Effects of hypoxia on energy state and pH in resting pulmonary and femoral arterial smooth muscles

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Effects of hypoxia on energy state and pH in resting pulmonary and femoral arterial smooth muscles. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L1051–L1060, 1998.—To determine the effects of hypoxia on energy state and intracellular pH (pHi) in resting pulmonary and systemic arterial smooth muscles, we used 31P nuclear magnetic resonance spectroscopy and colorimetric and enzymatic assays to measure pH;i; intracellular concentrations of ATP, phosphocreatine, creatine, and Pi; and phosphorylation potential in superfused tissue segments from porcine proximal intrapulmonary and superficial femoral arteries. Under baseline conditions (P0₂ 467 ± 12.1 mmHg), energy state and total creatine (phosphocreatine + creatine) concentration were lower and pHi was higher in pulmonary arteries. During hypoxia (P0₂ 23 ± 2.4 mmHg), energy state deteriorated more in femoral arteries than in pulmonary arteries. pHi fell in both tissues but was always more alkaline in pulmonary arteries. Reoxygenation reversed the changes induced by hypoxia. These results suggest that production and/or elimination of ATP and H⁺ was different in resting pulmonary and systemic arterial smooth muscles under baseline and hypoxic conditions. Because energy state and pHi affect a wide variety of cellular processes, including signal transduction, contractile protein interaction, and activities of ion pumps and channels, further investigation is indicated to determine whether these differences have functional significance.

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Cellular energy state is determined by the balance between production and utilization of high-energy substrates such as ATP, the immediate source of metabolic energy, and phosphocreatine (PCr), which provides ATP via the creatine kinase, or Lehmann, reaction (10). When the ratio of energy supply to energy demand decreases, as during severe hypoxia, homeostatic mechanisms attempt to match ATP production to ATP utilization. As a result, ATP concentration ([ATP]) can remain relatively constant and is therefore not a very sensitive index of the energy supply-demand condition (4, 13). More sensitive indexes include the ratios of PCr ([PCr]) or P, ([P]), concentration to [ATP] or creatine (Cr; [Cr]) concentration ([PCr]/[ATP], [P,]/[ATP], and [PCr]/[Cr]), phosphorylation potential (II = [ATP]/[ADP][P,], where [ADP] is ADP concentration), and adenylate charge ([ATP + 2ADP]/[ATP + ADP + AMP]). These ratios may also act as signals for homeostatic metabolic reactions and other adaptive responses (4, 13, 34).

The hypothesis that changes in energy state signal pulmonary vasomotor responses to hypoxia has been considered by many investigators, but the role of energy state in these responses remains unclear (30). In isolated pig lungs, we found that [ATP] and adenylate charge did not change during vasoconstrictor responses to moderate hypoxia or vasodilator responses to severe hypoxia (5). These findings did not support the possibility that pulmonary vasomotor responses were triggered by changes in energy state; however, a role for energy state was not ruled out because ATP and adenylate charge may not be the relevant energy state signals. Furthermore, measurements in the whole lung may not reflect energy state in the cells responsible for hypoxic responses.

The cell that triggers pulmonary vascular responses to hypoxia may be the pulmonary arterial myocyte itself. In support of this possibility, hypoxia was found to decrease potassium-channel conductance, increase membrane potential and intracellular calcium concentration, and cause contraction in isolated pulmonary arterial myocytes (29, 35, 44, 48). The signal responsible for these effects is unknown. Moreover, these hypoxic effects appear to differ from those in systemic arterial tissue where hypoxia typically causes smooth muscle relaxation, possibly due to deterioration of smooth muscle energy state and secondary inhibition of intracellular signal transduction (11, 18, 21, 28, 33, 38).

In addition to its effects on energy state, hypoxia can increase production of lactic acid (34) and thereby promote a decrease in intracellular pH (pHi). In vascular smooth muscle, changes in pHi can alter membrane potential, calcium homeostasis, and myosin light chain kinase activity (1, 9, 24, 31). Furthermore, interventions designed to alter pHi have been shown to change vasomotor tone in both systemic and pulmonary arteries (2, 24, 32) and to modify pulmonarypressor responses to hypoxia in isolated lungs (17, 37).

Despite the potential importance of smooth muscle energy state and pHi as determinants of vasomotor tone, it remains unclear how hypoxia affects these variables in pulmonary arterial smooth muscle or whether these effects differ from those in systemic arterial smooth muscle. Thus, in the present study, we used 31P nuclear magnetic resonance (NMR) and enzymatic and colorimetric assays to measure energy state and pHi in resting porcine intrapulmonary and femoral arterial smooth muscles. Our results demonstrate that...
energy state and pH differed markedly in these tissues under baseline conditions and that hypoxia decreased energy state more in femoral than in pulmonary arterial smooth muscle.

**METHODS**

Tissue preparation. Pigs weighing 28–35 kg were anesthetized with ketamine (20 mg/kg iv) followed by pentobarbital sodium (12.5 mg/kg iv) and killed by exsanguination from the carotid or femoral arteries. Proximal intrapulmonary (5–to 7-mm-ID) and superficial femoral (1–to 2-mm-ID) arteries were isolated, placed in oxygenated Ringer solution at 25°C, cleaned of adherent connective tissue, and cut into 4 × 4-mm segments. These vessels were chosen because they provided adequate amounts of tissue for 31P NMR spectroscopy and appeared to have similar wall thicknesses. In addition, proximal intrapulmonary arteries exhibited hypoxic vasodilation in vivo (20), and myocytes from proximal pulmonary arteries developed depolarization and reduced potassium-channel conductance during hypoxia in vitro (35, 48). We studied resting unloaded tissue because we were interested in the possibility that changes in energy state and pH1, caused changes in vasomotor tone rather than the reverse.

Approximately 25–35 segments of proximal intrapulmonary artery (total wet weight 0.94 ± 0.05 g; total dry weight 0.17 ± 0.007 g; n = 13) or superficial femoral artery (total wet weight 0.53 ± 0.04 g; total dry weight 0.11 ± 0.009 g; n = 11) were loosely arranged in the lower 3 cm of a glass NMR sample tube. Superfusates PO2, PCO2, and pH in the sample tube were measured with a blood gas analyzer (Radiometer, Copenhagen, Denmark).

31P NMR spectroscopy. The sample tube containing the superfused arterial tissue was placed in the bore of an 11.8-T vertical superconducting magnet containing a 31P-probe tuned to 202 MHz connected to a Bruker MSL-500 NMR spectrometer. Field homogeneity was optimized by shimming the free induction decay (FID) of protons at 500 MHz. Proton spectral line width was 15–35 Hz in pulmonary artery experiments and 5–20 Hz in femoral artery experiments. 31P NMR spectra were obtained with a 12-µs pulse width (60° flip angle) and the FID was acquired over 100 ms. For each spectrum, 904 scans were collected with a 1-s interpulse interval over 15 min. The FID was subjected to Fourier transformation after data were filtered to 202 MHz and baseline correction. Saturation factors determined in four pulmonary and three femoral artery experiments with an 8-s pulse interval were as follows: 1.5 ± 0.06 for PCr, 1.18 ± 0.07 for β-ATP, and 1.47 ± 0.01 for Pi in the pulmonary arteries and 1.57 ± 0.03 for PCr, 1.08 ± 0.01 for β-ATP, and 1.9 ± 0.05 for Pi in the femoral arteries.

Tissue energy state was assessed by integrating the areas under the PCr, β-ATP, and Pi peaks from spectra collected over 30 min. The baseline used to integrate the spectral peaks was determined by Fourier transformation of the summed FID from the total 5-h experiment, which produced a single spectrum with very low baseline noise. [PCr] [ATP] and [Pi] were calculated from these integrals after multiplication by appropriate saturation factors. Because tissue density in the coil varied from day to day, relative rather than absolute changes in intracellular [PCr], [ATP], and [Pi] were determined. Specifically, in each experiment, intracellular [PCr], [ATP], and [Pi] were expressed as a percentage of values measured during the baseline period (60–120 min of superfusion). Spectral resonance positions are expressed in parts per million (ppm) relative to that of PCr. pH was determined from the chemical shift difference (δs) between the PCr and Pi peaks according to the equation pH = pK1 + log((δs - δa)/(δb - δa)), where pK1, δa, and δb are constants equal to 6.70, 5.64, and 3.18, respectively (42).

Experiments in pulmonary (n = 9) and femoral (n = 7) arteries exposed to hypoxia consisted of an initial period of stabilization, followed by baseline (1-h), exposure (2-h), and recovery (1-h) periods. During the stabilization, baseline, and recovery periods, the perfusate was gassed with 93% O2-7% CO2. During the exposure period, the perfusate was gassed with 93% O2-7% CO2−balance N2. Data from the stabilization period were not included in the statistical analysis. Experiments in control pulmonary (n = 4) and femoral (n = 4) arteries were the same except that 1) the perfusate was gassed with 93% O2-7% CO2 during the exposure period and 2) after the recovery period, three control pulmonary arteries and three control femoral arteries were exposed sequentially for 30-min intervals to perfusate NaCN concentrations of 0.1, 1.0, and 10 mM. During this time, the perfusate was gassed with 93% O2-7% CO2.

Intracellular [ATP] and [Cr]. We performed additional experiments to determine the absolute intracellular [ATP] and [Cr] values in pulmonary and femoral arterial smooth muscles. Arterial segments were exposed to baseline conditions for 2 h (n = 11) or to baseline conditions for 2 h followed by hypoxia for 2 h (n = 8). During the last 30 min of these exposures, the tissue was freeze-clamped with aluminum tongs precooled in liquid nitrogen. The frozen tissue was rapidly weighed and ground to a powder under liquid nitrogen with 6% perchloric acid with a porcelain mortar and pestle. The frozen mixture of tissue and acid was homogenized for 30–60 s (Polytron, Brinkman Instruments, Westbury, NY) and centrifuged with a tabletop centrifuge (Centra 4B, International Equipment, Needham Heights, MA) at 4°C and 5,000 rpm for 10 min. The supernatant was removed, neutralized with 5 M K2CO3, centrifuged with a Centrifuge (Bellco, Cincinnati, OH) with the hexokinase-glucose-6-phosphate dehydrogenase method of Lamprecht and Traut- schold (26). Cr was measured with the α-naphthyl-diacyl colorimetric method of Ennor and Stocken (12), allowing 100 min at 0°C for color development as suggested by Wong (46). Results of these assays are expressed as micromoles per gram of wet tissue weight.

To express ATP and Cr as intracellular concentrations, we used the method of Soltoff et al. (41) to measure intracellular water volume per gram of wet tissue weight under baseline conditions in pulmonary and femoral arterial segments from 13 animals. Ten to twenty minutes after the addition of [1,2-3H]polyethylene glycol (mol wt 900; NEN Life Science Products, Boston, MA) to the superfusate (=1 µCi/ml) as an
extracellular marker, the tissue was weighed, dried to a constant weight at 50°C, and extracted with 1 N HNO₃ for 24 h. Intracellular water volume was calculated as the difference between total tissue water volume, measured as the difference between wet and dry tissue weights, and extracellular water volume, measured by dividing total tissue radioactivity by the concentration of radioactivity in the superfusate. Radioactivity was measured with a liquid scintillation counter (LS6000SC, Beckman Instruments) and corrected for attenuation. Intracellular [ATP] and [Cr] in millimoles per liter were determined by dividing the mean tissue content of intracellular water (in ml/g wet weight) into the total tissue [ATP] and [Cr] (in µmol/g wet weight).

Arterial wall thickness. We used a dissecting microscope (StereoZoom-4, Leica, Buffalo, NY) with a graduated calibrated eyepiece to measure wall thickness in two to three pulmonary and one to two femoral arterial segments from each of six animals after the segments were superfused under baseline conditions for 1–2 h. The resolution of this technique was 10 µm.

Statistical analysis. Data were analyzed by t-test or one-, two-, or three-factor analysis of variance, as appropriate, with a computer program (SuperANOVA, Abacus Concepts, Berkeley, CA). If significant variance ratios were obtained, pairwise comparisons of means were performed by calculating least significant differences (22). Differences were considered significant when P was <0.05. Values presented are means ± SE, and n is the number of animals studied.

RESULTS

In the 31P NMR experiments, control pulmonary and femoral arterial segments were superfused throughout the experiment with a physiological salt solution gassed with 93% O₂-7% CO₂. Under these conditions, PO₂ was 236 ± 12.1 mmHg, PCO₂ was 39.4 ± 3.4 mmHg, and pH was 7.41 ± 0.014 at the tissue in the sample tube. In arteries exposed to hypoxia, the perfusate was gassed with 0% O₂-7% CO₂ during the exposure period (third and fourth hours of the experiment), resulting in a sample tube PO₂ of 23 ± 2.4 mmHg. Perfusate PCO₂ and pH were unchanged. Differences between perfusate gas tensions measured in the sample tube and those predicted to exist in the reservoir were probably due to incomplete equilibration in the reservoir, diffusion of gas across the walls of perfusate tubing, or the difference in temperature between the reservoir and sample tube (50 vs. 37°C).

To optimize acquisition times for 31P NMR data, coefficients of variation for areas under the β-ATP, PCr, and Pi peaks were determined in control pulmonary and femoral arteries with data collected in 15-, 30-, or 60-min blocks (904, 1,808, and 3,616 scans, respectively) over the entire period of perfusion. As shown in Table 1, this index of variance, which includes both random and time-dependent physiological variations, was smaller in femoral than in pulmonary arterial tissue and decreased as acquisition time increased. On the basis of these results, we selected an acquisition time of 30 min as a reasonable compromise between adequacy of signal-to-noise ratio and ability to follow physiological changes occurring over time. Thus two 15-min scans were summed to produce spectra every 30 min.

Examples of spectra obtained in this manner are shown in Fig. 1, which demonstrates that 30-min acquisitions allowed acceptable signal-to-noise ratios to be achieved in both pulmonary and femoral arteries. The resonance positions of the γ-, α-, and β-ATP peaks

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**Table 1. Influence of acquisition time on coefficients of variation for PCr, β-ATP, and Pi spectral peak areas in control pulmonary and femoral arteries**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Acquisition Time, min</th>
<th>No. of Scans</th>
<th>Coefficient of Variation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>15</td>
<td>904</td>
<td>PCr: 26.3 ± 4.4, β-ATP: 12.3 ± 1.4, Pi: 26.1 ± 1.1</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>30</td>
<td>1,808</td>
<td>PCr: 16.9 ± 3.3, β-ATP: 9.4 ± 1.0, Pi: 18.8 ± 1.7</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>60</td>
<td>3,616</td>
<td>PCr: 12.6 ± 2.0, β-ATP: 6.4 ± 0.9, Pi: 16.1 ± 1.2</td>
</tr>
<tr>
<td>Femoral</td>
<td>15</td>
<td>904</td>
<td>PCr: 4.4 ± 1.1, β-ATP: 0.4 ± 0.1, Pi: 6.4 ± 0.2</td>
</tr>
<tr>
<td>Femoral</td>
<td>30</td>
<td>1,808</td>
<td>PCr: 4.8 ± 0.3, β-ATP: 4.1 ± 0.3, Pi: 13.8 ± 2.6</td>
</tr>
<tr>
<td>Femoral</td>
<td>60</td>
<td>3,616</td>
<td>PCr: 3.6 ± 1.0, β-ATP: 3.4 ± 0.4, Pi: 9.9 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 pulmonary and 4 femoral arteries. PCr, phosphocreatine. Spectral peak areas were measured by 31P nuclear magnetic resonance spectroscopy over 5 h.
relative to that of PCr were the same in pulmonary and femoral arteries (−2.43 ± 0.01, −7.54 ± 0.01, and −16.09 ± 0.01 ppm, respectively), as were those of the phosphomonoester and phosphodiester peaks (6.78 ± 0.02 and 2.97 ± 0.01 ppm, respectively). Only the position of the Pi peak differed between tissues (4.97 ± 0.007 and 5.02 ± 0.006 ppm in femoral and pulmonary arteries, respectively), indicating a difference in pHi as discussed below.

The time courses of energy state variables and pHi in control experiments are shown in Figs. 2 and 3. Comparison of control pulmonary and femoral arteries by analysis of variance indicated marked differences in [PCr]/[ATP] (0.410 ± 0.016 in pulmonary vs. 0.892 ± 0.015 in femoral arteries; P < 0.0001) and pHi (7.182 ± 0.005 in pulmonary vs. 7.201 ± 0.002 in femoral arteries; P < 0.01); however, [Pi]/[ATP] was not different between the tissues (0.467 ± 0.018 in pulmonary vs. 0.434 ± 0.019 in femoral arteries; P > 0.6). In control femoral arteries, none of the variables changed during the experiment. In control pulmonary arteries, [ATP], [Pi], [PCr], and [PCr]/[ATP] did not change, whereas [Pi]/[ATP] decreased and pHi increased slightly (P < 0.02 and 0.04, respectively); however, for no variable was the effect of time significantly different between control pulmonary and femoral arteries.

The effects of hypoxia were determined by comparing arteries exposed to hypoxia to control arteries (Figs. 2 and 3). Results obtained during the baseline period in experimental arteries confirmed the differences described above for control vessels, namely, lower [PCr]/[ATP] and higher pHi in pulmonary arteries. Hypoxia did not significantly alter [ATP], [Pi], [PCr], [PCr]/[ATP], or [Pi]/[ATP] in pulmonary arteries; however, pH, was markedly decreased (P < 0.001), achieving a value of 7.177 ± 0.026 by the end of exposure. This change was completely reversed on reoxygenation during the recovery period. In femoral arteries, hypoxia increased [Pi] (P < 0.03) and decreased [PCr] (P < 0.001) to 132 and 79.7%, respectively, of values measured in control vessels but did not alter [ATP]. As a result, [Pi]/[ATP] increased (P < 0.03) and [PCr]/[ATP] decreased (P < 0.0001). For example, during the last 30 min of the exposure period, [Pi]/[ATP] was 0.617 ± 0.046 and 0.433 ± 0.053 and [PCr]/[ATP] was 0.728 ± 0.059 and 0.887 ± 0.086 in hypoxic and control femoral arteries, respectively. As in pulmonary arteries, hypoxia markedly decreased pH, (P < 0.0001), which reached a value of 7.140 ± 0.010 by the end of exposure. All changes induced by hypoxia were reversed on reoxygenation. Analysis of variance indicated that the decrease in [PCr]/[ATP] induced by hypoxia was significantly greater in femoral arteries than in pulmonary arteries (P = 0.036). This comparison did not achieve significance for the other variables.
Table 2. Total tissue and intracellular [ATP] and [creatine] measured spectrophotometrically in pulmonary and femoral arteries

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Artery</th>
<th>Femoral Artery</th>
<th>P Value of F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Hypoxia</td>
<td>Condition</td>
</tr>
<tr>
<td>[ATP] µmol/g wet wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mM</td>
<td>0.379 ± 0.040</td>
<td>0.364 ± 0.031</td>
<td>0.613 ± 0.084</td>
</tr>
<tr>
<td>[Creatine] µmol/g wet wt</td>
<td>2.71 ± 0.285</td>
<td>2.60 ± 0.222</td>
<td>2.30 ± 0.316</td>
</tr>
<tr>
<td>mM</td>
<td>0.298 ± 0.030</td>
<td>0.395 ± 0.086</td>
<td>0.681 ± 0.089</td>
</tr>
<tr>
<td></td>
<td>2.13 ± 0.212</td>
<td>2.82 ± 0.616</td>
<td>2.55 ± 0.334</td>
</tr>
</tbody>
</table>

Values are means ± SE. [ATP], ATP concentration; [creatine], creatine concentration; NS, not significant. Measurements were made after 2 h of baseline conditions or 2 h of baseline conditions followed by 2 h of hypoxia.

[ATP] and [Cr] measured by enzymatic and colorimetric techniques during the last 30 min of the baseline and exposure periods are shown in Table 2. Expressed as total tissue concentration (in µmol/g wet weight), ATP was lower in pulmonary than in femoral arterial smooth muscle; however, expressed as intracellular concentration (in mM), ATP was the same. This occurred because intracellular water volume was lower in pulmonary arterial tissue (0.140 ± 0.047 vs. 0.267 ± 0.051 µmol/g wet weight; P = 0.04). Hypoxia did not affect [ATP] in either tissue. Cr, whether expressed as total tissue or intracellular concentration, was lower in pulmonary arteries and was increased equally by hypoxia.

To estimate the size of the total Cr pool in pulmonary and femoral arterial smooth muscle, we added the mean values of [PCr]/[ATP] determined by 31P NMR to the mean values of [Cr]/[ATP] determined spectrophotometrically during the last 30 min of the baseline and exposure periods. As shown in Fig. 4, baseline [Cr]/[ATP] and [PCr]/[ATP] values in pulmonary arterial smooth muscle were ~60 and 40%, respectively, of values measured in femoral arterial smooth muscle ([Cr]/[ATP] = 0.705 ± 0.097 vs. 1.21 ± 0.173, P < 0.01; [PCr]/[ATP] = 0.362 ± 0.047 vs. 0.906 ± 0.045, P < 0.0001). As a result, baseline [PCr + Cr]/[ATP] in pulmonary arterial smooth muscle was about one-half of that in femoral arterial smooth muscle. Hypoxia decreased [PCr]/[ATP] in femoral but not in pulmonary arterial smooth muscle and had no effect on [Cr]/[ATP] in either tissue. Consequently, [PCr + Cr]/[ATP] changed little, if at all.

The effects of NaCN administered to control pulmonary and femoral arteries after the recovery period are shown in Table 3. At concentrations of 1 and 10 mM, cyanide significantly decreased [PCr]/[ATP] and pH in both arteries. At 10 mM, cyanide decreased [ATP] in pulmonary and femoral arteries to 74 and 88%, respectively, of control values measured at the end of the recovery period; however, this change achieved significance only in the femoral arteries.

Vascular wall thickness averaged 671 ± 49 µm (range 463–911 µm) in pulmonary arterial segments and 487 ± 27 µm (range 339–607 µm) in femoral arterial segments. These values were significantly different (P < 0.01).

**DISCUSSION**

We used 31P NMR spectroscopy because it allowed nondestructive continuous determination of free intracellular [ATP], [PCr], [P], and H⁺ concentration in the same tissue sample. The major disadvantage of this technique is its low sensitivity. Consequently, large amounts of tissue, strong magnetic fields, and long spectral acquisition times are usually required to achieve adequate measurements. In previous studies of vascular tissue (19, 23, 42), relatively low magnetic field strengths and small amounts of tissue dictated acquisition times ≥ 1 h. In the present study, we used an 11.8-T magnet to examine 0.5–1 g of tissue and, as shown in Fig. 1 and Table 1, were able to obtain well-resolved spectra with acquisition times as short as 15 min in the femoral artery and 30 min in the pulmonary artery. Spectral peak positions were similar to those reported previously for vascular tissue (19, 23, 42). Line widths for the PCr and β-ATP peaks were small, and the Pᵢ peak was clearly resolved from the phosphomono- and diester peaks. Clear demarcation of the Pᵢ and PCr signals allowed pHᵢ to be monitored continuously from the difference in the
positions of the PCr and Pi peaks as described above. Because ATP, PCr, and Pi were not present in the extracellular fluid and smooth muscle cells contain virtually all of the intracellular fluid volume in vascular tissue (42), we assume that our measurements reflect changes occurring in arterial myocytes. These changes could occur by mechanisms intrinsic to myocytes or as a result of influences on myocyte energy state and pH deriving from other cell types.

Baseline conditions. In femoral arteries under baseline conditions, mean [ATP] (0.613 ± 0.084 µmol/g) and [PCr]/[ATP] (0.892 ± 0.015) were within the range of values previously reported for resting systemic vascular smooth muscle (6, 18, 23, 25, 28, 33). Mean [Pi]/[ATP] (0.434 ± 0.019) was comparable to previous values obtained by 31P NMR (23, 42) but lower than values obtained by enzymatic and chromatographic techniques (19, 21, 25), probably because the tissue extraction required by these techniques caused release of tissue-bound Pi and overestimation of free intracellular [Pi] (19, 45).

Another index of energy state is II, defined as

\[ II = \frac{[ATP]}{[ADP][Pi]} \]  

(1)

In our experiments, we measured [Pi]/[ATP] directly from the 31P NMR spectrum. Measurement of free [ADP] in this manner was not possible because of its low concentration (19); therefore, we estimated [ADP] with the creatine kinase reaction, which can be written as

\[ PCr + ADP \rightleftharpoons ATP + Cr \]  

(2)

The apparent equilibrium constant of this reaction \( K_{eq} \) varies with pH and free Mg2+ concentration \( ([\text{Mg}^{2+}]) \) (16) and is defined as

\[ K_{eq} = \frac{[ATP][Cr]}{[PCr][ADP]} \]  

(3)

Solving Eq. 3 for ADP and substituting into Eq. 1, we have

\[ II = \frac{([ATP][Pi])/([CR][ATP])}{(K_{eq}/[Cr])} \]  

(4)

The creatine kinase reaction is thought to be at equilibrium in resting and stimulated unloaded systemic vascular smooth muscles (10). The stability of [ATP], [PCr], [Pi], [ATP], and [Pi] shown in Figs. 2 and 3 indicates that equilibrium conditions existed in control femoral arteries. Given the measured value of pH and assuming that the free intracellular \([\text{Mg}^{2+}]\) was 0.5 mM (23), \( K_{eq} \) can be estimated at 89.4 (16). Substituting this value and the measured values of [Pi]/[ATP] and [PCr]/[ATP] (Fig. 3) and [Cr] (Table 2) into Eq. 4 yields a II of \( 7.3 \times 10^4 \text{M}^{-1} \) (Fig. 5). This value is somewhat higher than those reported for rat brain, skeletal muscle, and liver (1.6–3.0 \times 10^4 \text{M}^{-1}) but comparable to estimates reported for rabbit aorta and cardiac muscle (5–10 \times 10^4 \text{M}^{-1}) (21, 27, 45). Taken together, our results suggest that the energy state in femoral arterial smooth muscle under baseline conditions was stable and comparable to values reported previously for systemic vessels and other tissues.

Intracellular [ATP] in pulmonary arteries under baseline conditions was the same as that in femoral arteries (Table 2) but greater than the value (=1 mM) measured in rabbit aorta contracted with norepinephrine or KCl (11). Expressed as total tissue concentration, pulmonary arterial ATP was less than the values calculated for rat extrapulmonary arteries (1–2 µmol/g wet weight) but similar to the values obtained in systemic vascular smooth muscle (0.3–1.2 µmol/g wet weight) (6, 25, 39). These discrepancies may be related to differences in species, experimental conditions, measurement techniques, or vascular intracellular water volume.
We are unaware of previous measurements of [P]/[ATP] or [PCr]/[ATP] in pulmonary vessels; however, mean [P]/[ATP] in control pulmonary arteries (0.467 ± 0.018) was the same as that in control femoral arteries (0.434 ± 0.019). On the other hand, [PCr]/[ATP] was considerably lower (0.410 ± 0.016 vs. 0.892 ± 0.015; P < 0.0001). Equation 4 indicates that, at the same [P]/[ATP], pulmonary [PCr]/[ATP] would be less than femoral [PCr]/[ATP] if the pulmonary $K_\text{d}$ were higher or the pulmonary [Cr] or II were lower.

$K_\text{d}$ is a function of intracellular [Mg$^{2+}$] and H$^+$ concentration (16). A femoral-pulmonary difference in intracellular [Mg$^{2+}$] seems unlikely because the resonance position of the β-ATP peak, which depends on intracellular [Mg$^{2+}$] (19, 42), was the same in the two vessels. As discussed below, pH$_i$ was higher in pulmonary arteries (Fig. 3), but a higher pH should decrease, not increase, $K_\text{d}$ (16). [Cr] and [Cr]/[ATP] were lower in pulmonary arteries, and, as a result, the total intracellular Cr pool ([PCr + Cr]/[ATP]) was about one-half of that in femoral arteries (Table 2, Fig. 4). This difference must have contributed to the lower pulmonary [PCr]/[ATP], but it was not the whole explanation. As shown in Fig. 5, II in pulmonary arteries was $2.8 \times 10^4$ M$^{-1}$ under baseline conditions or ~40% of its value in femoral arteries. This difference is likely to be significant because the mean values of [PCr]/[ATP], [Cr], and pH$_i$ used to calculate II were significantly different. We conclude that [PCr]/[ATP] was less in pulmonary arteries not only because the Cr pool size was smaller but also because the energy state was lower.

The difference in baseline energy state between pulmonary and femoral arterial smooth muscles indicates that production or consumption of ATP was different under baseline conditions; however, it is unlikely that the difference in energy state was due to a difference in ATP consumption by actin-myosin interaction because the tissues were studied under resting, unloaded conditions. In addition, several considerations suggest that the energy state difference was not caused by decreased ATP production due to limitation of oxidative phosphorylation by oxygen diffusion. First, oxygen consumption in unstimulated unloaded vascular smooth muscle was very low (34). Second, because the tissues were exposed to a high flow of perfusate containing oxygen at high partial pressures, the gradient for oxygen diffusion was very high. Third, although vascular wall thickness was higher in pulmonary arteries (671 ± 49 vs. 487 ± 27 µm), the concentration of cells in the vascular wall was lower (intracellular water volume 0.140 ± 0.047 vs. 0.267 ± 0.051 ml/g); therefore, a larger oxygen diffusion distance was offset by a smaller oxygen consumption requirement. Fourth, phosphagen concentrations and pH$_i$ in control pulmonary and femoral arteries were extraordinarily stable throughout the 5-h observation period (Figs. 2 and 3). Finally, if oxygen diffusion were limiting ATP production under baseline conditions, energy state should rapidly deteriorate under hypoxic conditions. As discussed below, this did not occur in pulmonary arterial smooth muscle. Thus the pulmonary-femoral difference in baseline energy state must have some other explanation.

As shown in Fig. 3, pH$_i$ averaged 7.201 ± 0.002 in control femoral arteries and 7.246 ± 0.005 in control pulmonary arteries (P < 0.01). Our femoral arterial measurements are consistent with pH$_i$ values (7.01–7.26) previously measured with fluorescent dyes, NMR, or microelectrodes in systemic arterial tissue under similar conditions (1, 9, 23, 32). Only a few measurements of pH$_i$ have been made in pulmonary arterial tissue, and these yielded a similar range of values. For example, with fluorescence techniques, mean values of 7.28 ± 0.03 (36) and 6.9 ± 0.06 (14) were obtained in cultured pulmonary arterial smooth muscle from guinea pigs and ferrets, respectively, whereas an average of 7.09 was obtained in isolated segments of intrapulmonary arteries from dogs (24). To our knowledge, the only previous direct comparison of pH$_i$ in systemic and pulmonary arterial tissues was that of Vadula et al. (43), whose preliminary communication reported values of 7.08 ± 0.12 and 7.27 ± 0.22 in primary cultures of smooth muscle cells from cat cerebral and pulmonary arteries > 800 µm in diameter, respectively. These data are consistent with our own observations.

The pH$_i$ difference suggests lower H$^+$ production, higher H$^+$ extrusion, or greater intracellular buffering capacity in pulmonary arterial smooth muscle. Vascular smooth muscle is known to produce lactic acid via glycolysis at high rates, even under aerobic conditions (34), and to extrude acid via Na$^+$-H$^+$ exchange, Cl$^-$-HCO$_3^-$ exchange, and Na$^+$-HCO$_3^-$ cotransport (1, 14, 40, 47). Furthermore, intracellular buffering capacity in systemic vascular smooth muscle is appreciable (3). Which of these processes explains the baseline pH$_i$ difference is unknown; however, a lower rate of aerobic glycolysis in pulmonary arterial smooth muscle could explain both the higher pH$_i$ and lower II found in this tissue. A lower II could also signal a higher rate of oxidative phosphorylation (4, 13), which could secondarily increase the H$^+$ concentration gradient across the inner mitochondrial membrane and raise cytoplasmic pH (15).

Effects of hypoxia. In femoral arterial smooth muscle, hypoxia did not alter [ATP] (Fig. 2, Table 2), but it decreased [P] and [PCr]/[ATP] and increased [PI] and [P]/[ATP] (Figs. 2–4). Moreover, II decreased from $7.3 \times 10^4$ to $2.7 \times 10^4$ M$^{-1}$ (Fig. 5). These results indicate that hypoxia decreased the femoral arterial energy state.

Data on the energy state effects of hypoxia in resting systemic vascular smooth muscle are limited. Most previous studies (18, 21, 28, 38) were performed in stimulated or spontaneously contracting systemic vessels and yielded results similar to our own. For example, in rabbit aorta contracted with norepinephrine, Katayama et al. (21) and Scott and Coburn (38) found that short exposures to anoxic gas mixtures did not change [ATP] but increased [P] and decreased [PCr] and II.
The most likely explanation for deterioration of femoral arterial energy state during hypoxia is hypoxic inhibition of mitochondrial electron transport and oxidative phosphorylation. Previous observations (28) that oxygen consumption fell in vascular smooth muscle exposed to similar conditions support this possibility. The stability of [ATP] (Fig. 2, Table 2) implies that ATP production remained matched to ATP utilization despite inhibition of oxidative phosphorylation. This could occur because ATP utilization decreased or, as previously demonstrated in hypoxic vascular smooth muscle (28, 34), ATP production via glycolysis increased (the Pasteur effect). The decrease in pH, observed in femoral arteries during hypoxia (Fig. 3) is consistent with the latter possibility because lactic acid is also produced via glycolysis.

It is possible that factors other than enhanced lactic acid production contributed to the fall of femoral arterial pH during hypoxia. Decreased mitochondrial electron transport and proton pumping could lead directly to cytoplasmic acidification (15). Na\(^+\)-H\(^+\) exchange, an important component of pH regulation in vascular smooth muscle, requires a finite Na\(^+\) concentration gradient across the cell membrane, and this, in turn, depends on activity of the Na\(^+\)-K\(^+\) pump, which requires energy for operation (1, 36, 47). Thus deterioration of energy state could limit Na\(^+\)-K\(^+\)-ATPase activity, reduce the transmembrane sodium gradient, and decrease acid extrusion via Na\(^+\)-H\(^+\) exchange. Evidence that Na\(^+\)-K\(^+\)-ATPase preferentially utilizes ATP derived from glycolysis rather than from oxidative phosphorylation may argue against this possibility (7).

Hypoxia did not alter [ATP], [PCr], [P\(_i\)], [PCr]/[ATP], or [P\(_i\)]/[ATP] in pulmonary arterial smooth muscle (Table 2, Figs. 2 and 3). These results differ from those of Shigemori et al. (39), who found a progressive decrease in [ATP] in rat pulmonary arteries exposed to 0% oxygen for 10 min; however, because their vessels were stretched and precontracted with phenylephrine, energy utilization may have been greater and energy state more susceptible to hypoxia than those in unstretched, unstimulated tissue. As shown in Fig. 5, pulmonary arterial II was 1.6 \(\times\) 10\(^4\) M\(^{-1}\) at the end of the hypoxic exposure compared with a baseline value of 2.8 \(\times\) 10\(^4\) M\(^{-1}\). Of the variables used to calculate II (Eq. 4), only [Cr] was altered by hypoxia (Table 2). Although significant, this increase was small, and its effect on II was offset by a simultaneous increase in K\(_{\text{cr}}\) due to the decrease in pH (16), also caused by hypoxia (Fig. 3). Thus, in contrast to femoral arterial smooth muscle, we could not demonstrate that hypoxia altered the energy state in pulmonary arterial smooth muscle.

Because baseline energy state was lower in pulmonary than in femoral arteries, it could be argued that the effects of hypoxia were not significant in pulmonary arteries because the energy state in this tissue could not be further reduced. This explanation cannot be correct because cyanide given to pulmonary arteries under baseline conditions decreased the energy state more severely than hypoxia. For example, [PCr]/[ATP] fell from 0.447 \(\pm\) 0.031 to 0.057 \(\pm\) 0.017 after exposure to a cyanide concentration of 10 mM (Table 3) but did not change during hypoxia (Fig. 3).

Analysis of variance indicated that the decrease in [PCr]/[ATP] induced by hypoxia was significantly greater (\(P = 0.036\)) in femoral than in pulmonary arteries (Fig. 3), suggesting that hypoxia caused a greater deterioration of energy state in femoral arteries. The explanation for this difference is unknown. Possibly, pulmonary arterial smooth muscle was able to maintain a higher ATP production. Less impairment of oxygen diffusion could allow greater mitochondrial oxygen uptake by pulmonary arteries under hypoxic conditions; however, wall thickness (and therefore oxygen diffusion distance) was greater in pulmonary arteries. Perhaps pulmonary arteries had a greater capacity to increase glycolytic ATP production under hypoxic conditions; however, hypoxia decreased pulmonary and femoral pH\(_i\) by similar amounts (Fig. 3), suggesting similar increases in lactic acid production. Moreover, inhibition of oxidative phosphorylation by cyanide appeared to decrease energy state more severely in pulmonary than in femoral arteries (Table 3). This difference suggests that glycolytic capacity may have been smaller, not greater, in pulmonary arteries. Alternatively, pulmonary arterial smooth muscle may have had a greater ability to downregulate ATP utilization in the face of hypoxia (8) or a cytochrome oxidase of greater oxygen affinity. Further investigation will be required to determine which of these (or other) explanations is correct.

Summary. In this study of resting vascular smooth muscle, we found that 1) under baseline conditions, energy state and Cr pool size were lower and pH\(_i\) was higher in pulmonary than in femoral arteries; 2) during hypoxia, energy state deteriorated more in femoral than in pulmonary arteries; and 3) during hypoxia, pH\(_i\) fell in both vessels but was always more alkaline in pulmonary arteries. The mechanisms responsible for these differences are unknown.

Because energy state influences many cellular processes, including interaction of contractile proteins, Na\(^+\)-K\(^+\) pump activity, and signal transduction, a difference in energy state could be associated with a difference in contractile function. Higher II and [PCr] values are thought to characterize tissues routinely subjected to high-energy demand, such as cardiac and skeletal muscles. Because pulmonary and femoral arteries normally change caliber in the face of markedly different transmural pressures, the baseline pulmonary-femoral differences in II and [PCr] are consistent with this concept; however, [PCr] in vascular smooth muscle is very low compared with that in skeletal or cardiac muscle, where levels of 20–30 µmol/g are typical (6, 10, 13). This profound quantitative difference, the low-energy requirements for contraction in vascular smooth muscle (34), and the fact that our studies were conducted in resting smooth muscle suggest that the pulmonary-femoral difference in energy state was related to differences other than the potential load on the contractile machinery.

pH\(_i\) can also affect a wide variety of cellular processes. For example, increased pH\(_i\) has been found to
increase resting tone and intracellular calcium concentration in vascular smooth muscle, possibly due to the release of calcium from intracellular stores (2, 24, 32). In addition, higher pH has been associated with membrane depolarization (1) and increases in myosin light chain kinase activity (31), myosin phosphorylation (9), and calcium sensitivity of contraction (32).

These considerations suggest that the differences in energy state and pH, we observed between pulmonary and femoral arterial smooth muscles could have functional consequences. For example, they could play a role in vasomotor responses to hypoxia, which are typically constrictor in pulmonary arteries and dilator in systemic arteries. Further investigation will be required to determine whether this is true.

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