Intravital microscopy of the murine pulmonary microcirculation

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Tabuchi A, Mertens M, Kuppe H, Pries AR, Kuebler WM. Intravital microscopy of the murine pulmonary microcirculation. J Appl Physiol 104: 338–346, 2008. First published November 15, 2007; doi:10.1152/japplphysiol.00348.2007.—Intravital microscopy (IVM) is considered as the gold standard for in vivo investigations of dynamic microvascular regulation. The availability of transgenic and knockout animals has propelled the development of murine IVM models for various organs, but technical approaches to the pulmonary microcirculation are still scarce. In anesthetized and ventilated BALB/c mice, we established a microscopic access to the surface of the right upper lung lobe by surgical excision of a window of 7- to 10-mm diameter from the right thoracic wall. The window was covered by a transparent polyvinylidene membrane and sealed with α-cyanoacrylate. Removal of intrathoracic air via a transdiaphragmatic intrapleural catheter coupled the lung surface to the window membrane. IVM preparations were hemodynamically stable for at least 120 min, with mean arterial blood pressure above 70 mmHg, and mean arterial PO2 and arterial PCO2 in the range of 90–100 Torr and 30–40 Torr, respectively. Imaged lungs did not show any signs of acute lung injury or edema. Following infusion of FITC dextran, subpleural pulmonary arterioles and venules of up to 50-μm diameter and alveolar capillary networks could be visualized during successive inspiratory plateau phases over a period of at least 2 h. Vasoconstrictive responses to hypoxia (11% O2) or infusion of the thromboxane analog U-46619 were prominent in medium-sized arterioles (30- to 50-μm diameter), minor in small arterioles <30 μm, and absent in venules. The presented IVM model may constitute a powerful new tool for investigations of pulmonary microvascular responses in mice.

hypoxic pulmonary vasoconstriction; thromboxane analog U-46619

KEY CELL SIGNALING MECHANISMS regulating microvascular responses commonly involve coordinated signaling among cells of different phenotypes and critically depend on the local microenvironment of mechanical forces and metabolic factors. Cultured cell experiments fail to replicate this three-dimensional multicellular environment. In vivo, intravital microscopy (IVM) provides the methodological gold standard for the visualization and analysis of dynamic microvascular regulation.

Recently, microcirculatory studies have been extended to a large array of mouse models in which the rapidly growing availability of transgenic and knockout animals provides a new tool for mechanistic analyses in vivo. A variety of murine IVM models for microvascular studies in immotile organs, such as liver, brain, or the relaxed skeletal muscle, have been successfully established (16, 22). Yet the lung still poses a major challenge for IVM in mice due to its complex optical accessibility, its cyclic respiratory movements, and its high susceptibility to mechanical and proinflammatory interference.

The relevance of lung IVM is exemplified here by the analysis of the pulmonary microvascular response to hypoxia [hypoxic pulmonary vasoconstriction (HPV)]. Whereas studies in intact lungs, isolated pulmonary artery vessels, and isolated smooth muscle cells have provided important insights into underlying cellular mechanisms (25, 39), segmental distribution and time profile of HPV are still discussed controversially. Instead of a complete smooth muscle layer in the media, pulmonary microvessels with diameters of <100 μm only have scattered smooth muscle cells (15, 23). Pulmonary arterioles of <50 μm have been described as completely nonmuscular (12), while HPV has been observed in microvessels of as little as 30-μm (11) or even 20-μm (42) diameter in IVM studies of isolated, perfused lungs. Furthermore, HPV has been reported either to be highly conserved over time (36), to fade entirely within 80 min of hypoxia (28), or to show a characteristic biphasic response with an initial peak after 10 min, a pressure nadir at 20–30 min, and a secondary progressive increase in pulmonary arterial pressure (40, 41).

Here, we present a new model for IVM of the murine pulmonary microcirculation. The reported technique facilitates real-time visualization of pulmonary microvessels under closed chest conditions without direct application of mechanical traction forces on the observation area, signs of lung edema, or compromised gas exchange. Next, we applied this model to characterize the segmental and temporal distribution of HPV in lung microvessels in vivo. In the intact murine lung, HPV is primarily localized in arterioles >30 μm and maintained over 60 min.

MATERIALS AND METHODS

Animals

Male BALB/c mice of 23–30 g body weight (BW) were obtained from Charles River Laboratories (Charles River Wiga, Sulzfeld, Germany). All experiments were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, 7th edition, 1996). The study was approved by the animal care and use committee of the local government authorities.

Surgical Preparations

Mice were anesthetized by intraperitoneal injection of medetomidine (0.5 mg/kg BW, Domitor, Dr. E. Graeub AG, Basel, Switzerland), fentanyl (0.05 mg/kg BW, Janssen Cilag, Neuss, Germany), and midazolam (5 mg/kg BW, Dormicum, Roche, Basel, Switzerland), as previously described (14). Anesthetic depth was evaluated regularly

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by limb withdrawal to paw pinch, and anesthesia was maintained by repetitive injections of one-half of the dose necessary for initial anesthesia induction every 30-60 min.

Anesthetized mice were placed in the supine position on a heating pad (Animal Respirator Compact 4600 Series, TSE Systems, Bad Homburg, Germany), and body temperature was maintained via a feedback-coupled rectal thermoprobe at 37.0°C. Two catheters [Portex FineBore Polythene Tubing, 0.28 mm inner diameter (ID)/0.61 mm outer diameter (OD), Smiths Medical International, Keene, NH] were inserted into the right external jugular vein for the injection of drugs and fluorescent dyes, and for continuous fluid replacement (0.2 ml/h of 6% hydroxyethyl starch, 6% 200/0.5; Fresenius, Bad Homburg, Germany). A catheter was placed in the right common carotid artery for arterial blood-gas analyses and continuous monitoring of arterial blood pressure (DasyLab 32; DasyLab, Moenchengladbach, Germany).

Following catheter placement, mice were tracheostomized, intubated with a polyethylene tube (Portex FineBore Polythene Tubing, 0.58 mm ID/0.96 mm OD; Smiths Medical International), and positioned in the left decubitus position. Animals were ventilated with room air at 100 breaths/min and end-inspiratory and end-expiratory pressures of 11 and 1 cmH2O, respectively (Animal Respirator Compact 4600 Series, TSE Systems, Bad Homburg, Germany). The skin over the right rib cage was removed, and the medial and caudal ends of the underlying muscles were dissected to expose ribs and intercostal muscles of the right anterior-lateral thoracic wall. By partial resection of the third to fifth rib, a 7- to 10-mm circular window was excised, in the center of which the lower margin of the upper right lung lobe was exposed (Fig. 1). Special care was taken to avoid any mechanical contact with the intact lung surface. After placement of a transdiaphragmatic catheter (Portex FineBore Polythene Tubing, 0.58 mm ID/0.96 mm OD; Smiths Medical International) into the right intrapleural space, the window was covered with a transparent polyvinylidene membrane (New Kure Wrap, Kureha, Tokyo, Japan) and tightly sealed with α-cyanoacrylate glue (Pattex; Henkel, Duesseldorf, Germany). Intrapleural air was removed via the intrapleural catheter, thus establishing direct coupling of the lung surface to the transparent window membrane. After air removal, the intrathoracic pressure was reconstituted at −3 mmHg and recorded continuously thereafter (DasyLab 32; DasyLab).

Lung IVM

After completion of the surgical preparation, mice were transferred to an upright microscope (Axiotechvario 100HD; Zeiss, Jena, Germany) on a custom-built, computer-controlled stage. At the beginning of a 30-min stabilization period, a bolus of the plasma marker FITC dextran (0.2 ml of a 1% solution; molecular mass 150 kDa, Sigma Chemical, St. Louis, MO) was infused intravenously to allow for the visualization of pulmonary arterioles, capillaries, and venules on the surface of the right upper lung lobe. In some experiments, carboxy-fluorescein diacetate-labeled platelets or fluorescent microspheres (15 μm FluoSpheres; Molecular Probes, Eugene, OR) were infused intravenously and imaged following their sequestration in pulmonary microvessels. Fluorescence was excited at 495 nm by monochromatic illumination (Polychrome IV; T.I.L.L. Photonics, Martinsried, Germany), collected through appropriate objectives (Achromplan 10/0.30 W and Achromplan ×20/0.50 W, both Zeiss) and dichroic and emission filters (FT 510, LP520; Zeiss), imaged by a silicon intensified tube camera (CF 8/4 FMC; Kappa, Gleichen, Germany) and recorded on digital videotape (DVCAM, DSR-25, Sony Deutschland, Berlin). Subpleural pulmonary arterioles and venules identified by their divergent and convergent flow pattern, respectively, were viewed and recorded during the expiratory plateau phase. The focal plane was adjusted to the midplane of the observed vessels by maximizing the apparent vessel diameter. Imaging sequences were recorded from all microvessels with diameters between 20 and 50 μm, which could be visualized within the observational field. On average, between two and four pulmonary arterioles and venules could be imaged at all experimental time points and entered subsequent diameter analyses. Image analysis was performed offline by use of a custom-made image-processing system (30). In brief, video images were digitized, and cursors were manually superimposed on opposing margins of lung microvessels. Vessel diameters were measured in triplicate at midsegmental positions between the proximal and distal vessel bifurcation. The distance between the two cursors was calculated based on the overall magnification of the optical system, which was determined by use of a recorded length reference. The precision of the length measurement is restricted to a dimension equivalent to one pixel length, which corresponds to 0.41 μm in the current setting (30).

Diameters were determined either during end expiration at ventilation rates of 100 breaths/min, or, in some cases of strong respiratory motion artifacts, during prolonged expiratory plateau phases of up to 5 s. Based on the internal diameter measured under baseline conditions, lung microvessels were differentiated into medium-sized (diameter range 50–30 μm) and small (30–20 μm) arterioles and venules. Larger arterioles and venules of >50 μm in diameter are not accessible to IVM due to their deeper position in the lung parenchyma (18, 37).

Gel Chromatography

Stability of FITC binding to dextran was tested by gel chromatography, as previously described (20). In brief, FITC dextran dissolved for 2 h in either 0.9% NaCl or murine plasma (100 μl) was added to a gel filtration column (Sephadex G-25; Sigma-Aldrich, St. Louis, MO), consisting of 3 ml void volume and >5-kDa molecular exclusion. Samples eluted from the column were sequentially collected as 1 ml per fraction and analyzed by fluorescence spectrometry (650–105; Perkin Elmer, Waltham, MA).

Experimental Protocols

Stability of the model. After completion of surgical preparation, mice were ventilated for 30 min at an inspiratory O2 fraction (FiO2) of 0.3 to warrant postsurgical stability of hemodynamics, as assessed by
continuous monitoring of arterial blood pressure. Thereupon, intravital microscopic image sequences were recorded, arterial blood pressures measured, and arterial blood gases analyzed at times $t = 0$ min (baseline), 60 min, and 120 min, respectively ($n = 5$ each). At the end of experiments, lungs were excised, and wet-to-dry weight ratio was determined for left and right lungs individually, as previously described (9). Wet-to-dry weight ratio from lungs of control mice without thoracotomy ($n = 5$) was quantified after the identical period of mechanical ventilation.

**HPV.** Following baseline IVM recordings at an $FIO_2$ of 0.3, the $FIO_2$ was reduced to 0.11. IVM recordings of the identical vessel segments were replicated after $t = 10$, 30, and 60 min of hypoxia ($n = 9$ each), i.e., at the proposed peak of the initial vasoconstrictive response, at the subsequent nadir, and during the secondary progressive pressure increase (40). Arterial blood gases were analyzed at baseline and after 60 min of hypoxia, and wet-to-dry weight ratio was determined at the end of the experiments.

**U-46619-induced pulmonary vasoconstrictor response.** To compare the spatial and temporal profile of HPV to the effects of a hypoxia-independent, pharmacological vasoconstrictor, we determined the vasoactive response to the endoperoxide analog of thromboxane, U-46619. Following baseline IVM recordings, U-46619 (Sigma Chemical) was successively infused at rates of 50 and 100 ng/kg/min, and IVM was repeated after 10 min of each infusion ($n = 6$ each).

**Statistics**

All data are given as means ± SE. Repeated-measures and dependent groups were analyzed by the Friedman and Wilcoxon test, and independent groups by Mann-Whitney U-test (Statview; SAS Institute, Cary, NC). Statistical significance was assumed at $P < 0.05$.

**RESULTS**

**IVM**

Using a ×10/0.30 W Achromplan objective, the intravital microscopic model allowed for visualization of large arteriolar and venular vessel trees on the surface of the right lung in ventilated mice (Fig. 2A). On average, four to five different arteriolar and two to three venular vessel trees with diameters of up to 50 μm could be visualized along the lower margin of the right upper lung lobe. After switching to a ×20/0.50 W Achromplan objective, individual FITC dextran-perfused capillaries within the membrane and septa of single alveoli could be identified as bright tubular networks against a darker background (Fig. 2B). During the respiration cycle, the lung surface shifted, but areas of interest always returned into the focus plane at the identical position during successive expiratory plateau phases. Imaging of sequestered carboxyfluorescein diacetate-labeled platelets revealed a steady return of the lung surface into the focus plane in successive expiratory plateau phases and a shift in the xy plane of <5 μm over 30 min (data not shown). Intraindividual variability of vessel diameter measurements, as determined by repetitive analyses in consecutive plateau phases, was 1.24 ± 0.11%, while interindividual variability between two independent investigators was 1.05 ± 0.15%. Representative video recordings from the upper right lung lobe of a ventilated mouse are provided in the online supplement (see online version of this article) and show subpleural alveoli and lung microvessels visualized by ×10 and ×20 objectives, respectively.

After surgical preparation and stabilization, no macroscopically visible signs of hemorrhage or atelectases were detectable in the area of observation or at the edges of the window rim for at least 2 h. Blinded histological analyses of hematoxylin-eosin-stained tissue slides from the subpleural areas of observation did not reveal any differences between lungs harvested 2 h after window implantation and lungs from control mice without thoracotomy ($n = 3$ each). During experiments, mice were hemodynamically stable for at least 120 min, with mean arterial blood pressure above 70 mmHg and mean partial pressures of oxygen ($P_{O_2}$) and carbon dioxide ($P_{CO_2}$) in the range of 90–100 Torr and 30–40 Torr, respectively (Fig. 3, A and B). A tendency of arterial blood pressure to decrease from 80 ± 5 mmHg at baseline to 73 ± 5 mmHg after 2 h did not reach significance. Lung wet-to-dry weight ratio after window preparation and IVM did not differ from lungs of control rats (Fig. 3C). Similarly, there was no difference between right and left lungs following thoracotomy and IVM of the right lung.
Diameters of pulmonary arterioles (baseline diameters 44.5 ± 1.7 μm for medium sized and 24.9 ± 0.7 μm for small) and venules (35.3 ± 1.4 for medium sized and 25.9 ± 1.5 for small) did not change over 120 min in the absence of hypoxia or U-46619 infusion (n = 5, Fig. 4A). Additional experiments were performed to verify the stability and accuracy of diameter measurements in lung microvessels visualized by the plasma marker FITC dextran over a 2-h time course. Representative chromatography responses in Fig. 4B show that all of the FITC dextran fluorescence occurred in the void volume (fractions 1 and 2), while free FITC appeared considerably later (fractions 8–12) during gel chromatography. Hence, over a period of 2 h, FITC does not dissociate from its complex with dextran, neither in 0.9% NaCl solution nor in murine plasma. To rule out that progressive accumulation of FITC dextran at the vessel wall may affect diameter measurements in a time-dependent manner, we analyzed the thickness of the highly fluorescent vessel fringe, which did not change over 2 h (Fig. 4C). Accuracy of distance measurements was tested in vivo by imaging of fluorescent microspheres with a nominal diameter of 15 μm and a coefficient of variation of ~1%, which had been infused intravenously and became trapped in subpleural lung microvessels (Fig. 4D). Image analysis of sequestered microspheres yielded measured diameters of 15.39 μm, with a coefficient of variation of 1.47%. Hence, absolute measured values exceeded nominal diameters by approximately one pixel length, while measurement variation was on average one-half a pixel length.

**Vasoactive Responses**

Reduction of FIO2 from 0.3 to 0.11 decreased PaO2 by 94.1 ± 7.8 Torr and mean arterial blood pressure by 9.8 ± 1.9 mmHg, while PaCO2 and pH remained unchanged (Table 1). Medium-sized arterioles (baseline diameters 43.0 ± 1.2 μm) decreased in diameter within 10 min of hypoxia (Fig. 5). This response was slightly abated after 30 min, while attenuation was no longer evident after 60 min (Fig. 6A). Small arterioles (baseline diameters 26.4 ± 0.6 μm) exhibited a trend toward reduced diameters during hypoxia, which only reached significance after 30 min (Figs. 5 and 6A). In contrast, no diameter changes in response to hypoxia were evident in small or medium-sized venules (baseline diameters: 24.0 ± 1.1 and 38.8 ± 2.2 μm, respectively). Lung wet-dry-weight ratios determined after 60 min of hypoxia did not differ from normoxic controls, indicating that arteriolar diameter changes did not result from interstitial edema formation (Fig. 6B).

Infusion of 50 ng·kg⁻¹·min⁻¹ of the thromboxane analog U-46619 reduced primarily vessel diameters of medium-sized, but also to a lesser extent of small, arterioles (baseline diameters 40.8 ± 1.5 and 25.7 ± 0.7 μm, respectively), but did not evoke significant diameter changes in small or medium-sized venules (baseline diameters: 24.2 ± 1.0 and 33.1 ± 0.9 μm, respectively) (Fig. 7) and did not increase arterial blood pressure (Table 2). Further elevation of the U-46619 concentration to 100 ng·kg⁻¹·min⁻¹ did not increase the lung microvascular response, but raised systemic blood pressure.

**DISCUSSION**

We present a new model for IVM of the pulmonary microcirculation in the ventilated mouse lung. Resealing of a surgically excised optical access to the right lung with a transparent membrane created a thoracic window for intravital lung microscopy under closed-chest conditions. Direct application of mechanical tension or traction forces to the observation field was avoided, thus allowing for regular respiratory movements of the lung surface. IVM observations were performed during the expiratory plateau phase. For at least 2 h, the model maintains stable arterial blood gases and mean arterial blood pressure above 70 mmHg in the absence of signs of lung injury or edema. Subpleural pulmonary arterioles, capillaries, and venules can be visualized at a high temporal and spatial.

Fig. 3. Stability of the intravital microscopy (IVM) model. Mean arterial blood pressure (A) and arterial PO2 (PaO2), pH, and arterial PCO2 (PaCO2) (B) in the ventilated mouse during 120 min of lung IVM (n = 5 each). C, wet-to-dry lung weight ratio in right and left lungs of mice following 120 min of lung IVM and of ventilated control mice (n = 5 each). No significant differences were detected over time (A and B) or between groups (C).
resolution, allowing for quantitative assessment of dynamic microvascular responses, e.g., the time course and longitudinal profile of vasoactive responses. Hence, the presented model provides a stable murine pulmonary IVM preparation under closed thorax conditions, with no signs of impaired gas exchange or edema formation. Due to its applicability in transgenic and knockout mice, it may present a new powerful technique for translational studies of pulmonary microvascular physiology and pathology.

**IVM of the Mouse Lung**

IVM of the pulmonary microcirculation in transgenic mice may provide a powerful tool to gain novel mechanistic insights into the regulation of pulmonary vascular tone and perfusion, cell-cell interactions, and cell migration. Yet IVM of murine lungs poses several methodological challenges. In larger animals, such as dogs (37), rabbits (19), or rats (21, 29), visual access to the lung has previously been created by surgical implantation of cylindrical, flanged steel windows into the thoracic wall of mice ventilated with 100% O2. Respiratory window previously established in rats (21) into the right thoracic wall of mice ventilated with 100% O2. Yet care should be taken when observation periods are extended even further, since the constant motion of the lung surface over the edges of the chest wall may ultimately irritate or even damage the area of observation. The lack of lung surface fixation confines imaging of lung microvessels to the expiratory plateau phase, which allowed
tively large access area required for IVM. In addition, flanges protruding into the thoracic cavity may interfere mechanically with the lung surface. To counteract resulting motion artifacts, negative pressure is typically applied via suction manifolds and a series of bore holes (19, 37). While potential mechanical traumas at the lung surface due to suction and flanges occur remote from the microscopic observation field in dog and rabbit models with large thoracic windows, these areas may come into close proximity in a mouse model. Recently, Razavi and coworkers (31) reported the intravital imaging of fluorescently labeled neutrophils in the murine lung. The authors implanted the miniaturized modification of a flange-suction window previously established in rats (21) into the right thoracic wall of mice ventilated with 100% O2. Respiratory motions of the observation field were minimized by repositioning the animals into a right lateral decubitus position and intravital imaging of the dependent lung via an inverted microscope (31).

In the present study, we chose a different approach by directly sealing the excised window with a transparent membrane. Coupling of the lung surface to the membrane was achieved by removal of intrathoracic air via a transdiaphragmatic intrapleural catheter, and lungs were allowed to move freely during the respiratory cycle. Direct contact of the lung surface with the cut edges of the partially removed ribs is prevented by the intermediate layer of parietal pleura and subpleural fascia, and neither structural nor functional defects in the subpleural area of observation were detectable over an observation period of 2 h. Yet care should be taken when observation periods are extended even further, since the constant motion of the lung surface over the edges of the chest wall may ultimately irritate or even damage the area of observation.
for stable visualization for ~300 ms, corresponding to 15 half images per respiratory cycle. Temporary ventilator stop facilitated single-observation periods of up to 5 s in the prolonged, expiratory plateau phase.

The extension of the lung IVM technique to the mouse model is expected to promote translational studies by use of transgenic and knockout animals. Yet these advances come at the cost of anatomical miniaturization, which impedes simultaneous determinations of basic hemodynamic conditions, such as pulmonary arterial pressure and flow. Thoracic instability as a result of a relatively large hemithoracotomy and the prerogative of adequate oxygenation necessitate constant mechanical

Fig. 5. Fluorescence microscopic images of lung microvascular response to hypoxia. Representative images show arterioles (top and center) and venules (bottom) at baseline [inspiratory O2 fraction (FiO2) = 0.3; left] and after 10 min of hypoxia (FiO2 = 0.11; right). Solid lines reflecting baseline diameters of medium-sized (30–50 μm; black lines) and small (20–30 μm; white lines) microvessels are of identical length in baseline and respective hypoxia images. Reduced vessel diameters in hypoxia are evident as lines protruding out of the vessel in medium-sized arterioles (arrows and detail magnification), but not in small arterioles or venules.

Fig. 6. Temporal and spatial profile of hypoxic pulmonary vasoconstriction. A: bar graph gives relative diameter change in response to hypoxia (FiO2 = 0.11) in medium-sized (30- to 50-μm diameter; n = 31) and small arterioles (20–30 μm; n = 19) and venules (20–30 μm, n = 10; 30–50 μm, n = 8) determined 10, 30, and 60 min after FiO2 reduction. Data are from n = 9 mice. *P < 0.05 vs. baseline (FiO2 = 0.3). #P < 0.05 vs. 10 min of hypoxia. B: wet-to-dry weight ratios in lungs of mice following 60 min of hypoxia (FiO2 = 0.11; n = 5) did not differ significantly from values in control lungs (FiO2 = 0.30).
ventilation compared with preparations in larger animals in which lung IVM has been performed successfully during spontaneous breathing (37).

A potential limitation imminent to all intravital microscopic analyses is the restriction to microvascular networks on the organ surface, which are not necessarily representative for interior blood vessels. In the lung, subpleural pulmonary networks are less dense and consist of larger capillaries compared with the interior pulmonary microvasculature (32, 35). Elegant histological studies, as well as analyses of regional blood flow by the use of radiolabeled microspheres, have shown that subpleural microvessels adequately reflect basic principles of vascular regulation, such as capillary recruitment characteristics (35) or blood flow redistribution during regional hypoxia (7). Yet caution should be taken when vascular responses are numerically extrapolated from the subpleural region to the entire lung, since quantitative differences in vascular tone and vasoactive responses may exist (8).

The applied ventilatory rate of 100 breaths/min is typically used in anesthetized mice (33), but is considerably slower than spontaneous respiratory rates in BALB/c mice, which are ~300 breaths/min (4). Over an observation period of 2 h, the applied ventilator settings maintained stable blood gases within the normal range reported for mice (5). Mean arterial blood pressure ranged between 70 and 80 mmHg during the observation period, which is in agreement with reported data for anesthetized mice (44), but falls below physiological blood pressure values in mice during sleep by ~10–15 mmHg (17). Bearing these potential limitations in mind, the reported intravital microscopic approach enables visualization of murine pulmonary arterioles, capillaries, and venules, with high spatial and temporal resolution at stable systemic hemodynamics and arterial blood gases in the absence of detectable signs of acute lung injury or edema.

**HPV**

When we imaged pulmonary arterioles and venules of up to 50 μm in diameter, a marked response to hypoxia was only detectable in the medium-sized arterioles of 30- to 50-μm diameter, with a concomitant minor diameter reduction in small arterioles. An essentially similar longitudinal distribution was observed in response to infusion of 50 ng·kg⁻¹·min⁻¹ U-46619, a frequently applied model for acute experimental pulmonary hypertension (13). Higher doses of the thromboxane analog caused loss of pulmonary selectivity, as indicated by an increase of systemic arterial pressure, but did not change the spatial response pattern within the pulmonary microvasculature.

The detected minor response in small arterioles and the lack of response in venules were not attributable to limitations of the optical system. In the applied microscope setting, the nominal optical resolution using the ×200/0.5 W Achromat objective is 0.61 μm at a pixel length of 0.41 μm. Measurements of fluorescent microspheres and detection of even slight diameter changes in small arterioles following U-46619 infusion demonstrate the accuracy of length measurements and the detectability of diameter changes within the optical boundaries of the system. Hence, our findings confirm the notion that marked vasoconstrictive responses in the lung microvasculature are primarily restricted to vessels of more than 30-μm diameter (43). Murine pulmonary arterioles of 30 μm or more stain positive for α-smooth muscle actin (28), and HPV responses in pulmonary arterioles of 30- to 50-μm diameter have previously been demonstrated in isolated, perfused canine and feline lungs by video microscopy and micropuncture measurements, respectively (11, 26). Pulmonary arterioles with diameters of <30 μm may contain pericytes and so-called “intermediate cells,” which show morphological features of smooth muscle-like cells and are considered as contractile cells (2, 38). These vascular segments may constrict in response to very severe hypoxia, as suggested by confocal microscopic studies of isolated rat lungs exposed to 2% O₂ (42), but only showed a mild diameter response to hypoxia or U-46619 infusion in the present study. No detectable diameter changes were observed in small or medium-sized venules, consistent with the previously reported finding that only single α-smooth muscle actin-positive cells are occasionally detectable in murine pulmonary venules of <50 μm in diameter (28). In contrast, canine pulmonary venules of the same caliber have 9% of their diameter accounted for by muscular media (23), which may explain their differential reactivity to hypoxia, as previously demonstrated by video microscopy in isolated dog lungs (11). It is important to note that the present findings do not preclude an important contribution of larger pulmonary veins to lung vascular responses. In fact, tension recordings of isolated pulmonary arterioles and venules of 30- to 50-μm diameter have shown that HPV is an important contributor to pulmonary vascular tone (39).
vessels and direct micropuncture studies have clearly identified pulmonary veins as a major site of vasoconstriction in response to hypoxia or thromboxane (6).

In a series of control experiments, we could rule out time-dependent effects, as well as dissociation or progressive accumulation of the fluorescent dye along the vessel wall as potential factors contributing to the observed diameter responses. Yet it should be noted that diameter responses indicate, but do not prove, active vasoconstriction. A decrease in diameter may similarly result from reduced microvascular distension pressures, interstitial edema formation, or inward redistribution of blood flow. Because distensibility of arterioles and arteries in canine and rat lungs (10, 24) and of the pulmonary vasculature in mice (36) ranges between 2.5 and 3.2%/mmHg, a “passive” diameter decrease by 6.5% in medium-sized arterioles would require a reduction in distension pressure of ~2.0–2.5 mmHg. Yet direct measurements by micropuncture revealed that hypoxia rather increases lung microvascular pressure in medium-sized arterioles by ~4 mmHg or 20%, respectively (26). The notion that the hypoxia-induced decrease in arteriolar diameters was attributable to active vasoconstriction is further supported by previous studies in isolated, perfused canine lobes, in which microvascular pressure was held constant during the switch from normoxia to hypoxia by adjustment of the pump flow rate and the height of the venous outflow reservoir (11). In these experiments, arteriolar diameters decreased by ~20%, suggesting that, compared with the in vivo situation, the diameter response may be even more pronounced when transmural pressures are kept constant. Potentially, inward distribution of blood flow during hypoxia may selectively reduce microvascular distension pressures in subpleural microvessels, yet no apparent redistribution of flow was evident in previous microsphere studies in intact rabbit or isolated rat lungs (7, 11). Interstitial edema is an unlikely cause for the observed decrease in vessel diameters, since hypoxia did not increase lung wet-to-dry weight ratios and IVM did not reveal any detectable signs of perivascular cuffing.

According to Poiseuille’s law, the observed diameter reduction by ~7% should result in a 34% increase in segmental vascular resistance. In vivo data on the change in murine pulmonary vascular resistance in response to 11% O2 are lacking due to methodological constraints, and the strength of the hypoxic response differs between species (3). However, available data from calves (1), sheep (27), or rabbits (34) demonstrate pulmonary vascular resistance increases by ~40–60% in response to a comparable hypoxic stimulus. Hence the HPV response may be even more pronounced in larger or more central vascular segments than those visualized in the present study. Medium-sized pulmonary arterioles sustained their HPV response over 60 min with a minor attenuation after 30 min. In contrast, the pressure response to hypoxia in isolated, perfused mouse lungs reveals a characteristic biphasic profile with an initial peak after 10 min and a marked pressure nadir after 30–60 min (40, 41). If a similar biphasic pressure profile exists in the present in vivo model, the reported findings may point to a differential temporal response pattern in different vascular segments of the lung in that medium-sized arterioles maintain a high vascular tone, while larger vessels undergo a phasic response. This hypothesis is in agreement with a recent study in precision-cut slices from murine lungs, demonstrating that some vessels maintain HPV for 120 min, while others return to baseline values after 80 min of hypoxia (28). Since a differential regulation of the acute and the sustained phase of HPV has been proposed (40, 41), this notion may also point to a diverse longitudinal distribution of HPV mechanisms in the pulmonary vasculature.

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