Imaging of the pulmonary circulation in the closed-chest rat using synchrotron radiation microangiography

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1Department of Biochemistry, National Cardiovascular Center Research Institute, Suita, Osaka, Japan; 2Department of Physiology, and Monash Centre for Synchrotron Science, Monash University, Melbourne, Australia; 3Faculty of Health Sciences, Hiroshima International University, Hiroshima; and 4Japan Synchrotron Radiation Research Institute, Hyogo, Japan

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Schwenke DO, Pearson JT, Umetani K, Kangawa K, Shirai M. Imaging of the pulmonary circulation in the closed-chest rat using synchrotron radiation microangiography. J Appl Physiol 102: 787–793, 2007. First published October 12, 2006; doi:10.1152/japplphysiol.00596.2006.—Structural changes of the pulmonary circulation during the pathogenesis of pulmonary arterial hypertension remain to be fully elucidated. Angiography has been used for visualizing the pulmonary circulation, conventional angiography systems have considerable limitations in providing images of small blood vessels with diameters of <200 μm, particularly within a closed-chest animal model. In this study we assess the effectiveness of monochromatic synchrotron radiation (SR) for microangiography of the pulmonary circulation in the intact-chest rat. Male adult Sprague-Dawley rats were anesthetized, and a catheter was positioned within the right ventricle, for administering isotonized contrast agent (Iomeron 350). Subsequently, microangiography of pulmonary arterial branches within the left lung was performed using monochromatic SR. Additionally, we assessed dynamic changes in vessel diameter during acute hypoxic (10% and 8% O2 for 4 min each) pulmonary vasconstriction (HPV). Using SR we were able to visualize pulmonary microvessels with a diameter of <100 μm (the 4th generation of branching from the left axial artery). Acute hypoxia caused a significant decrease in the diameter of all vessels less than 500 μm. The greatest degree of pulmonary vasconstriction was observed in vessels with a diameter between 200 and 300 μm. These results demonstrate the effectiveness of SR for visualizing pulmonary vessels in a closed-chest rat model and for assessing dynamic changes associated with HPV. More importantly, these observations implicate SR as an effective tool in future research for assessing gross structural changes associated with the pathogenesis of pulmonary arterial hypertension.

is required to fully understand the structural and functional properties of the microcirculation in the normal lung as well as understand the dynamic changes that occur in pathological conditions (e.g., PAH).

Angiography has been used for visualizing the pulmonary circulation of laboratory animals. However, conventional angiography systems have considerable limitations in providing images of small blood vessels with diameters of <200 μm in vivo (30, 33). Shirai et al. (25) designed a unique X-ray TV system for directly visualizing pulmonary vessels in the cat with diameters 100–500 μm, although such measurements were only possible in open-chest animal models (24, 27). In the last decade, technological advances in microangiography have utilized monochromatic synchrotron radiation (SR) as a powerful X-ray source and, when coupled with a high-resolution and high-speed imaging system, provide the ability to study microvessels of various animal organs ex vivo and in vivo (18). Tanaka et al. (28) were able to visualize coronary vessels (>50 μm) in the exposed dog heart. Moreover, Yamashita et al. (33) used SR to study mouse coronary arteries in beating hearts in vivo, and Tokiya et al. (30) were able to observe the microvasculature of tumors (ex vivo) with vessel diameters as small as 20–30 μm.

This study demonstrates for the first time the ability to clearly visualize pulmonary microvessels in an anesthetized closed-chest rat model using SR. Furthermore, we demonstrate the effectiveness and reliability of SR angiography to detect dynamic changes in vessel diameter during acute hypoxia [i.e., acute hypoxic pulmonary vasoconstriction (HPV)].

MATERIALS AND METHODS

Animals. Experiments were conducted on seven male Sprague-Dawley rats (8 wk old; body wt ~250–350 g). All rats were on a 12:12-h light-dark cycle at 25 ± 1°C and were provided with food and water ad libitum. All experiments were approved by the local Animal Ethics Committee and conducted in accordance with the guidelines of the Physiological Society of Japan.

Anesthesia and surgical preparation. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Supplementary doses of anesthetic were periodically administered (~15 mg·kg⁻¹·h⁻¹ ip). Throughout the surgery, body temperature was maintained at 37°C using a rectal thermostor coupled with a thermostatically controlled heating pad.

The trachea was cannulated and the lungs ventilated with a rodent ventilator (SN-480–7, Shinano, Tokyo, Japan). The inspire gas was

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enriched with O₂ (~50% O₂), and the ventilator settings were ad-
justed [tidal volume ~3.5 ml; frequency ~70 breaths/min; these
settings were previously shown to maintain arterial PCO₂ normocap-
ic]. A 20-gauge BD Angiocath catheter (Becton Dickinson), with the
tip at a 30° angle, was inserted into the jugular vein and advanced into
the right ventricle for administering contrast agent as well as inter-
mittently measuring right ventricular pressure (RVP).

The rat was securely fastened to a clear Perspex surgical plate,
which had a single window directly beneath the thorax area. The
surgical plate was then fixed in a vertical position in front of the beam
pathway so that the synchrotron beam would pass perpendicular to the
sagittal plane from anterior to posterior through the rat thorax, with
the aid of the window in the Perspex plate, and ultimately to a
SATICON X-ray camera described below (Fig. 1).

Microangiographic system. The pulmonary circulation was visual-
ized using SR microangiography at the SPring-8 BL28B2 beam line
facility, Hyogo, Japan. The microangiographic system has previously
been described in detail (11, 30).

SR has a broad and continuous spectrum from the infrared to the
X-ray regions. A single-crystal monochromator selects a single energy
of SR, and the X-rays of a very narrow energy bandwidth (an energy
bandwidth of 0.02–0.03 keV) are used for imaging. This SR system
comprises a monochromatic 33.2-keV X-ray source, just above the
iodine K-edge energy for maximal contrast. The monochromatic
X-ray beam from the synchrotron source is highly collimated. Fur-
thermore, the small size of the X-ray source and the very long
source-to-object distance ensures that image size distortion is avoided
(i.e., measurement of vessel diameter does not change at different
depths within the lung). The distance between the X-ray source and
object was 4,610 cm, and the distance between the object and detector
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The biomedical imaging SATICON X-ray camera has a resolution
of 1,050 scanning lines and can record images at a maximum speed of
30 frames/s for up to 30 s. The shutter open time used in this study
was 2.6–3.0 ms/frame. The detector features a 9.5-μm equivalent
pixel size projected onto the input area and an input field size of 9.5 ×
9.5 mm. High-resolution images were stored in a digital frame
memory system with 1,024 × 1,024 pixel format and 10-bit resolu-
tion.

Experimental protocol. The rat was positioned in front of the beam
line so that the upper segment of the left lobe was positioned in front
of the SATICON X-ray camera in alignment with the 9.5 × 9.5 mm
imaging field (i.e., between the 2nd and 3rd rib; see Fig. 2). Subse-
sequently, baseline heart rate (HR) and RVP data were collected.
Immediately before vessel imaging, the three-way stopcock on the
right ventricle catheter was opened to a clinical autoinjector (Nemoto
Kyorindo, Tokyo, Japan), which was used to inject a single bolus of
contrast agent (Iomeron 350; Eisai, Tokyo, Japan) at high speed (0.4
ml at 0.4 ml/s) so as to provide a concentrated dose of agent within the
pulmonary vasculature during the first second of the 2-s period of
imaging, thus ensuring optimal visualization of the pulmonary mi-
crovessels using angiography. For each 2-s period of scanning, 100
frames were recorded. Rats were given at least 10 min to recover from
each injection of contrast agent. Regular inspection between contrast
injections confirmed that the pulmonary vasculature was clear of
agent within this period of time. However, it is possible that not all of
the contrast agent had been expelled by the rat, since some residual
agent has been observed in the kidney of rats 10 min after adminis-

Fig. 2. Schematic drawing of the arterial circulation of the left
lung in a rat. The square box represents the position of the
9.5 × 9.5-mm imaging window used for microangiography of
the pulmonary microvessels.
X-ray flux at the position of the sample (subject) was \( \frac{1}{100} \times 10^{10} \) photons \( \cdot \text{mm}^{-2} \cdot \text{s}^{-1} \) at an energy of 33.2 keV. Therefore, the dose of radiation is 1.4 mGy per one frame at an exposure time of 2 ms, resulting in a total dose of 140 mGy in one scan of 100 consecutive frames. The dose value is \( \sim 2 \) times higher than that in clinical coronary angiographic imaging. However, it is possible to reduce the number of images per scan by half to be acceptable for repeated scans in the future.

Following baseline imaging, rats were randomly exposed to two levels of acute hypoxia (8% O\(_2\) and 10% O\(_2\) in N\(_2\)), each for 4 min. There was a period of 10–15 min between each hypoxic test. During acute hypoxia, HR and RVP data were continuously recorded until the third minute, after which recording of RVP was stopped, and the catheter was switched from the pressure transducer to the clinical injector for imaging. Lung microangiography was performed on the hypoxic lung after the fourth minute of each level of hypoxia.

**Data acquisition and analysis.** The RVP signal was detected using a Deltran pressure transducer (Utah Medical Products), amplified, and then sampled at 500 Hz with a DAQ pad data-recording system (National Instruments 6052E, Austin, TX) using DAQ-Universal 2004 custom software (courtesy of Geoff Head, Baker Heart Institute). HR was derived from the right ventricular systolic peaks.

During the 2-s period of image collection, pulmonary vessels pulsed, and vessel caliber fluctuated, due to the mechanical forces associated with systole and diastole. Consequently, only individual frames recorded at, or near, end systole were used for assessing and comparing pulmonary vessel diameter between baseline and hypoxic conditions. It was possible to identify the image at, or near, end systole by splitting the RVP signal and relaying one of the signals to an oscilloscope (the other signal was directed to the DAQ pad).
Innovative Methodology

**Table 1. Changes in HR and systolic RVP in response to two levels of acute hypoxia (10% and 8% O2) in anesthetized rats**

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>Systolic RVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>388±31</td>
<td>20.1±2.7</td>
</tr>
<tr>
<td>10% O2</td>
<td>392±29</td>
<td>30.9±2.2*</td>
</tr>
<tr>
<td>8% O2</td>
<td>381±37</td>
<td>27.9±3.0*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 5. HR, heart rate; RVP, right ventricular pressure. *Significantly different from control values (P < 0.05).

Separate Hamamatsu charge-coupled device (CCD) camera (Hamamatsu Photonics) was synchronized with the angiography SATICON X-ray pickup camera so that for every angiogram image recorded, a corresponding image of the oscilloscope was also captured as a separate tiff file. At a heart rate between 350 and 400 beats/min and at 30 frames/s, there were 5.8–6.7 beats/s, equivalent to approximately 5–6 frames within each cardiac cycle. Therefore it was possible to select an image at, or very close to, end systole. One frame per scan (one scan = 100 frames) was selected for image enhancement and analysis. Where possible, two to four vessels of each branching generation (2nd to 4th generation) were analyzed to ensure that a wide variety of vessel sizes was selected for measurement from each imaging scan. A total of 80 vessels was measured from seven rats, during normoxia and then again for each level of hypoxia. Six clearly identifiable vessels had a diameter <100 μm; 28 vessels had a diameter between 100 and 200 μm; 22 vessels had a diameter between 200 and 300 μm; 12 vessels had a diameter between 300 and 500 μm; and 12 vessels had a diameter >500 μm.

**Image analysis.** The computer-imaging program Image Pro-plus (version 4.1, Media Cybernetics) was used to enhance contrast and the clarity of angiogram images. To enhance images, a temporal subtraction operation was performed for flat-field correction using summation results of 10 consecutive frames acquired before contrast-agent injection. The summation image taken before injection was subtracted from a single raw image taken after injection to eliminate the superimposed background structure (Fig. 3). The pixel intensities of the raw image were first multiplied by a factor of 10. When the contrast between the foreground (vessels) and background is increased by averaging the background, boundary segmentation best clarifies the objects of interest.

Image Pro-plus was also used to evaluate the vessel internal diameter. A 100-μm-thick tungsten filament, which had been placed directly across the corner of the detector’s window, appeared in all recorded images and was subsequently used as a reference for calculating vessel diameter (μm). The line-profile function of Image Pro-Plus was used to measure changes in pixel intensity (brightness) along manually drawn segments perpendicular to the vessel, which span 10–40 pixels on either side of the vessel (see Fig. 4C). The first edge of the vessel was determined as the pixel at which intensity decreased below that of the preceding 10–40 pixels (depending on space between vessels). The opposite criterion was applied to ascertain the distal edge of the vessel. This boundary segmentation procedure was performed at two different points along the length of the vessel, and the average of the two values was used for data collation. To assess reproducibility, the procedure for measuring vessel width was repeated by a second observer.

All numerical data are presented as means ± SE. One-way ANOVA (factorial) was used to test for differences in vessel diameter during normoxia compared with acute hypoxia. A P value ≤ 0.05 was predetermined as the level of significance for all statistical analysis.

**RESULTS**

**Baseline.** Using SR, we were able to clearly visualize pulmonary microvessels in the left lung of an intact-chest rat. Figure 4A shows the branching pattern of the pulmonary artery from the main axial artery of the left lobe to the fourth generation of branching (within the 9.5 × 9.5-mm imaging window). Vessel caliber tended to decrease according to each generation of branching, as well as the distance away from the main axial artery toward the periphery. In general, the axial artery was ~1,200 μm at the point of branching in Fig. 4A. Thereafter, the first primary branch was ~600–800 μm, and the second branch was ~400–600 μm. By the fourth generation of branching, vessel caliber was sometimes <100 μm in diameter (the 100-μm-diameter tungsten wire was the reference). The smallest vessels that could be clearly measured were ~80 μm. These vessels, however, were not visible (or the vessel edges were too opaque to be accurately detected by segmentation) during acute exposure to hypoxia and so were excluded from analyses for the acute hypoxic response (see below).

**Responses to acute hypoxia.** Rats were exposed to 10% and 8% O2, each for 4 min with time to recover in between each hypoxic test. The changes in HR and systolic RVP in response to acute hypoxia are presented in Table 1. Hypoxia did not alter HR, but it did significantly increase systolic RVP (40–50% increase). Since systolic RVP is a reflection of systolic pulmonary arterial pressure (PAP), the observed increase in systolic RVP indicates that HPV is likely to have occurred. This assumption was confirmed by microangiography.

Acute hypoxia caused a significant decrease in the internal diameter of all vessels <500 μm (Figs. 4 and 5). The magnitude of constriction tended to increase as vessel caliber decreased, except for those vessels with a diameter of 100–200 μm (Fig. 5). The greatest degree of pulmonary vasoconstriction was observed in vessels with a diameter between 200 and 300 μm. These vessels were generally of the third to fourth generation of branching. In addition, there was no significant difference in the magnitude of hypoxic pulmonary vasoconstriction for 10% O2 and 8% O2 for all vessel sizes.

**Evaluation of accuracy of measurement.** To estimate a margin of error for accurately detecting the edge of any given vessel containing pure contrast agent, we assessed the variability in our measurements of the width (in pixels) of

![Fig. 5. The relationship between vessel size and the magnitude of pulmonary vasoconstriction (% decrease in vessel diameter) in response to 2 levels of acute hypoxia (10% and 8% O2) in a closed-chest rat model (n = 7). *Significant response to acute hypoxia (P < 0.05). There was no significant difference in the magnitude of response between 10% and 8% O2.](http://jap.physiology.org/Downloadedfrom/10.2353/ajpheart.2007.102.2.247)
the reference wire (known diameter of 100 μm). From one individual image frame, we measured wire width at 40 random points along its length. The mean width of this reference wire was 12.99 pixels (SD ± 1.12). This equates to an average pixel size of 7.69 μm (95% confidence interval of 7.496–7.908 μm). Consequently, the 100-μm tungsten wire could accurately be measured to within ~5 μm (range of 97.4–102.7 μm).

Measurement of the reference wire represents ideal X-ray absorption and contrast. In this study, however, the iodine contrast agent was injected into the right ventricle so that the iodine concentration within the pulmonary circulation would have been diluted, which is likely to have attenuated X-ray absorption and potentially the accuracy of measuring vessel width. In an earlier preliminary study phantom vascular images were recorded to assess the relationship between X-ray absorption and iodine concentration (K. Umetani, unpublished observations). Using the same experimental set-up as described in MATERIALS AND METHODS, eight tubes with an internal diameter of 200 μm were filled with various concentrations of iodinated contrast material ranging from pure agent (370 mg/cm³ iodine, Nihon Schering) to distilled water (i.e., 0 mg/cm³). In addition, a 5-mm-thick aluminum mask was overlaid to simulate signal attenuation by the animal body.

The widths of tubes containing an iodine concentration between 370 and 32 mg/cm³ could be accurately measured with a small margin of error; e.g., the width of the tube containing 32 mg/cm³ iodine was measured at 40 points and had a 95% confidence interval of 196.84–203.26 μm. This is a similar margin of error as that obtained for multiple measurements of the tungsten reference wire (described above).

These results show that measurements of 200 μm in diameter can be made precisely for those vessels with an iodine concentration of >32 mg/cm³. Moreover, it seems reasonable to suppose that measurements of ~100 μm in diameter can be made with the required precision for those vessels with an iodine concentration of 64 mg/cm³ or greater.

In our study, therefore, even though the bolus contrast agent was diluted within the right ventricle, the iodine concentration within the pulmonary arteries was likely to have been sufficient to detect well-defined edges for those vessels as small as ~100 μm.

One limitation of this study is that it was not possible to make multiple measurements across vessels ~80–150 μm in diameter because of the short length of these peripheral branches. Nevertheless, on the basis of the boundary segmentation procedure used, intra- and interobserver analyses were highly reproducible for each vessel determination. A total of 40 vessels from various branching generations was assessed by two different observers. Regression analysis indicated that the measurements from both observers were highly correlated (y = -4.829 + 0.997x, r² = 0.979) and had an average difference of 9 ± 2 μm (for 100- to 200-μm vessels) to 14 ± 7 μm (for 300- to 500-μm vessels).

**DISCUSSION**

This study demonstrates, for the first time, the ability to visualize the pulmonary circulation in a closed-chest rat model and the dynamic changes associated with HPV using SR microangiography.

Although significant advances in the treatment of pulmonary disorders have been made in recent decades, the underlying mechanisms governing the pathogenesis of PAH remain to be fully elucidated. Before the underlying mechanisms can be examined, however, meticulous information concerning the normal pulmonary vessels is essential to accurately assess the changes in pulmonary arteries in experimental pulmonary hypertension. Yet, ever since the pioneering work of Reid and colleagues (5, 7, 8, 13, 14, 17), there has been a paucity of studies describing the anatomic and structural features of the normal pulmonary vasculature.

One of the primary characteristics of PAH is vascular remodeling and medial thickening of thin muscular-walled vessels (100–300 μm), as well as the formation of new muscle around nonmuscular or partially muscular vessels (50–150 μm) (8, 13, 14, 20). The irreversible remodeling of the pulmonary vasculature (3, 31) decreases the caliber of the pulmonary vessels and causes a sustained increase in PAP. Additionally, sustained hypoxia, which occurs in numerous pulmonary disorders, can cause adverse structural changes in the pulmonary vascular bed, such as pruning of small vessels, contributing to an increase in vascular resistance (5, 7, 16), although more recent evidence suggests that angiogenesis is also occurring in the chronic hypoxia rat lung (9). Ultimately PAH increases the workload of the heart and is, therefore, closely associated with heart failure and increased mortality.

In this study, we were able to clearly visualize pulmonary vessels, with a diameter as small as 80 μm. Although smaller vessels exist within the pulmonary vascular bed, we were unable to clearly detect the border of these vessels, suggesting that the resolution limit of the pulmonary microvessels in this study was close to 80 μm. Determining the exact spatial resolution limit in vivo is difficult in a vessel because of the possibility of nonuniform constrictions or uneven iodine contrast distribution within the smaller vessels, which would increase the signal-to-noise variability between neighboring pixels of any single image.

To enhance the ability to measure vessel diameter in our study, we performed temporal subtraction whereby the summation of 10 consecutive preinjection frames was subtracted from a single postinjection raw image. Only a single postinjection image could be used for subtraction because a summed image would be blurred because of movement of pulmonary vessels (by heart palpitations) during iodine injection. Studies that use vessels that remain static during iodine injection, however, can summate images after contrast injection, as well as before. The final subtracted image not only eliminates background structures but also eliminates uneven iodine distribution and magnifies the contrast between vessel and background. Indeed, such image enhancement allows visualization of vessels <50 μm, and as small as 20–30 μm, within the microvasculature of tumors (in vitro), exposed heart (28), liver (12), and brain (11, 29) of experimental animals.

In our study, we were limited to measuring vessels with a diameter ≥80 μm in the rat lung. Despite this limitation, the vessels that are most susceptible to pathological disorders have an internal diameter >100 μm, i.e., resistance vessels that are at least partially muscular (8). Therefore, the results of our study demonstrate the suitability of SR microangiography for
visualizing the small peripheral pulmonary vessels that are normally susceptible to adverse alterations in various pathological conditions.

An advantage of the superior definition achieved with SR is the ability to visualize the pulmonary microvessels within a natural physiological milieu (i.e., within the intact chest), a technique not previously possible with conventional X-ray systems. Conventional X-ray systems with a small focal spot have previously used an X-ray direct-conversion type VIDICON camera as a two-dimensional high-resolution detector (22, 26). This type of X-ray system, however, has limitations when providing images of blood vessels with a diameter <50 μm (even in vitro) because images cannot be magnified without focal spot blurring. Furthermore, polychromatic light from conventional X-ray systems will scatter differently according to wavelength. In addition, a conventional angiography system incorporating an X-ray image intensifier and a video camera is not intended for detection of small vessels of 200–300 μm diameter or less because it is designed for large-field digital angiographic imaging based on a 1024 × 1024-pixel format.

In contrast to conventional systems, SR is characterized by high brilliance and extreme collimation (28), allowing enhanced sensitivity to contrast material and superior image quality in terms of spatial and density resolution because divergence and scatter of X-ray photons are eliminated. The collimation is possible because of bending magnets in the synchrotron storage ring that maintain beam trajectory and minimize X-ray dispersion. Consequently, this allows vessels at different depths to be measured without magnification artifacts.

One concern expressed about this angiography approach is the likelihood of a non-synchrotron source with similar spatial and temporal resolution will be available in the immediate future for conventional bench-top X-ray systems. The SPring-8 facility is currently the largest synchrotron source available, but access to synchrotron sources is available in many regions of the world. Therefore, most research groups will have sufficient access to be able to conduct intensive experiments.

HPV in the rat. In this study we were also able to assess dynamic changes in vessel caliber during acute hypoxia, i.e., the well-documented HPV phenomenon. We showed that all vessels with a diameter <500 μm constricted in response to hypoxia and that those vessels with a diameter of 200–300 μm showed the greatest magnitude of HPV.

HPV has been reported to preferentially occur in vessels with an internal diameter of ~150–300 μm in cats, and rabbits (10, 26, 27) and up to ~600 μm in dogs (2). During hypoxia, as PAP increases, vessels larger than 300–500 μm are likely to be subjected to a higher distending pressure (assuming no change in cardiac output), suggesting that constriction of larger vessels (i.e., >500 μm) may be underestimated by a high distending pressure. However, Shirai et al. (27) demonstrated that, when the increase in PAP during hypoxia is prevented, the magnitude of vasoconstriction tends to be accentuated, albeit slightly, equally for all pulmonary vessel sizes in the cat.

Several studies have used a severe level of hypoxia (i.e., ~8% O2) to elicit significant pulmonary vasoconstriction in the rat, rabbit, and cat (10, 21, 22, 27). In this study, we tested severe hypoxia (8% O2) and moderate hypoxia (10% O2) expecting that the magnitude of pulmonary vasoconstriction may be dependent on the severity of hypoxia. Yet the vascular responses to 10% and 8% O2 were similar (assessed by angiography and right ventricular systolic pressure). Shirai et al. (27) also assessed the response of the pulmonary vasculature of the cat lung to moderate (10% O2) and severe (5% O2) hypoxia using angiography. They reported that the magnitude of vasoconstriction was greater for 10% O2 compared with 5% O2 only if the hypoxia was global (i.e., whole body). If the hypoxic stimulus was restricted to just the lung (i.e., regional hypoxia), the magnitude of vasoconstriction was proportional to the degree of hypoxia (i.e., greatest for 5% O2). Hypoxia is a potent activator of pulmonary sympathetic nerve activity (SNA), especially when the inspired level of O2 is below 8% O2 (23, 27). Shirai et al. (27) were able to subsequently demonstrate that the vasoconstrictor effect of global hypoxia was attenuated by a sympathetic β-receptor-mediated vasodilator effect. Although cats were used in that study (27), the importance of a β-receptor-mediated vasodilator effect for modulating HPV has also been demonstrated in the rat (4, 5).

In our study, we exposed rats to global hypoxia. It is reasonable, therefore, to speculate that the response to 8% O2 (severe hypoxia) comprised a strong neural response (i.e., a potent increase in a sympathetic β-receptor-mediated vasodilatation) that countered the local humoral vasoconstrictive response of hypoxia on the pulmonary vasculature. In contrast, the response to 10% O2 (moderate hypoxia) may have comprised mainly the local vasoconstrictive response of hypoxia (without eliciting a significant increase in SNA).

Limitations of this study. In the SR imaging system, the smallest-diameter vessel seen for the measurements is primarily determined by the concentration of iodinated contrast material. The iodine concentration depends on the type and dose of the contrast material, the injection technique, and the dilution curve and the vessel size under study. One of the limitations of this study was the inability to inject contrast agent directly into the pulmonary circulation of the left lung. Instead, the contrast agent was injected into the right ventricle. Therefore, not all of the contrast agent was delivered into the left lung, and, furthermore, the contrast agent would have been diluted, at least to some extent, by venous return. However, the rate at which contrast agent was injected (0.4 ml at 0.4 ml/s) is approximately equivalent to cardiac output of an anesthetized rat (~24 ml/min). Therefore, even if the bolus dose were diluted by as much as 1:5 in the right ventricle during ejection (equivalent to an iodine concentration of 70 mg/cm3), the preliminary phantom study indicates that vessel edge detection should be accurate and reproducible for vessels as small as 100–200 μm.

Because of the fact that only one frame could be used for measuring vessel caliber, and that the iodine contrast agent was diluted, it was not possible to find an image frame whereby the pulmonary vessels were completely filled with contrast agent. Consequently, this often resulted in images with less-than-perfect contrast. Nevertheless, as mentioned above, despite this limitation, it was still possible to enhance a single image for the purpose of accurately measuring vessel caliber >80 μm.

In this study we could not view the circulation of the whole lung but rather were restricted to a relatively small field of view (FOV) of 9.5 × 9.5 mm. Before the 1990s, video cameras consisted of a pickup tube to capture images. However, advances in technology have meant that pickup tubes have largely been replaced by a CCD. Some pickup tubes cameras, such as the X-ray SATICON camera used at SPring-8, are still used for special purpose applications in scientific and industrial fields. The pickup tube is a glass cylinder maintained under vacuum; the front
end of the tube is a flat plate, the inside of which is coated with a photosensitive material. The FOV limit is imposed by the diameter of the glass cylinder, a standard size of 1 in. The 28B2 experimental beam line at SPring-8 produces an X-ray beam of approximately 150 by 100 μm at the sample. Therefore it is not possible to use a larger detector at this specific beam line without increasing the distance between the source and subject significantly. It is anticipated that an X-ray direct-conversion type solid-state image sensor will be available in the near future for special purpose applications (e.g., scientific). This type of sensor will be able to remove restrictions on the FOV, while retaining temporal and spatial resolution at so-called long beam lines with larger beam dimensions.

In summary, we have demonstrated the effectiveness of SR for visualizing pulmonary vessels in a closed-chest rat model and for assessing dynamic changes associated with HPV. Despite some limitations, the observations from this study unlock a wide range of possibilities for using SR in pulmonary microangiography. Of particular importance is the application of SR in future studies for assessing gross structural changes in pulmonary vessel density, and changes in microvessel diameter, associated with the pathogenesis of PAH.

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GRANTS

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REFERENCES