutions were titrated to pH 7.4 with 1 M HCl before use.

Effects of endothelial denudation on hypoxic responses in pulmonary arteries. Pulmonary arteries from seven animals were subjected to endothelial denudation. The experimental protocol was the same as that described above for arteries exposed to hypoxia.

Statistical analysis. Data were analyzed by t-test or one- or two-factor ANOVA as appropriate. When significant F ratios were obtained with ANOVA, least significant differences were calculated to allow pairwise comparison of means. Differences were considered significant when $P < 0.05$. Values are means ± SE.

RESULTS

Typical $^{31}$P-NMR spectra collected over 30 min in rings prestimulated with phenylephrine are illustrated in Fig. 1. Spectral resonance positions of the $\gamma$-,$\alpha$-, and $\beta$-ATP peaks (−2.44 ± 0.01, −7.52 ± 0.01, and −16.08 ± 0.01 ppm, respectively) and the PME and PDE peaks (6.77 ± 0.01 and 2.97 ± 0.01 ppm, respectively) were the same in pulmonary and femoral arteries; however, the resonance position of P$_i$ was different (5.01 ± 0.01 and 4.96 ± 0.01 ppm in pulmonary and femoral arteries, respectively, P < 0.001). Coefficients of variation for $\beta$-ATP, PCr, and P$_i$ peaks areas, determined over 6 h in control rings, were 12.1 ± 1.2, 15.9 ± 1.2, and 16.9 ± 1.8%, respectively, in pulmonary arteries and 5.3 ± 1.0, 8.9 ± 0.6, and 13.6 ± 2.6%, respectively, in femoral arteries.

The effects of hypoxia were determined by comparing arteries exposed to hypoxia during hours 3 and 4 of the 6-h protocol with control arteries exposed to baseline conditions throughout the experiment (Fig. 2). In control pulmonary arteries, [ATP] and [PCr] did not change, however, tension and pH increased and [P$_i$] decreased slightly over time (P = 0.02). During the first hour of hypoxia, experimental pulmonary artery rings contracted briefly to 125 ± 6.4% of baseline before relaxing to 56.4 ± 12.0%. Vasorelaxation was accompanied by falls in [PCr] to 38.9 ± 13.9% and pH, from 7.26 ± 0.019 to 7.112 ± 0.021. During the second hour of
hypoxia, tension rapidly recovered to control levels (117 ± 12.3% at 4 h), accompanied by increases in [PCr] (85.5 ± 9.7%) and pHi (7.16 ± 0.015). During the recovery period, reoxygenation caused another rapid fall in tension (22.2 ± 9.1% after 15 min). Thereafter, tension rose progressively toward control levels (68.4 ± 15.6% at 6 h). [PCr] and pHi remained at control levels during the recovery period (93.4 ± 13.6% and 7.24 ± 0.017, respectively, at 6 h). [ATP] and [Pi] were not altered during the experiment.

In femoral arteries under baseline conditions, tension and [PCr]/[ATP] were higher and pHi and [Pi]/[ATP] were lower than in pulmonary arteries (Table 1). In control femoral arteries, no variable changed significantly as a function of time (Fig. 2). In experimental femoral arteries, the first hour of hypoxia induced profound relaxation to 18.3 ± 14.9% of baseline after a transient contraction (118 ± 8.2%) as in pulmonary arteries (Fig. 2); however, unlike pulmonary arteries, vasorelaxation persisted throughout hypoxic exposure. Corresponding falls in [PCr] and pHi also persisted, and at the end of hypoxia, [PCr] and pHi, averaged 52.0 ± 6.6% and 7.00 ± 0.049, respectively. [Pi] increased to 150 ± 10.7% during the first 30 min of hypoxia and remained elevated. With reoxygenation during the recovery period, tension gradually returned to 81.3 ± 13.5% of baseline at 6 h, accompanied by progressive recovery of [PCr] and pHi (95.3 ± 4.8% of baseline and 7.20 ± 0.008, respectively). During the first 30 min of recovery, [Pi] fell to 61.7 ± 12.9% but then returned to control levels (97.1 ± 3.7% at 6 h). [ATP] did not change during the experiment.

ANOVA revealed that the effects of hypoxia on tension, [PCr], and [Pi] were significantly different in pulmonary and femoral arteries (P = 0.0001, 0.038, and 0.0037, respectively). The pHi was higher in pulmonary arteries during baseline and exposure periods (P < 0.01); however, ANOVA did not detect a between-vessel difference in the effects of time on pHi (P = 0.125). Because inspection of the data (Fig. 2) suggested that pHi might differ in pulmonary and femoral arteries at the end of hypoxia (3.5–4 h), we repeated ANOVA on data obtained only at this time. This analysis suggested that, at the end of hypoxia, pHi was closer to its control value in pulmonary arteries (P = 0.060). When the analysis was repeated on intracellular H+ concentration, calculated from values of pHi, the statistical significance of this between-vessel difference increased (P = 0.034).

Thus, hypoxic responses of pulmonary and femoral arteries were similar in that initial vasoconstriction and vasorelaxation were accompanied by falls in [PCr] and pHi but no change in [ATP]. They were different in that hypoxic vasorelaxation in femoral arteries was sustained throughout exposure in association with increased [Pi] and decreased [PCr] and pHi, whereas pulmonary arteries exhibited a late hypoxic contraction in association with recovery of [PCr], and possibly pHi, toward control levels. In addition, pulmonary arterial...
[Pi] did not change during hypoxia and pHi remained higher than femoral arterial pH throughout exposure. In contrast to hypoxia, glucose depletion did not alter tension, [ATP], [PCr], [P], or pHi (Fig. 3). Pulmonary arteries exposed to hypoxia and glucose depletion initially exhibited responses similar to arteries exposed to hypoxia alone: early transient contraction followed by relaxation accompanied by decreases in [PCr] and pHi but no change in [ATP]. Continued exposure to this combined stress, however, revealed major differences. Glucose depletion abolished late hypoxic contraction and recovery of [PCr] and pHi during the second hour of hypoxia; for example, tension, [PCr], and pHi, at the end of exposure averaged 1.12 ± 0.29 g (32.4 ± 11.5% of baseline), 16.7 ± 8.8% of baseline, and 7.003 ± 0.007, respectively, all of which were significantly different from baseline.

Table 1. Isometric tension, [PCr]/[ATP], [P]/[ATP], and pHi during baseline period in phenylephrine-contracted artery rings with and without endothelium

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<th>Pulmonary Artery</th>
<th>Femoral Artery</th>
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<tr>
<td></td>
<td>E+</td>
<td>E-</td>
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<tr>
<td>n</td>
<td>22</td>
<td>7</td>
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<tr>
<td>Tension, g</td>
<td>3.31 ± 0.11</td>
<td>3.87 ± 0.23</td>
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<tr>
<td>[PCr]/[ATP]</td>
<td>0.423 ± 0.017</td>
<td>0.479 ± 0.030</td>
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<tr>
<td>[P]/[ATP]</td>
<td>0.801 ± 0.043</td>
<td>1.45 ± 0.56*</td>
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<tr>
<td>pHi</td>
<td>7.223 ± 0.006</td>
<td>7.219 ± 0.007</td>
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Values are means ± SE; n, no. of rings; [PCr], [ATP], and [P], phosphocreatine, ATP, and P, concentrations, respectively; pHi, intracellular pH; E+, endothelium intact; E-, endothelium denuded. *P < 0.05 vs. E+: pulmonary artery.
from values measured in arteries exposed to hypoxia alone (3.30 ± 0.64 g, 85.5 ± 9.7%, and 7.162 ± 0.015, respectively). Moreover, [ATP] fell to 50.1 ± 7.5% and [P_i] rose to 256 ± 4.4% of baseline, whereas no significant changes were observed during hypoxia alone. During the recovery period, [PCr] returned rapidly to baseline, whereas the other variables returned more gradually.

The effects of NaCN on pulmonary arteries are shown in Fig. 4. At 0.1 mM, NaCN caused slight vasorelaxation (tension = 3.09 ± 0.78 g or 76.7 ± 8.1% of baseline at 3 h) but no significant change in [ATP], [PCr], [P_i], or pH_i. On exposure to 1 mM NaCN, tension initially decreased to 2.35 ± 0.58 g (66.6 ± 12.9% of baseline) in association with a decrease in pH_i (7.167 ± 0.052). This relaxation was followed by partial recovery of tension to 3.11 ± 0.09 g (87.6 ± 16.0%), but pH_i remained slightly below control values. [ATP], [PCr], and [P_i] were not different from control at the end of this exposure. At 10 mM, NaCN caused vasorelaxation to 1.22 ± 0.23 g...
(37.8 ± 15.5% of baseline) at 5 h in association with decreases in [ATP], [PCr], and pH, to 61.4 ± 6.8%, 34.8 ± 17.2%, and 7.067 ± 0.003, respectively, at 5 h and an increase in [Pi] to 113 ± 20.5%. During recovery, tension, [Pi], and pH returned toward control, but [ATP] and [PCr] remained depressed.

Baseline [Pi]/[ATP] was higher in pulmonary arteries without endothelium than in pulmonary arteries with endothelium, but baseline tension, [PCr]/[ATP], and pH, were not different (Table 1). On exposure to hypoxia (Fig. 5), pulmonary arteries without endothelium exhibited vasorelaxation similar to that seen in intact arteries (tension = 1.50 ± 0.28 g or 37.4 ± 3.2% of baseline after 30 min); however, early transient and late sustained hypoxic vasoconstriction did not occur. Endothelial denudation did not alter the effects of hypoxia on [ATP], [PCr], [Pi], or pH (Fig. 5).

DISCUSSION

Comparison of precontracted pulmonary and femoral arteries. In precontracted femoral artery rings (Fig. 2), hypoxia decreased [PCr] and pH, increased [Pi], but had no effect on [ATP]. These results are consistent with findings of other investigators in systemic arteries (22, 25, 34, 50) and are most likely explained by hypoxic limitation of mitochondrial electron transport and oxidative phosphorylation (34), leading to compensatory upregulation of glycolysis (the Pasteur effect) sufficient to maintain [ATP] and increase lactic acid concentration (3).

In association with these changes, hypoxia caused marked, sustained femoral artery relaxation (Fig. 2). This association supports the long-considered hypothesis that smooth muscle energy state regulates vasomotor tone. Using hypoxia and other interventions to inhibit energy production in rabbit aortas precontracted with norepinephrine, Scott and colleagues (50, 51) found a consistent relationship between vasorelaxation and the ratio of [PCr] to free creatine concentration. Subsequent studies suggested that hypoxic relaxation was more closely correlated with increased [Pi] or decreased phosphorylation potential, further implicating cytochrome-c oxidase as the oxygen sensor signaling this response (25). Because hypoxia at equivalent [PCr] caused less relaxation in rings precontracted with KCl than in rings precontracted with norepinephrine, it was proposed that energy state regulated vasomotor tone at energy-dependent steps in receptor-linked transduction pathways upstream from myosin light chain kinase (14). Demonstration that hypoxia increased concentrations of phosphatidylinositol cycle intermediates proximal to the ATP-requiring phosphatidylinositol phosphate kinase reaction was consistent with this possibility (38). Alternatively, decreased energy state could activate sarcolemmal ATP-dependent K$^+$ channels (15, 46) or inhibit ATP-dependent Ca$^{2+}$ channels (32, 55), leading to decreased Ca$^{2+}$ influx, a fall in intracellular Ca$^{2+}$ concentration, and vasorelaxation.

Hypoxic vasorelaxation may also have resulted from changes in variables closely linked to energy state. For example, intracellular acidosis has been associated with activation of sarcolemmal K$^+$ channels (7, 15, 46), hyperpolarization (2, 45), and decreases in vasomotor tone (1, 4, 39, L-type Ca$^{2+}$ channel availability (26), inward Ca$^{2+}$ currents (23), intracellular Ca$^{2+}$ concentration (1, 40, 45), and Ca$^{2+}$ sensitivity of contraction (39). In embryonic cardiomyocytes, prolonged hypoxia decreased the affinity of cytochrome-c oxidase for oxygen, leading to increased mitochondrial production of reactive oxygen species, which signaled suppression of contractile activity (8, 12, 16). Reactive oxygen species are well-recognized modulators of vasomotor tone (48), but their role in hypoxic vasorelaxation remains to be determined.

Fig. 5. Time course of isometric tension, [ATP], [PCr], [Pi], and pH, measured by $^{31}$P-NMR spectroscopy in pulmonary arteries with (E+) and without (E−) endothelium before, during, and after exposure to hypoxia for 2 h (shaded area). NMR variables are expressed as percentages of values measured during 2nd h of experiment.
Precontracted pulmonary arteries had lower [PCr]/[ATP] and higher [P_i]/[ATP] at baseline than femoral arteries (Table 1), suggesting lower baseline energy state. This difference was probably not due to greater ATP utilization in pulmonary arteries, since phenylephrine-induced tension was less in these vessels. Lower ATP production due to diffusion limitation of oxygen uptake also seems unlikely, since energy state in control pulmonary arteries was stable under baseline conditions for 6 h (Fig. 2) and, as discussed below, hypoxia (which should worsen diffusion limitation) caused less deterioration of energy state in pulmonary than in femoral arteries. Perhaps more likely is a lower rate of glycolysis, thought to be a significant source of ATP in vascular smooth muscle (44). Because glycolysis also produces lactic acid, the higher baseline pH in pulmonary arteries (Table 1) would be consistent with this possibility.

The initial effects of hypoxia in precontracted pulmonary arteries were similar to those in femoral arteries (Fig. 2) and were presumably caused by the same mechanisms. The only difference was an unchanging [P_i] in pulmonary arteries (Fig. 2), suggesting less deterioration of energy state in these vessels. During the 2nd h of hypoxia, however, marked differences emerged. Recovery of [PCr] and pH indicated that energy state improved in pulmonary arteries. This improvement was probably not due to decreased energy utilization because tension rose. The rise in pH argues against increased ATP production via glycolysis, since lactic acid production would also be increased; however, pH is determined not only by acid production but also by intracellular buffering and H⁺ extrusion. In vascular smooth muscle, the latter is thought to occur predominantly via sarcolemmal Na⁺/H⁺ exchange (18, 47, 52). Although Na⁺/H⁺ exchange does not require energy, its effectiveness depends on the magnitude of the transmembrane Na⁺ gradient, which is increased by Na⁺-K⁺-ATPase. Because this pump preferentially utilizes ATP produced by glycolysis (9), increased glycolytic ATP production during hypoxia could improve energy state and promote extrusion of H⁺ in amounts sufficient to maintain or increase pH in the face of increased lactate production.

Alternatively, oxidative phosphorylation may have been restored in pulmonary arteries despite continued hypoxia. In their “near equilibrium” hypothesis, Erecinska and Wilson (17) proposed that mitochondrial ATP production is determined by the rate of the irreversible reaction of reduced cytochrome c with oxygen, which in turn depends on the reaction’s rate constant and the concentrations of oxygen and reduced cytochrome c. If electron transport reactions upstream from cytochrome c are in near equilibrium, the concentration of reduced cytochrome c will be directly proportional to the ratio of the mitochondrial concentration of NADH to NAD⁺ and inversely proportional to cytoplasmic [ATP][ADP][P_i]. On this basis, mitochondrial ATP production and cytoplasmic energy state could increase in persistently hypoxic pulmonary arterial smooth muscle because the ratio of the mitochondrial concentration of NADH to NAD⁺ or the rate constant of the cytochrome c reaction increased. Because glycolysis is rate limited in vascular smooth muscle by sarcolemmal glucose transport (37), the former could result from a hypoxia-induced enhancement of glucose transport as occurs in skeletal muscle (10), leading to acceleration of glycolysis and tricarboxylic acid cycle activity. The latter could result from altered affinity of cytochrome-c oxidase for oxygen (11, 12). If mitochondrial electron transport recovered during hypoxia, secondary downregulation of lactic acid production and increased proton utilization by ATP synthase due to restoration of proton gradients across mitochondrial membranes could also increase pH (19).

Differences between precontracted pulmonary and femoral arteries shown in Table 1 and Fig. 2 were similar to those we reported previously for resting arterial tissue (31). For example, under baseline conditions, energy state was lower in resting pulmonary arteries than in resting femoral arteries. During hypoxia, energy state did not change in resting pulmonary arteries but deteriorated in resting femoral arteries. Thus, despite a baseline disadvantage, pulmonary arteries tolerated hypoxia better than femoral arteries under resting and precontracted conditions. This suggests that the regulation of ATP production and consumption was intrinsically different in these vessels. During hypoxia in precontracted pulmonary arteries, recovery of energy state was clearly associated with an increase in tension (Fig. 2), again suggesting that vasomotor tone was regulated by energy state or some closely related variable. To further evaluate this possibility and to assess mechanisms by which hypoxia altered energy state, we determined the effects of glucose depletion and NaCN.

Effects of glucose depletion and cyanide in pulmonary arteries. By itself, glucose depletion had no effect (Fig. 3), confirming previous studies in vascular smooth muscle (5, 36, 41, 42) and indicating that nonhypoxic pulmonary arterial smooth muscle can maintain energy state and tension in the absence of extracellular glucose by utilizing endogenous fats, amino acids, or glucose derived from intracellular glycogen as substrates for ATP production. During the first hour of hypoxia, glucose depletion had little influence on the changes in tension, energy state, and pH elicited by hypoxia. During the second hour, however, glucose depletion caused profound vasorelaxation accompanied by severe deterioration of energy state and pH (Fig. 3). These results suggest that late hypoxic contraction and recovery of energy state required ATP production from extracellular glucose. Because extracellular glucose is metabolized primarily to lactate in vascular smooth muscle (36), energy state recovery was probably due to increased glycolysis rather than increased oxidative phosphorylation. Recent observations that late hypoxic contraction varied directly with glucose concentration (29) and was abolished by glucose-free conditions or iodoacetate, but not by rotenone (56), are consistent with this possibility. Thus differences between pulmonary and femoral arteries might be due to a greater
ability of pulmonary smooth muscle to upregulate glycolysis during hypoxia.

Cyanide blocks mitochondrial electron transport by binding to the heme iron of cytochrome-c oxidase. Previous studies in vascular smooth muscle used 0.05–2 mM cyanide to achieve this effect (34, 50, 53, 54). As shown in Fig. 4, 0.1–1 mM NaCN had relatively little influence on vasomotor tone, energy state, and pH i, similar to the effects of hypoxia at the end of exposure (Fig. 2). If oxidative phosphorylation were strongly inhibited at these concentrations, as would be predicted (34, 50, 53, 54), these data imply that 1) pulmonary arterial energy state was maintained by glycolysis alone and 2) decreases in tension, energy state, and pH i at higher cyanide concentrations (10 mM) were caused by cyanide toxicity, perhaps secondary to enhanced production of reactive oxygen species (49). Interestingly, the combined effects of hypoxia and glucose depletion (Fig. 3) increased [P i] and decreased pH i by greater amounts than even 10 mM NaCN (P < 0.001 and 0.04, respectively). This comparison again suggests that glycolysis was the major source of ATP in hypoxic pulmonary arteries.

The effects of glucose depletion and cyanide (Figs. 3 and 4) support the conclusion that deterioration of pulmonary arterial smooth muscle energy state and pH i during the first hour of hypoxic exposure (Fig. 2) was due to hypoxic inhibition of oxidative phosphorylation, whereas recovery of energy state and pH i during the second half of hypoxic exposure was due to enhanced ATP production via glycolysis. In addition, the consistent relationships between vasomotor tone and energy state or pH i, during exposures to hypoxia, glucose depletion, and NaCN (Figs. 2–4) suggest that energy state, pH i, or some closely related variable regulated tone in pulmonary arterial smooth muscle.

Role of endothelium in pulmonary arterial responses to hypoxia. We previously demonstrated that late hypoxic contraction in pulmonary arteries was endothelium dependent, whereas hypoxic vasorelaxation was not (27, 30). To determine whether this endothelium dependence was due to endothelial effects on smooth muscle energy state and pH i, we measured hypoxic responses in pulmonary arteries subjected to endothelial denudation.

Baseline [P i]/[ATP] was higher in endothelium-denuded than in endothelium-intact pulmonary arteries (Table 1), suggesting a lower tissue energy state in denuded arteries; however, because [PCr]/[ATP] and pH i were not different, it may be more likely that endothelial denudation injured some myocytes. Injured or dead cells could have very high [P i] and absent or very low [ATP] and [PCr], leading to an increase in whole tissue [P i]/[ATP] without alteration of [PCr]/[ATP]. Similarly, pH i could be relatively uninfluenced, since it was estimated from the spectral position of the highest point (rather than the centroid of the area) of the P i peak, which would be determined by the larger, more homogeneous population of uninjured myocytes.

The correlation between vasomotor tone and energy state and pH i observed in endothelium-intact pulmonary arteries exposed to hypoxia, glucose depletion, or cyanide was not present in endothelium-denuded pulmonary arteries exposed to hypoxia. Specifically, endothelial denudation abolished early and late hypoxic contractions but did not alter hypoxic relaxation or the effects of hypoxia on [ATP], [PCr], [P i], and pH i (Fig. 5). These results indicate that recovery of smooth muscle energy state and pH i during hypoxia was caused by mechanisms intrinsic to vascular smooth muscle. The dissociation of this recovery from changes in tension has two important implications regarding the mechanism of late hypoxic contraction, which distinguishes hypoxic responses of pulmonary arteries from those of femoral arteries (Fig. 2). First, late hypoxic contraction was not due to reversal of energy- or pH-dependent depression of phenylephrine-induced contraction. Second, late hypoxic contraction was at least partly due to increased endothelium-derived contractile factor activity and/or decreased endothelium-derived relaxing factor activity. The former may be more likely, since inhibitors of nitric oxide synthase and cyclooxygenase did not prevent late hypoxic contraction in isolated pulmonary arteries (27, 30).

Compartmentation of metabolism and function in pulmonary arterial smooth muscle. Our results should also be interpreted in light of accumulating evidence that metabolism and function are compartmentalized in vascular smooth muscle (20, 21, 24, 35, 36, 43). According to this model, contraction preferentially utilizes ATP produced from endogenous substrates via oxidative phosphorylation (the “oxidative” compartment). Sarcolemmal functions, such as activity of Na + -K + -ATPase (9, 37) and ATP-dependent Ca 2 + - and K + channels (32, 33), preferentially utilize ATP produced by metabolism of extracellular glucose to lactate via glycolysis (the “glycolytic” compartment).VASORELAXATION INDUCED IN PRECONTRACTED PULMONARY AND FEMORAL ARTERIES BY HYPOXIA IN THE PRESENCE OF GLUCOSE (Fig. 2) AND THE PERSISTENCE OF HYPOXIC VASORELAXATION IN ENDOTHELium-DENUDED PULMONARY ARTERIES (Fig. 5) ARE CONSISTENT WITH HYPOXIC INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT AND SECONDARY DECREASES OF ENERGY STATE IN THE OXIDATIVE COMPARTMENT, WHICH INHIBITED TRANSDUCTION PATHWAYS UPSTREAM FROM MYOSIN LIGHT CHAIN KINASE THROUGHOUT HYPOXIC EXPOSURE. PRESUMABLY, DOWNSTREAM PATHWAYS COULD STILL FUNCTION BECAUSE ENERGY STATE IN THE OXIDATIVE COMPARTMENT WAS NOT SUFFICIENTLY REDUCED TO LIMIT DOWNSTREAM ATP-DEPENDENT REACTIONS OR BECAUSE THESE REACTIONS HAD ACCESS TO ATP PRODUCED IN THE GLYCOLYTIC COMPARTMENT. THE DEPENDENCE OF LATE HYPOXIC CONTRACTION AND RECOVERY OF PULMONARY ARTERIAL ENERGY STATE ON EXTRACELLULAR GLUCOSE SUGGESTS THAT HYPOXIA CAUSED ACCELERATION OF GLUCOSE TRANSPORT INTO THE GLYCOLYTIC COMPARTMENT, LEADING TO INCREASED ATP PRODUCTION VIA GLYCOLYSIS AND SECONDARY IMPROVEMENT OF COMPARTMENTAL ENERGY STATE. RECENT FINDINGS THAT GLUCOSE UPTAKE BY RAT PULMONARY ARTERIES INCREASED 346% DURING LATE HYPOXIC CONTRACTION SUPPORT THIS POSSIBILITY (29). HOWEVER, BECAUSE MITOCHONDRIAL ELECTRON TRANSPORT REMAINED LIMITED BY HYPOXIA, CONTRACTILE PATHWAYS IN THE OXIDATIVE COMPART-
ment upstream from myosin light chain kinase remained inhibited. The dependence of late hypoxic contraction (but not recovery of smooth muscle energy state or pH) on endothelium suggests that recovery of energy state and pH in the glycolytic compartment permitted transduction of an endothelial contractile effect or that hypoxic endothelium required glucose to produce and release a contractile factor. Further investigation is required to determine whether these speculations have merit.

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