

Fig. 3 - Changes in MCA caliber expressed as a percentage of basal values. Marks on the graph indicate statistically significant changes as compared with the basal condition. † $p < 0.01$; ‡ $p < 0.005$.

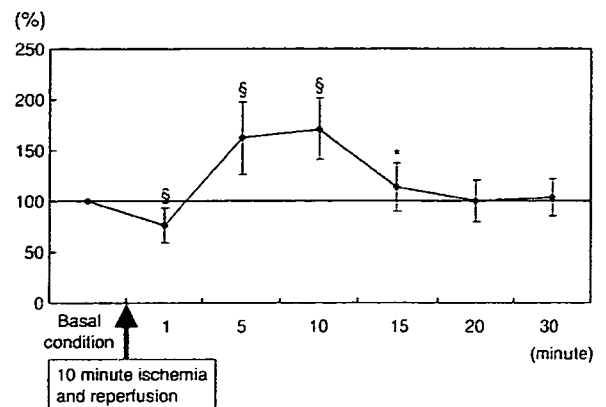


Fig. 5 - Changes in pial arteriole caliber expressed as a percentage of basal values. Marks on the graph indicate statistically significant changes as compared with the basal condition. * $p < 0.05$; § $p < 0.00001$.

angiography system. The major findings of the present study can be summarized as follows: first, the pial arterioles were constricted, whereas the striate arteries were dilated at 1 min after reperfusion; second, the pial arterioles and striate arteries were dilated from 5 to 10 min after reperfusion; third, early venous filling was observed at the same time as the vessels dilatation.

In a four-vessel occlusion rat model of 15-min ischemia, Pinard et al. (2000) reported that changes in the caliber of arterioles of the cortical surface were not significant for 5 min after reperfusion, but significantly increased from 5 to 15 min after reperfusion. Their findings were almost consistent with our findings of pial arteriolar behaviors after reperfusion. In contrast, the striate arteries in the present study were dilated significantly 1 min after reperfusion. Although under normal circumstances the autoregulatory responses of the intracerebral vessels, which are inaccessible to direct observation, are thought to be accu-

rately gauged by studying the small pial arterioles (Kontos et al., 1978), in the case of an ischemia and reperfusion model this may not be true. Accordingly, care should be taken in estimating the responses of the central branches of the cerebral vessels from those of cortical branches during ischemia and reperfusion.

The corpus striatum, nourished by the striate arteries, is reported to be more vulnerable to ischemia and reperfusion than the neocortex, which is perfused by the pial arterioles (Garcia et al., 1995; Pulsinelli et al., 1982). Liachenko et al. (2001) reported that transient hyperperfusion after 12 min of circulatory arrest in rats started from the thalamus and hypothalamus and later shifted to the cortex. In their experiments, the cerebral blood flow (CBF) of the cortex remained at about 10% of the basal condition 2.5 min after a circulatory arrest of 12 min, whereas the CBF within the thalamus increased about 150%. Therefore, we can infer that the constriction of the pial arterioles and the dilatation of the striate arteries at 1 min of reperfusion in the present study

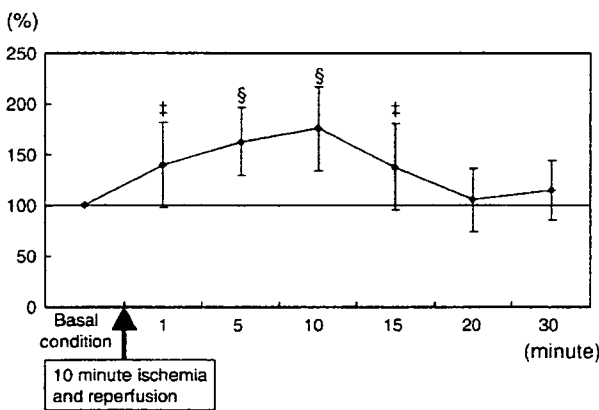


Fig. 4 - Changes in striate artery caliber expressed as a percentage of basal values. Marks on the graph indicate statistically significant changes as compared with the basal condition. † $p < 0.005$; § $p < 0.00001$.

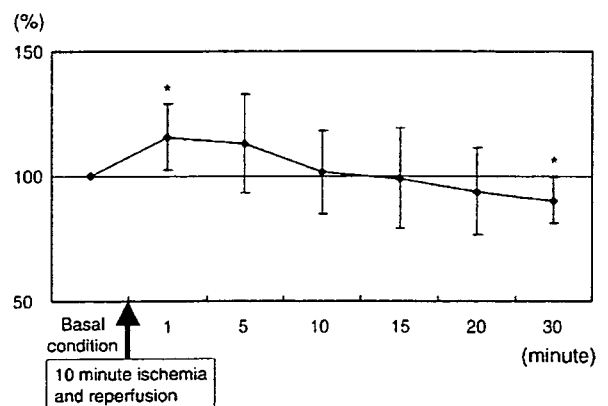


Fig. 6 - Changes in ICA caliber expressed as a percentage of basal values. Each asterisk (*) shows $p < 0.05$ as compared with the basal condition.

were accompanied by hypoperfusion and hyperperfusion, respectively. In other words, the response of the pial arterioles to an abrupt increase in intravascular pressure after ischemia is constriction at 1 min of reperfusion, whereas that of the striate arteries is dilatation. This means that the striate arteries in themselves may be more vulnerable to ischemia and reperfusion than the pial arterioles, leading to hypoxic fragility of the corpus striatum. This might also explain why hemorrhagic transformation is most often seen in the corpus striatum in nonhuman primates after proximal middle cerebral artery occlusion (del Zoppo et al., 1998). Another explanation depends on the presence of collateral vessels (Bär et al., 1978; del Zoppo et al., 1998): the pial vessels have abundant collaterals on the cortical surface, but the striate arteries have few collaterals because they are the end of the artery.

Early venous filling, which was defined as early angiographic appearance of the venous structures from the arterial phase, was observed in the present study during reperfusion. Ohta et al. (2004) reported that there was a significant correlation between the appearance of angiographic early venous filling during intra-arterial reperfusion therapy and post-therapeutic hemorrhagic complications. Although its appearance seems to be a good predictor of hemorrhagic complications after reperfusion therapy, the pathophysiologic basis of early venous filling is not completely understood. Clear detection of early venous filling in rats with the MRS angiography system could lead to its clarification.

There were some limitations to the present study. There may be some vascular response of the blood vessels induced by the injection of contrast medium. In conventional cerebral angiography in rats, a relatively large amount of contrast medium compared to the total blood volume is injected. In the present study, a small amount (0.3 ml) of contrast medium was sufficient for imaging, and clear images were obtained after repeated imaging. Recently, Kidoguchi et al. (2006) successfully performed *in vivo* X-ray angiography in the mouse brain using the MSR angiography system. Thirty microliters of contrast agent was injected, the equivalent volume per body weight (g) used in the present study. Exposure to the synchrotron can be another disadvantage. To minimize this effect, irradiation was blocked during the intervals between angiography recordings. Eight consecutive angiography recordings were conducted every 5 min with normal rats, yielding clear images without significant changes in the vascular diameters.

If the pixel location of a high contrast background object should move during mask-mode subtraction, this misregistration between the mask and the opacified vessel image will result in the introduction of an artifact. In Figs. 2A–D, motion artifacts are shown in the upper left corners and the middle of the left side, and there are residual subtraction artifacts associated with the tungsten wire and the tympanic bulla, respectively. However, it is not difficult for observers to distinguish between misregistration artifacts and the opacified blood vessels.

In conclusion, we clearly demonstrated responses in cerebral vessels of various sizes (from 20–30 μm to 500 μm in diameter) during ischemia and reperfusion using the MSR angiography system.

4. Experimental procedures

4.1. Animals

Eight male Wistar rats weighing 280–350 g were used in this study. The rats had access to food and water *ad libitum* and were housed in individual cages. All experimental procedures were performed in accordance with the Animal Care and Use Committee of the Kawasaki Medical School (03-018, 04-018).

4.2. Microangiographic system

The present study was performed at the SPring-8 BL28B2 beamline of the Japan Synchrotron Radiation Research Institute. The illustration of the experimental arrangement at the BL28B2 beamline is shown in Fig. 7. Synchrotron radiation was derived from a storage ring of electrons with an accelerated energy of 8 GeV and an average beam current of 99 mA. This synchrotron radiation was monochromatized by a silicon crystal that was placed in front of the rat. The monochromatic X-ray energy was adjusted to 33.2 keV just above the iodine K-edge energy to produce the highest contrast image of the iodine contrast material. The distance between the X-ray source and the bending magnet and the detector was about 45 m and the distance between the rat and the detector was about 20 cm. A nearly parallel X-ray beam was used for imaging without image blur because of the small size of the X-ray source and the very long source-to-object distance. An X-ray SATICON camera (Hitachi Denshi Techno-System and Hamamatsu Photonics) was used as the detector. This camera has resolution of 1050 scanning lines and can record images at a maximum speed of 30 frames/s. Sequential images were obtained with an input field of view of 7 mm \times 7 mm. High-resolution images were stored in a digital frame memory system with a 1024 \times 1024 pixel format and a 12-bit resolution.

The best three frames were selected from a sequential digital image of 50 frames and used for this examination. The microvessel inner diameter was measured using a tungsten wire 100 μm in diameter as a standard.

4.3. Animal preparation

The rats were prepared for transient global ischemia using the four-vessel occlusion model (Pulsinelli and Brierley, 1979). On the day before the experiment, the rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The skin and muscles over the first two cervical vertebrae were incised and separated from the midline. Both vertebral arteries were electrocauterized after exposure of the alar foramina under a surgical microscope.

On the day of the experiment, the rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and then underwent mechanical ventilation (SAR-830, CWE Inc.) using room air O₂ with a tidal volume of 1 ml per 100 g body weight and 40 strokes per minute after tracheal intubation and muscle relaxation (vecuronium bromide, 0.5 mg/kg). Anesthesia was maintained with hourly injection.

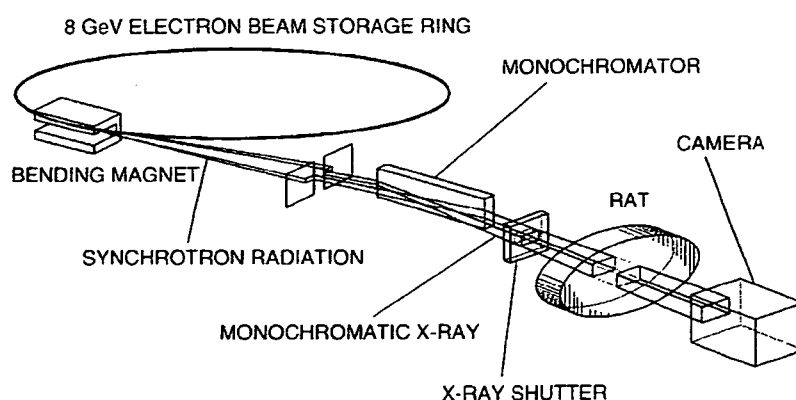


Fig. 7 – Schematic presentation of the experimental setup. Synchrotron radiation is generated by bending the path of electrons at relativistic speeds using a bending magnet. The storage ring maintains the electron beam for many hours. A single silicon crystal selects a single-energy synchrotron radiation. The digital images were acquired through analog-to-digital conversion.

tions of sodium pentobarbital (10 mg/kg). The bilateral common carotid arteries (CCA) were isolated from surrounding tissues through a midline neck incision and silk ligatures were placed loosely around them. Femoral artery and vein catheters were inserted to monitor ABP to measure arterial blood gases (i-STAT, Abbott Laboratories), minerals (sodium and potassium) (i-STAT, Abbott Laboratories) and hematocrit (i-STAT, Abbott Laboratories) and for infusion of anesthetics. Another arterial catheter (PE-50) was placed in the left extra carotid artery for angiography. A temperature sensor (ATB-1100, Nihon Kohden) was inserted into a temporal muscle. The duration of the whole preparation was 1 h from the start of anesthesia to induction of global ischemia.

4.4. Global ischemia

Each rat was placed in front of the MSR angiography system on a handmade stereotaxic device to which the head of the rat was certainly fixed by holding both external acoustic meatuses and the incisor in place. The arterial catheter was connected to a pressure transducer (BSM-2301, Nihon Kohden) for the continuous recording of ABP. The body temperature was kept at 37 °C by a blanket and heater (ATB-1100, Nihon Kohden). Global ischemia was induced by occlusion of the bilateral CCA by tightening the silk sutures. Completion of ischemia was checked by the appearance of mydriasis. After 10 min of ischemia, reperfusion was performed by release of the silk sutures. Microangiography was performed before occluding the bilateral CCA, and 1, 5, 10, 15, 20 and 30 min after releasing the silk ligatures by a bolus injection of 0.3 ml of the contrast medium iopamidol, a nonionic water-soluble contrast medium (iodine concentration of 370 mg/ml; Nihon Schering K.K.).

Since each rat had to be manipulated when releasing the silk ligatures of the bilateral CCA, there was a possibility that the rat might shift from the control position. Therefore, the rat was re-positioned by reconfirming the rat's skull image on the camera within 5 min after releasing the ligatures, but

not 1 min after releasing the ligatures because of a lack of enough time.

4.5. Vessel diameter measurement

Using the NIH Image Device, the microvessel caliber was measured using a 100- μ m diameter as the standard. The vascular caliber of the internal carotid artery (ICA) immediately before it branched off the posterior cerebral artery, in the horizontal region of the middle cerebral artery (MCA) immediately after it branched from the ICA, and in the pial arterioles (PA) and striate arteries (SA) immediately after they branched off. The diameters of the PA and SA that were clearly observed on all images were measured (Fig. 2).

Confirming good visualization of the respective vessels in all images, we outlined the region including each respective vessel in each image and converted the gray scale image to an image consisting of only black and white pixels. Then we measured the caliber of the respective vessels. In the case of ICA and MCA, calibers of three different portions that were a few pixels apart from each other were measured. In the case of the PA and SA, calibers of two to four predetermined vessels of the PA and SA were measured.

4.6. Statistical analysis

The changes with time are expressed as the means \pm SD. The mean basal value was calculated in all vessels. A statistical analysis was performed using the paired t-test. The first value was taken as the control value. Values of $p < 0.05$ were considered to be significant.

Acknowledgment

The synchrotron radiation experiments were performed in the BL28B2 experimental unit at SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (Proposal Nos. 2003B0090, 2004A0128 and 2004B0622).

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In Vivo Cerebral Artery Microangiography in Rat and Mouse Using Synchrotron Radiation Imaging System

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Microangiography with spatial resolution in the micrometer range was carried out to depict vascular responses of the cerebral artery and arterioles in rats and mice using a real-time imaging system and a third generation synchrotron radiation source at SPring-8. An X-ray direct-conversion type detector with 6- μm spatial resolution was developed for real-time biomedical imaging. The X-ray image is converted directly into an electrical signal in the photoconductive layer without image blurring. In synchrotron radiation radiography, a long source-to-object distance and a small source spot can produce high-resolution images. Microangiographic images were obtained without image blurring and were stored in a digital frame memory system with a 1024 \times 1024-pixel, 10-bit format. In imaging experiments, vasoconstriction and vasodilatation of small cerebral arteries were visualized in response to hypercapnia, hemorrhagic hypotension, and vasoactive agents after iodine contrast agent injection into the carotid artery.

I. INTRODUCTION

MEDICAL imaging using synchrotron radiation has been investigated since intravenous coronary angiography research was begun at the end of 1970s. This safe intravenous angiography technique is useful in place of conventional selective coronary arteriography. Research groups in several synchrotron radiation facilities have improved imaging systems for human studies [1–6]. In addition to intravenous coronary angiography, an intra-arterial microangiography system was developed at the Photon Factory for laboratory animal studies to obtain high-resolution images of small blood vessels: the penetrating transmural coronary arteries [7] and vasodilatation of the arterial circle of cerebrum and its branches [8] in dogs, collateral microvessels following therapeutic angiogenesis in rats [9], and tumor-derived angiogenic vessels in mice [10].

In place of synchrotron radiation, an X-ray generator with a small focal spot has been employed for microangiography using an X-ray direct-conversion type VIDICON camera as a two-dimensional high-resolution detector [11,12]. However, the X-ray generator system has limited capability for

displaying images of blood vessels smaller than 50- μm diameter: images cannot be magnified without focal-spot blurring. Furthermore, a conventional diagnostic angiography system incorporating an X-ray image intensifier and a video camera is not intended for detection of vessels smaller than 200 μm diameter because it is designed for large-field digital angiographic imaging with a 1024 \times 1024-pixel format [7].

At SPring-8, a digital microangiography system with spatial resolution as high as 6 μm was developed using an X-ray direct-conversion type detector incorporating an X-ray SATICON pickup tube for depiction of tumor-derived angiogenic vessels in a rabbit model of cancer [13]. The imaging system was also applied to clarify the basal tone and microvascular reactivity in endothelial NO synthase (eNOS)-overexpressing transgenic mice [14].

For this study, we performed cerebral microangiography in rats and mice and particularly undertook radiographical evaluation of changes in small arteries that had not been observed previously. Small arteries branching directly from major trunk vessels, known as perforators, supply arterial blood to the basal nucleus. These perforators are important arteries when considering the effects of systemic disease on cerebral vessels. Vasoconstriction and vasodilatation of the small cerebral arteries were visualized in response to hypercapnia, hemorrhagic hypotension, and vasoactive agents after injection of iodine contrast agent into the carotid artery [15,16].

II. IMAGING SYSTEM

A. Synchrotron Radiation

A useful source of synchrotron radiation is a storage ring, which maintains an electron beam at relativistic speeds in a closed trajectory for many hours using bending magnets. By bending the path of electrons at relativistic speeds, X-rays are emitted at each bending magnet in a direction tangential to beam trajectory. Fig. 1 shows an experimental arrangement at the SPring-8 BL28B2 beamline for X-ray imaging using monochromatic synchrotron radiation X-rays. Microangiographic imaging for depiction of tumor-derived angiogenic vessels in rabbits [13] and microvascular reactivity in transgenic mice [14] were performed at the BL20B2 beamline. Cerebral microangiographic imaging, however, was carried out at the BL28B2 because this beamline, employing a single crystal monochromator,

Manuscript received March 29, 2007. This study was supported in part by a grant-in-aid for scientific research ((C) (18500359)) to K. Umetani from the Ministry of Education, Science, Sports, and Culture of Japan.

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produces higher-flux X-rays than those at the BL20B2, which uses a double-crystal monochromator.

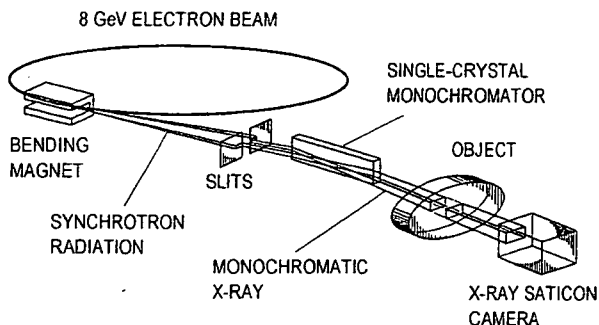


Fig. 1. An illustration of an experimental arrangement for synchrotron radiation microangiography.

Synchrotron radiation generated by bending magnets produces a fan-shaped beam and has a broad and continuous spectrum from infrared to the X-ray region. The single crystal monochromator selects a single energy of synchrotron radiation. Consequently, X-rays with a small energy bandwidth are used for imaging. A rotating-disk X-ray shutter, which is situated between the monochromator and the object, produces pulsed monochromatic X-rays (Fig. 1). The shutter consists of two disks with radial slots rotating about an axis parallel to the X-ray beam. The radial slot width can be changed to adjust the duration of X-ray pulses according to the rotation of one disk with another one. A large amount of disk metal is used to block the beam, resulting in considerably higher attenuation of X-rays, while maintaining low inertia in the disks. The disks rotate to match timing with synchronous signals of a video camera.

Pulsed monochromatic X-rays transmitted through the object are detected using the X-ray direct-conversion type detector, which incorporates the X-ray SATICON pickup tube. The distance between the point-source in the bending magnet and the detector was about 46 m. A nearly parallel X-ray beam was used for imaging without image blur because of the small size X-ray source and the extremely long source-to-object distance. The storage ring was operated at 8 GeV electron beam energy and the beam current was 100 mA. Monochromatic X-ray energy was adjusted to 33.2 keV, which was slightly above the iodine K-edge energy to produce the highest contrast image of the iodine contrast agent. X-ray flux at the object position was ca. 1×10^{10} photons/mm²/s in imaging experiments.

B. Real-Time Detector

The camera system shown in Fig. 2 comprises a camera head incorporating the X-ray SATICON pickup tube and a camera control unit with an analog-to-digital converter for digital signal output. X-rays enter the SATICON tube through an aluminum window. Fig. 3 shows that absorbed X-rays in

the photoconductive layer are converted directly into electron-hole pairs. Charge carriers generated by X-rays are transported across the photoconductive layer by an electric field. Then, a charge-density pattern is formed on the photoconductive layer surface. The electrostatic image on the surface is read out by a scanning beam of low-velocity electrons to produce a video signal. In Fig. 3, X-ray absorption depends on the photoconductive layer thickness. Thickness of amorphous selenium is 25 μm , yielding a detection efficiency of 14.2% for 33.2 keV X-rays.

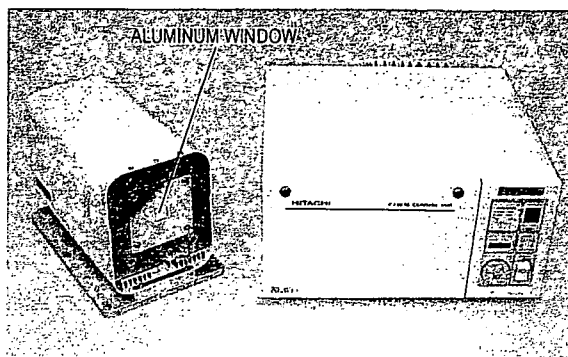


Fig. 2. Photograph of the X-ray SATICON camera system consisting of a camera head (left) and a camera control unit (right).

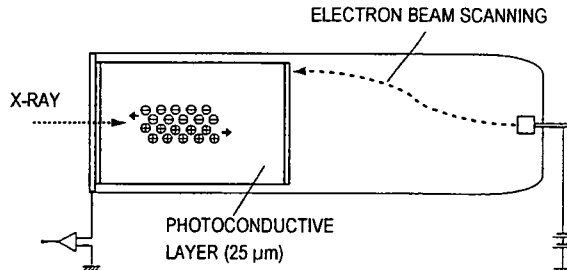


Fig. 3. Schematic cross-section of the SATICON tube. The tube is a glass cylinder maintained under vacuum; the front end of the tube is a flat plate with a diameter of 1 inch, the inside of which is coated with a 25- μm photoconductive material.

In a normal imaging mode with the detector's input field of view of 9.5 mm \times 9.5 mm, an equivalent pixel size is 9.5 μm in the case of a 1024 \times 1024-pixel format. On the other hand, in zoom imaging modes, the input fields are, respectively, 7.0 mm \times 7.0 mm and 4.5 mm \times 4.5 mm with pixel size of 7.0 and 4.5 μm . The camera can take sequential images at a maximum speed of 30 images per second. Then the image signals are converted to digital data with the 1024 \times 1024-pixel and 10-bit format using an analog-to-digital converter installed in the camera control unit shown in Fig. 2. Digital images are stored in 2 GB RAM of a custom-designed frame memory system after analog-to-digital conversion. Image storage is synchronized with electron-beam scanning

in the pickup tube. A personal computer system controls the entire imaging system, including the camera control unit and the frame memory system.

C. Microangiography

Performance of the direct-conversion type detector was evaluated by taking images of a custom-designed gold resolution chart. The chart thickness was 5 μm ; the bar pattern widths were 5.0–12.1 μm . After that, male 6-month-old rats weighing 450–600 g and male 10–12-week-old mice weighing 26–29 g were used for microangiographic imaging experiments.

The animals were anesthetized using sodium pentobarbital intraperitoneally. The right external carotid artery was cannulated in the retrograde direction, with the top of the cannula placed close to the common carotid artery so that all the injected contrast agent went into the common carotid artery without affecting the normal blood flow in the internal carotid artery. The rats and mice were held rigidly on a frame with a specially shaped head holder and ear bars. The frame was then fixed vertically to the X-ray beam to obtain axial views of the cerebral arteries.

All animal experiments conformed to the SPring-8 Guide for Care and Use of Laboratory Animals, and all animal experiments were conducted according to the guidelines for animal experiments at Kobe University Graduate School of Medicine.

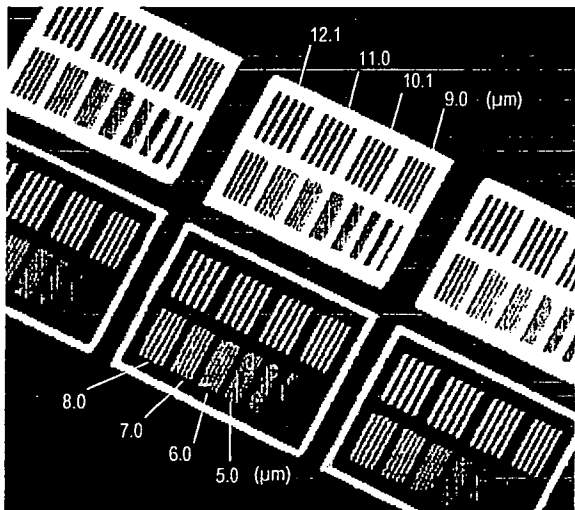


Fig. 4. X-ray image of the central area of the 5- μm -thick chart. Numerical values show bar widths. The limiting spatial resolution is around 6.0 μm .

III. RESULTS AND DISCUSSION

An X-ray image of the chart with micrometer-scale bar patterns was obtained in the zoom-imaging mode of the detector with the 4.5 mm \times 4.5 mm field of view at an X-ray

energy of 20.0 keV. Fig. 4 shows an image of the 5- μm -thick chart; the 6.0- μm -wide bars are visible. The limiting spatial resolution is about 6.0 μm in the zoom imaging mode. The 6.0 μm bar width is comparable to the capillary blood vessels' size. Images of the capillaries are obtainable at an X-ray energy of 33.2 keV just above the iodine K-edge energy to produce the highest contrast image of the iodine contrast agent if capillaries are opacified using a high-density contrast agent. However, the contrast agent is diluted substantially in the blood flow.

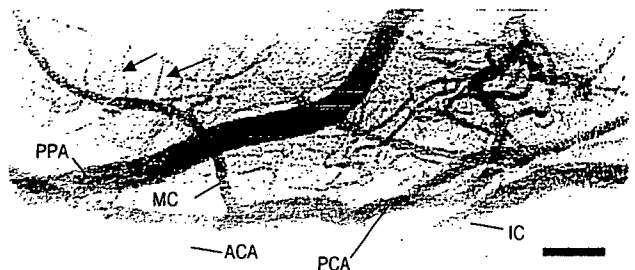


Fig. 5. Synchrotron radiation angiography of a rat showing the anatomy of the cerebral arteries in the brain hemisphere. Black arrows indicate perforating arteries; MCA, middle cerebral artery; ICA, internal carotid artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; PPA, pterygopalatine artery. Selective imaging was performed in the ICA territory. The scale bar shows 1 mm.

Angiography was performed selectively in the internal carotid artery (ICA) territory. A representative image of the rat cerebral arteries in Fig. 5 was obtained in the normal imaging mode of the detector with the 9.5 mm \times 9.5 mm field of view. The smallest detectable vessels were branches of the middle cerebral artery (MCA), which were ca. 30- μm diameter. We confirmed the right ICA, the anterior cerebral artery (ACA), the posterior cerebral artery (PCA), and the pterygopalatine artery (PPA) entirely. We specifically examined the large perforators, which were mostly found arising from the middle cerebral artery trunk. Vasoconstriction and vasodilatation of the large perforators were visualized in response to hypercapnia, hemorrhagic hypotension, and vasoactive agents after iodine contrast agent injection into the carotid artery [15,16].

Fig. 5 depicts a summation result image of 10 consecutive frames in the microangiographic sequence. Original frames, however, were obtained at a rate of 30 frames per second. Image summation was necessary to increase the signal-to-noise ratio and to detect small blood vessels. Furthermore, 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 raw images taken after injection to eliminate the superimposed background structure.

We developed a novel cerebral angiography procedure for rats and mice using monochromatic synchrotron radiation X-rays and obtained images of the cerebral perforating arteries for the first time. These results demonstrate the importance of hemodynamic studies in deep brain vessels that had previously been unattainable.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Tadaaki Hirai and Mr. Toshiaki Kawai of Hamamatsu Photonics K.K., Mr. Sadao Takahashi of Hitachi Denshi Techno-System, Ltd., and Mr. Norio Iwanaga of Zenisu Keisoku Inc. for development of the camera and frame memory system. Synchrotron radiation experiments were performed at the SPring-8 BL28B2 beamline with the approval of the Japan Synchrotron Radiation Research Institute (Acceptance Nos. 2002A0079, 2002B0312, 2004A0313, 2004B0767, 2005A0524, and 2005B0675).

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Microscopic X-ray imaging system for biomedical applications using synchrotron radiation

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ABSTRACT

An X-ray direct-conversion type detector with a spatial resolution of 10-11 μm was developed for real-time biomedical imaging. The detector incorporates the X-ray SATICON pickup tube with a photoconductive target layer of amorphous selenium. For high-resolution imaging, the X-ray image is directly converted into an electric signal in the photoconductive layer without image blur. Microangiography experiments were carried out for depicting angiogenic vessels in a rabbit model of cancer using the direct-conversion detector and a third generation synchrotron radiation source at SPring-8. In synchrotron radiation radiography, a long source-to-object distance and a small source spot can produce high-resolution images. After transplantation of cancer cells into the rabbit auricle, small tumor blood vessels with diameters of 20-30 μm in an immature vascular network produced by angiogenesis were visualized by contrast material injection into the auricular artery at a monochromatic X-ray energy of 33.2 keV just above the iodine K-edge energy. The synchrotron radiation system is a useful tool to evaluate the micro-angioarchitecture of malignant tumors in animal models of cancer for *in vivo* preclinical studies.

Keywords: X-ray SATICON, microangiography, synchrotron radiation, monochromatic X-ray, preclinical study, angiogenesis, malignant tumor

1. INTRODUCTION

Medical imaging using synchrotron radiation has been investigated since dual-energy subtraction coronary angiography was started in 1979 [1]. Differential X-ray absorption by iodine contrast material at energies just above and just below the iodine K-edge energy has been used for high-sensitivity imaging of the diluted contrast material. Research groups at several synchrotron radiation facilities have improved dual-energy imaging systems for human studies [2-5]. Such systems are composed of two single-dimensional line detectors and two fan beams, one just above and the other just below the iodine K-edge energy. For a two-dimensional image, a patient is scanned vertically through the two fan beams and two line detectors.

In Japan, the first human study of intravenous coronary angiography was performed using a combination of one single-energy beam and one two-dimensional detector [6]. In this approach, a monochromatic X-ray beam with a large cross-section size and energies just above the iodine K-edge energy was used to produce two-dimensional images without a patient scan. The two-dimensional detector approach has also been applied to intra-arterial microangiography [7]. Aside from coronary angiography for human studies, the intra-arterial microangiography system has been investigated as a preclinical imaging tool for circulatory disorders and early stage malignant tumors using laboratory animals [8].

An X-ray generator with a small focal spot has been employed for conventional microangiography combined with an X-ray direct-conversion type VIDICON camera as a two-dimensional high-resolution detector [9]. The X-ray generator system, however, has limitations when providing images of small blood vessels with diameters of less than 50 μm because images cannot be magnified without focal spot blurring. In addition, a diagnostic angiography system incorporating an X-ray image intensifier and a video camera is not intended for detection of small vessels having diameters of 200 μm or less because it is designed for large-field digital angiographic imaging with a 1024 \times 1024-pixel format.

At SPring-8, the digital microangiography system with a spatial resolution down to 10-11 μm was developed using a direct-conversion type detector incorporating the X-ray SATICON pickup tube [10]. Microangiography experiments were carried out for depicting angiogenic vessels in a rabbit model of cancer at a monochromatic X-ray energy of 33.2

keV just above the iodine K-edge energy. Sequential images of tumor blood vessels induced by transplanted VX2 cancer cells in the rabbit auricle were obtained after iodine contrast material injection to the auricular artery and then the tumor-induced small blood vessels with diameters of 20-30 μm were visualized.

2. METHODS

2.1 Image detector

The X-ray direct-conversion type SATICON tube consists of a photoconductive target layer of amorphous selenium in Fig. 1. Absorbed X-rays in the photoconductive layer of the tube are directly converted into electron-hole pairs. Charge carriers generated by X-rays are transported across the photoconductive layer by an electric field. Then, a charge-density pattern is formed on the photoconductive layer surface. The electrostatic image on the surface is read out by a scanning beam of low velocity electrons to produce a video signal.

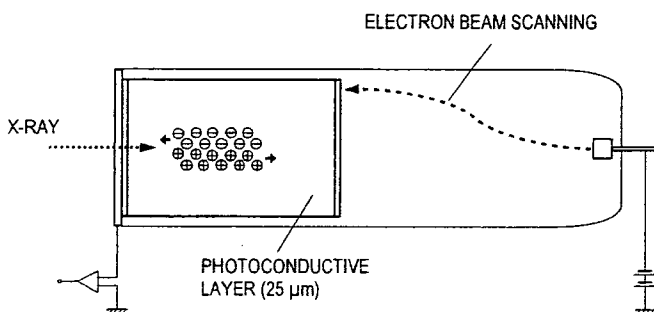


Fig. 1. Schematic cross-section of the X-ray direct-conversion type pickup tube consisting of a photoconductive target layer.

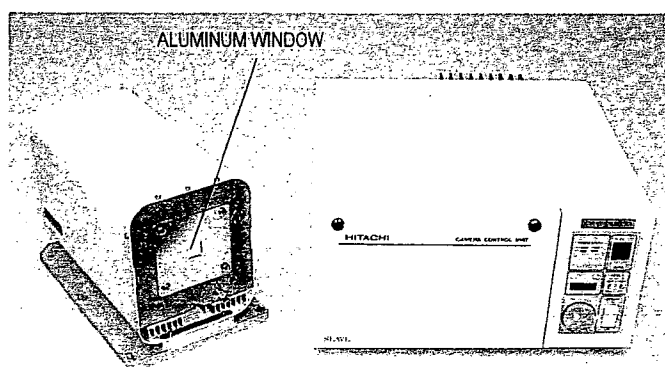


Fig. 2. Photograph of the SATICON camera system: a camera head (left) and a camera control unit (right).

The X-ray direct-conversion type pickup tube was developed in the 1960's using a PbO photoconductive layer. The image detector was combined with a high-power X-ray generator for observation of real-time topography images in material science fields. Photoconductive materials were improved for high-resolution imaging. In place of the PbO photoconductive layer, the X-ray SATICON tube with an amorphous photoconductive Se-As alloy target was developed for use in synchrotron radiation experiments of live topography at the Photon Factory [11]. After that, the direct-conversion camera was much improved through introduction of a high-definition television (HDTV) system, which increased resolution to 1050 scanning lines.

The new X-ray SATICON camera in Fig. 2 for biomedical imaging has identical resolution of 1050 scanning lines. The camera can take images at a maximum speed of 30 images/s. Sequential images were obtained with an input field of view of 9.5 mm × 9.5 mm. Digital images were stored in a digital-image acquisition system after analog-to-digital conversion, synchronizing timing with electron beam scanning in the pickup tube. An equivalent pixel size projected onto the input window was 9.5 μm in the case of the 1024 × 1024-pixel format.

2.2 Imaging system

The medium length bending-magnet beamline BL20B2 at SPring-8 in Fig. 3 is dedicated mainly to basic medical science using biological specimens and small animals. Synchrotron radiation has a broad and continuous spectrum from the infrared to the X-ray region. A double crystal monochromator selects a single energy of synchrotron radiation, and X-rays with a small energy bandwidth are used for imaging. In Fig. 3, the storage ring and the beamline consist of vacuum tubes. The monochromatic X-ray comes out from the vacuum tube into the atmosphere by passing through a beryllium window that is located in the down stream of vertical slits. The X-rays transmitted through the object are detected by the imaging detector.

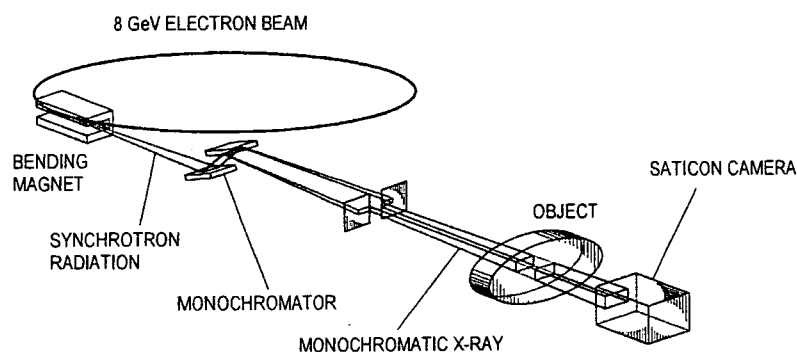


Fig. 3. Illustration of the experimental arrangement at SPring-8 BL20B2 beamline.

The distance between the source point in the bending magnet and the detector was about 210 meters. The nearly parallel X-ray beam was used for imaging without geometrical unsharpness because of the small size of the X-ray source and the very long source-to-object distance. The storage ring was operated at 8 GeV electron beam energy and the beam current was 100 mA. The energy of monochromatic X-ray was adjusted to 33.2 keV just above the iodine K-edge energy to produce the highest contrast image of the iodine contrast material. X-ray flux at the object position was around 4×10^7 photons/mm²/s.

2.3 In vivo imaging

We conducted an experimental study using rabbit VX2 carcinoma in the rabbit auricle to evaluate growth of tumor-induced small blood vessels. VX2 carcinoma is a rapidly growing solid tumor. The tumor cells are easily transplanted into other rabbits. Japanese white rabbits weighing about 3 kg were used for microangiographic imaging. The rabbits were anesthetized with an intravenous injection of pentobarbital sodium. Then, the VX2 cancer cells were subcutaneously injected into the rabbit auricle.

Microangiography experiments were performed at one, three, and seven days after transplantation. After the rabbit was similarly anesthetized, an intracatheter was inserted into the auricular artery. The rabbit was placed in lateral posture on a stage and the auricle was put in the X-ray field of view. The contrast material used for imaging was 0.8 ml per one injection at a speed of 0.2 ml/s. The contrast material was injected into the auricular artery by an auto injector. The timing of the injection was controlled by the digital-image acquisition system.

Microangiographic images were obtained sequentially at a speed of 5 images/s. The exposure time per image was 0.2 s. Images were stored in the digital-image acquisition system with the 10-bit resolution and 1024 × 1024-pixel format. The

total number of acquired frames was 50 and the total imaging time was 10 s. The animal protocol used was approved by the Animal Care and Use Committee of Kawasaki Medical School. All of our experiments on animals conformed to the SPring-8 Guide for the Care and Use of Laboratory Animals.

3. RESULTS

Performance of the direct-conversion detector was evaluated by taking an image of a spatial resolution chart. We used a specially ordered gold bar chart with up to 8.8- μm -width patterns. The thickness of the gold chart was 23 μm and pattern widths were 8.8-31.4 μm . The limiting spatial resolution was 10-11 μm and nearly equal to 9.5 μm , which is equivalent to Nyquist frequency [10]. A conventional angiography system using an X-ray image intensifier and a video camera has the limiting spatial resolution of around 100 and 200 μm when a circular input field of the X-ray image intensifier is 4.5 and 9 inches in diameter, respectively. Our small-field imaging system using the direct-conversion detector achieved the limiting spatial resolution of 10-11 μm , which is 10-20 times higher resolution than that of the conventional diagnostic system designed for large-field digital angiographic imaging.

A tumor stimulates growth of small blood vessels for feeding the tumor itself. It has been proposed that tumor-induced small blood vessels are an intrinsic part of tumor development and progression. The growth rate of tumors is slow before blood vessel formation and rapid after vessel formation [12,13]. Radiographic depiction of tumor-induced small vessels that feed a space-occupying lesion is a useful tool for preclinical studies of malignant tumors.

Representative images from a microangiographic sequence is shown in Fig. 4. Sequential images of the transplanted tumor were taken at three days after transplantation by the direct-conversion detector after contrast material injection into the auricular artery. Each image in Fig. 4 is a summation result of four consecutive frames in the angiographic sequence. The image summation was needed to reduce quantum noise and to visualize small blood vessels. A temporal subtraction operation was also performed for flat field correction. The summation of four consecutive frames, which were taken just before the contrast material injection, was subtracted from the summation of four iodine injection frames to eliminate the superimposed background structure.

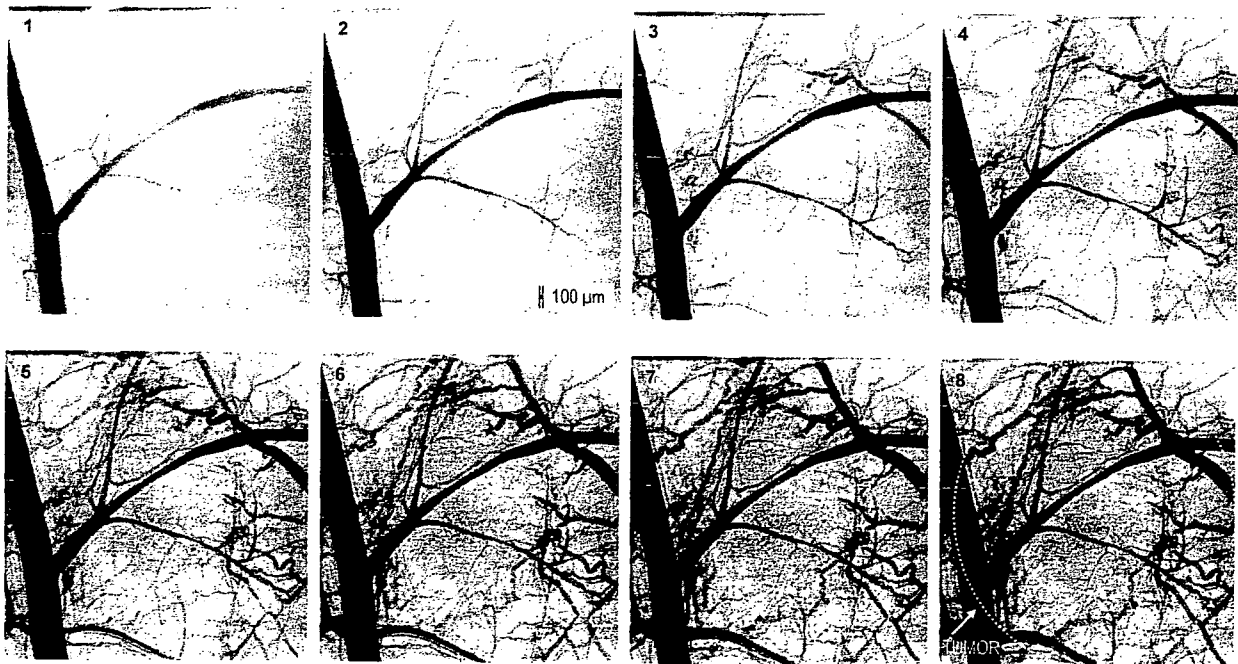


Fig. 4. Sequential images of tumor blood vessels (dotted circle) after iodine injection at three days after tumor transplantation.

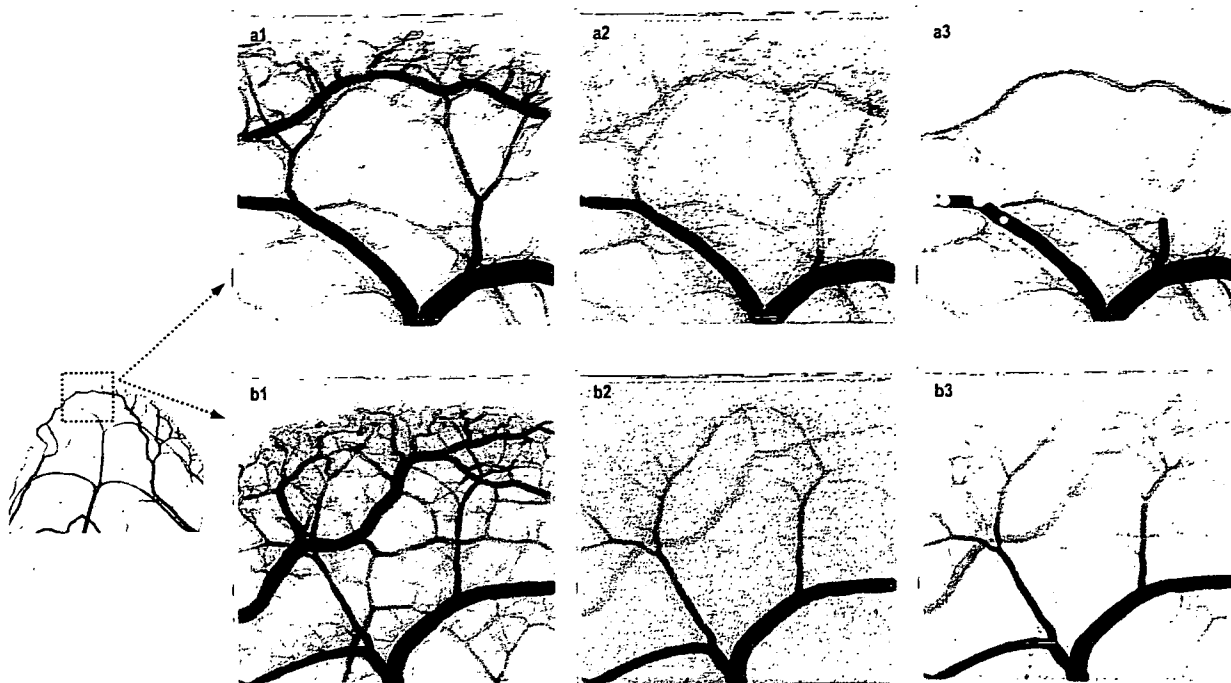


Fig. 5. Two sets of normal blood vessel images in the marginal region of the auricles showing the effects of transcatheter arterial embolization: (a1 and b1) before embolization, (a2 and b2) under embolization, and (a3 and b3) after embolization. Embolization materials are particles with diameters of (a1-a3) 100-300 μm and (b1-b3) 90-180 μm .

Small particles clog small blood vessels and block blood flow to the tumor. Arterial embolization is the selective blocking of arteries by introducing embolization materials to treat certain types of cancer. In this procedure, a mixture of the contrast material and a synthetic material that is designed to occlude the blood vessels was injected for blocking and imaging. We tested two types of particulate embolization materials: polyvinyl alcohol (PVA) particles and trisacryl gelatin microspheres (TGM) with respective diameters of 100-300 μm and 90-180 μm . Figure 5 shows the effect of transcatheter arterial embolization on normal blood vessels in marginal region of the rabbit auricles.

Results of testing the particulate materials with diameters of about 100-300 μm (PVA) and 90-180 μm (TGM) are shown respectively in Fig. 5 (top row) and Fig. 5 (bottom row). The first images were obtained before the embolization procedure (left column), the second ones during the procedure (center column), and the third ones after the procedure (right column). The first and third images in Fig. 5 were taken using a conventional injection material comprising only the contrast material, whereas the second images were taken using a mixture of the contrast material and the embolization material under the procedure.

4. DISCUSSION

At three days after transplantation, the tumor size was 5-6 mm in diameter. In Fig. 4, a dotted circle shows the immature vascular network in the transplanted solid tumor. The first-third images in Fig. 4 mainly show the network of tumor-feeding arteries originating from the first-order branch of the auricular artery; some veins are slightly visible in the top right of the image because of arteriovenous malformations. Small blood vessels of 20-30 μm diameter are depicted in these image. The number of vessels in the solid tumor increased remarkably compared with that of the normal area in the auricle. The tumor derived blood vessels exhibit many irregular veins, which have the characteristic appearance of an undulating shape lacking side branches. The vascular network also shows abnormal staining.

The X-ray generator system with the small focal spot can visualize images of small blood vessels with diameters of more than 50 μm . The synchrotron radiation system, however, can provide images of blood vessels with diameters of 20-30 μm , which is 2-3 times higher detection than that of the X-ray generator system. The detection can improve by the operation of the camera with a zoom imaging mode [10].

In testing of the embolization materials in transcatheter arterial embolization, microangiographic images show the microscopic mechanism of the blood vessel occlusion. In particular, Fig. 5 (top right panel) shows individual PVA particles with 100–300 μm diameter wedging in the auricular artery. Images also illustrate that the levels of occlusion and collateral blood supply were controlled by particle sizes. In Fig. 5 (top right panel), almost all small arteries were blocked with PVA particles (100–300 μm), whereas TGM particles (90–180 μm) did not clog arteries with diameters of more than 100–200 μm in Fig. 5 (bottom right panel).

5. CONCLUSIONS

X-ray imaging with a spatial resolution of 10-11 μm was carried out using the direct-conversion detector and the nearly parallel X-ray beam provided at SPring-8. In synchrotron radiation radiography, the long source-to-object distance and the small source spot can overcome geometric unsharpness. The imaging system can visualize the immature vascular network in the transplanted malignant tumor, and it is useful for the depiction and quantification of angiogenic vessels in the rabbit model of cancer. Furthermore, microangiographic images show the microscopic mechanism of the blood vessel occlusion in testing of the embolization materials in transcatheter arterial embolization. The synchrotron radiation imaging system will be a useful tool for evaluating the micro-angioarchitecture of malignant tumors for preclinical studies.

Before the 1990s, video cameras consisted of a pickup tube to capture images and then the pickup tube has largely been replaced by a charge-coupled device (CCD). Some pickup tube cameras such as the X-ray SATICON camera are still used for special purpose applications in scientific and industrial fields. However, all of the present pickup tubes will be replaced by solid-state image sensors in the near future. The 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 field-of-view limit is imposed by a diameter of the glass cylinder, a standard size of one inch. If an X-ray direct-conversion type solid-state image sensor with spatial resolution in the micrometer range is produced for special purpose applications, the sensor will be able to remove restrictions on the field of view, while retaining temporal and spatial resolution.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Tadaaki Hirai and Mr. Toshiaki Kawai of Hamamatsu Photonics K.K. for development of the X-ray SATICON tube. The authors also thank Mr. Sadao Takahashi of Hitachi Denshi Techno-System, Ltd. for providing the camera system. The authors also thank Mr. Norio Iwanaga of Zenisu Keisoku Inc. for development of the frame memory system.

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Changes in macrovessel pulmonary blood flow distribution following chronic hypoxia: assessed using synchrotron radiation microangiography

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Submitted 10 June 2007; accepted in final form 22 October 2007

Schwenke DO, Pearson JT, Kangawa K, Umetani K, Shirai M. Changes in macrovessel pulmonary blood flow distribution following chronic hypoxia: assessed using synchrotron radiation microangiography. *J Appl Physiol* 104: 88–96, 2008. First published October 25, 2007; doi:10.1152/jappphysiol.00610.2007.—Structural and functional changes of the pulmonary circulation, particularly during the pathogenesis of pulmonary arterial hypertension (PAH), remain to be fully elucidated. In this study, we utilized monochromatic synchrotron radiation (SR) microangiography to assess changes in pulmonary arteriole blood flow in the intact-chest rat after 4 wk of chronic hypoxia. Sprague-Dawley rats were exposed to normoxia (N-rats) or chronic hypoxia (10% O₂; CH-rats) for 28 days. Rats were anesthetized, and microangiography was performed on the left lung to assess 1) the branching distribution of pulmonary arteriole blood flow (internal diameter >80 μm) and 2) dynamic changes in vessel lumen diameter during acute hypoxic (8% O₂ for 4 min) pulmonary vasoconstriction (HPV) before and after β-adrenoceptor blockade (2 mg/kg iv propranolol). Using SR angiography, we observed that the number of opaque third- and fourth-generation vessels (100–300 μm) for CH-rats was significantly fewer than the number for N-rats. The magnitude of HPV was not different between CH-rats and N-rats. β-Adrenoceptor blockade accentuated the HPV in 200- to 300-μm vessels for CH-rats, but even more so in N-rats. However, in CH-rats, β-adrenoceptor blockade also accentuated the HPV in 100- to 200-μm vessels. In summary, we utilized SR to assess gross blood flow changes and functional changes (i.e., HPV) of the pulmonary circulation in PAH. These results highlight the benefits of SR for assessing pulmonary circulatory pathology. Of particular importance, future use of SR will provide an effective method for assessing potential therapeutic treatments for PAH.

pulmonary microvessels; hypoxia; intact chest; rat

ALVEOLAR HYPOXIA, A POTENTIAL adverse complication associated with numerous respiratory disorders, causes pulmonary vasoconstriction, which is reversible on reoxygenation. However, when sustained, the elevated shear stress within the pulmonary vasculature causes endothelial cell injury dysfunction (2), ultimately leading to the pathogenesis of pulmonary arterial hypertension (PAH). PAH is characterized by irreversible 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) (12, 23, 24, 31). The ensuing decrease in vessel internal caliber increases pulmonary vascular resistance and, consequently, increases the workload of the heart, enhances the

risk of heart failure, and is, therefore, closely associated with an increased mortality.

Several studies have indicated that the increase in pulmonary vascular resistance is further exacerbated during chronic hypoxia by a reduction in the number of perfused blood vessels within the pulmonary vascular bed, due to either vessel occlusion (i.e., extensive medial thickening) or the loss of vessels, also known as “rarefaction” or “pruning” of small vessels (8, 11, 25). More recent evidence, however, has cast doubt on this paradigm, with some reports now indicating that angiogenesis is evident within the pulmonary circulation during chronic hypoxia (13, 14, 16).

The underlying mechanisms governing the pathogenesis of PAH remain to be fully elucidated. One of the limitations in understanding the pathology of the lung has been the inability to clearly visualize blood flow within the pulmonary vascular bed. More conventional methods of assessing the vascular anatomy of the diseased lung necessitate removal of the lung from the animal (i.e., *in vitro*) for angiography (i.e., X-ray) or microsection analysis. Conventional angiography methods have considerable limitations in visualizing the vessels that are most susceptible to pathological changes, i.e., <200 μm (37, 40). Therefore, a technique for visualizing the pulmonary circulation within a closed-chest model, i.e., under intact neurohumoral regulation, is required to better understand the structural and functional changes *in vivo* and, ultimately, to assess specific treatments for lung disorders more reliably.

In a recent study, we (32) demonstrated the validity and accuracy of synchrotron radiation (SR) microangiography for visualizing pulmonary blood flow within vessels in a closed-chest rat model and for assessing dynamic changes in vessel caliber associated with acute hypoxic pulmonary vasoconstriction (HPV). Although resolution limitations of SR only enabled vessels with an internal diameter >80 μm to be visualized, many vessels that are susceptible to pathological changes during chronic hypoxia are well within the resolution capabilities of SR. In contrast to conventional angiography systems, SR is characterized by high brilliance and extreme collimation (36), 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 primary aim of this study was to utilize SR microangiography to effectively highlight changes in pulmonary blood flow

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distribution, following the pathogenesis of chronic hypoxia-induced PAH. Because of the resolution limitations of SR, we only assessed those pulmonary arterioles with an internal diameter $>80 \mu\text{m}$. The structural changes in the pulmonary circulation associated with chronic hypoxia have been shown to alter the functional properties of the pulmonary vasculature, in particular, the vasoconstriction response to acute hypoxia (i.e., HPV) (19, 22). This altered HPV has been attributed, at least in part, to the modulatory effects of the sympathetic nervous system on the pulmonary vasculature (33, 35). Therefore, we also aimed to utilize the high definition of SR to assess the adverse changes in pulmonary reactivity to acute hypoxia and, furthermore, to distinguish the local intrinsic response (i.e., HPV) from that of the extrinsic neural response (i.e., sympathetic modulation) by using propranolol to inhibit β -adrenoceptor activation.

MATERIALS AND METHODS

Animals. Experiments were conducted on 10 male Sprague-Dawley rats (10 wk old; ~ 220 – 320 g body wt). All rats were on a 12:12-h light-dark cycle at $25 \pm 1^\circ\text{C}$ and were provided with food and water ad libitum. Rats were housed in standard normoxic conditions ($n = 5$) or were continuously housed in a hypoxic chamber ($10 \pm 0.1\%$ O_2) for 4 wk ($n = 5$), except for a 10-min interval each day when the chamber was cleaned. The hypoxic gas mixture was prepared from N_2 (gas cylinders) and compressed air and was continuously delivered to the hypoxic chamber (30 liter capacity) at a flow rate of ~ 8 l/min. 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 ($\sim 15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ip). Throughout the experimental protocol, body temperature was maintained at 37°C using a rectal thermistor coupled with a thermostatically controlled heating pad.

The trachea was cannulated, and the lungs were ventilated with a rodent ventilator (SN-480-7; Shinano, Tokyo, Japan). The inspirate gas was enriched with O_2 ($\sim 50\%$ O_2), and the ventilator settings were adjusted (tidal volume ~ 3.5 ml; frequency of ~ 70 /min). A femoral artery and vein were cannulated for measurement of systemic arterial blood pressure (ABP) and drug administration, respectively. A 20-gauge BD Angiocath catheter (Becton Dickinson), with the tip at a 30-degree angle, was inserted into the jugular vein and advanced into the right ventricle for administering contrast agent and intermittently measuring right ventricular pressure (RVP).

The rat was securely fastened to a clear Perspex surgical plate, which had a single window opening 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 could pass perpendicular to the sagittal plane from anterior to posterior through the rat thorax and ultimately to a SATICON X-ray camera described below.

Microangiographic system. The pulmonary circulation was visualized with SR microangiography at the SPring-8 BL28B2 beam-line facility (Hyogo, Japan). The use of SR for visualizing the pulmonary microcirculation in the closed-chest rat has previously been described in detail (32).

In brief, SR has a broad and continuous spectrum from the infrared to the X-ray regions. A single crystal monochromator was used to select a single energy of SR, producing X-rays of a very narrow energy bandwidth for imaging. This SR system comprised a monochromatic 33.2-keV X-ray source, just above the iodine K-edge energy for maximal contrast.

X-rays transmitted through the rats were detected by an X-ray detector (Hitachi Denshi Techno-System, Tokyo, Japan) incorporat-

ing a SATICON X-ray pickup tube (Hamamatsu Photonics, Shizuoka, Japan). 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 $9.5\text{-}\mu\text{m}$ 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 $1,024 \times 1,024$ pixel format and 10-bit resolution.

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; Fig. 1). Subsequently, baseline heart rate (HR), RVP, and ABP data were collected. Immediately before vessels were imaged, 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/s). 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.

After baseline imaging was completed, rats were exposed to acute hypoxia (8% O_2 in N_2) for 4 min. During acute hypoxia, ABP, HR, and RVP data were continuously recorded until the 3rd min, 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 4th min of hypoxia.

After recovery from the acute hypoxic test, rats were administered the β -receptor blocker propranolol (2 mg/kg iv). After 10–15 min was allowed for all cardiovascular variables to stabilize, pulmonary microangiography was repeated before and after acute hypoxia.

Data acquisition and analysis. The RVP and ABP signals were detected by separate Deltran pressure transducers (Utah Medical Products), and the signals were relayed to PowerLab bridge amplifiers (ML117, ADInstruments) and then continuously sampled at 500 Hz with an eight-channel MacLab/8s interface hardware system (ADInstruments) and recorded on a Macintosh Power Book G4 using Chart software (version 5.0.1, ADInstruments). HR was derived from the arterial systolic peaks.

From the 2-s period of image collection, one frame per scan (one scan = 100 frames) was selected for image enhancement and analysis. Furthermore, only those frames recorded at, or near, end systole were used for assessing and comparing pulmonary vessel diameter between baseline and hypoxic conditions.

All imaged vessel branches were counted. Where possible, the widths of two to four vessels of each branching generation (2nd to 4th

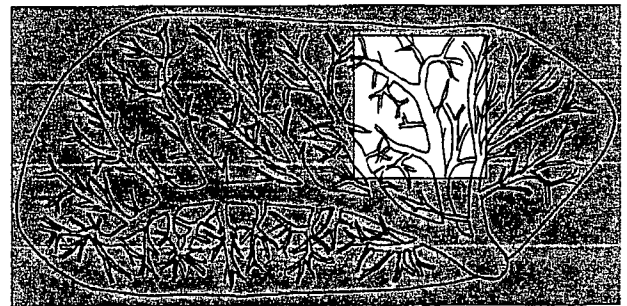


Fig. 1. A schematic drawing of the arterial circulation of the left lung in a rat. The square box represents the position of the 9.5×9.5 -mm imaging window used for microangiography of the pulmonary microvessels. [Reprinted from Schwenke et al. (32).]

generation) were measured to ensure that a wide variety of vessel sizes was selected from each frame. Vessels were categorized according to internal diameter: 100–200, 200–300, 300–500, and >500 μm . The internal diameter of individual vessels was measured before and after acute hypoxia, with and without β -receptor blockade (i.e., propranolol treatment).

Image analysis. The computer-imaging program Image Pro-plus (version 4.1, Media Cybernetics) was used to enhance the contrast and 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.

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 of the 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 spanning 10–40 pixels on either side in the direction perpendicular to the vessel (see Fig. 2). The first edge of the vessel was determined as the pixel at which intensity decreased by 1.5 standard deviations 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. Of the total number of vessels analyzed (91 vessels from normoxic and chronic hypoxic rats; see RESULTS), 50 vessels from various branching generations were randomly selected from the appropriate angiograph image for analyses by a second observer. The value for each vessel width that was analyzed by both observers was highly reproducible. Regression analysis indicated that the measurements from both observers were highly correlated ($y = 6.752 + 0.983x$, $r^2 = 0.975$) and had an average difference of $6 \pm 3 \mu\text{m}$ (for 100- to 200- μm vessels) to $9 \pm 4 \mu\text{m}$ (for 300–500 μm).

Evaluation of accuracy of measurement. We (32) have previously described in detail the method of evaluating the accuracy of measurement. In brief, we estimated a margin of error for detecting the edge of a vessel by assessing pixel variability of the reference wire (known diameter of 100 μm). Before commencing the study, 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 $\sim 5 \mu\text{m}$ (range of 97.4–102.7 μm).

In this study, 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 the vessel width measurement. We previously performed preliminary phantom measurements to assess the relationship between X-ray absorption and iodine concentration. The phantom measurements consisted of filling nine tubes, with an internal diameter of 200 μm , with various concentrations of iodinated contrast material ranging from pure agent (370 mg/cm^3) to distilled water (i.e., 0 mg/cm^3). The line-profile function of Image Pro-plus was used to assess the magnitude of absorption (i.e., brightness) for each concentration of iodinated contrast material (Fig. 2).

The widths of tubes containing an iodine concentration between 32 and 370 mg/cm^3 could be accurately measured with a small margin of error, e.g., the width of the tube containing 32 mg/cm^3 iodine was measured at 40 points and had a 95% confidence interval of 196.84–

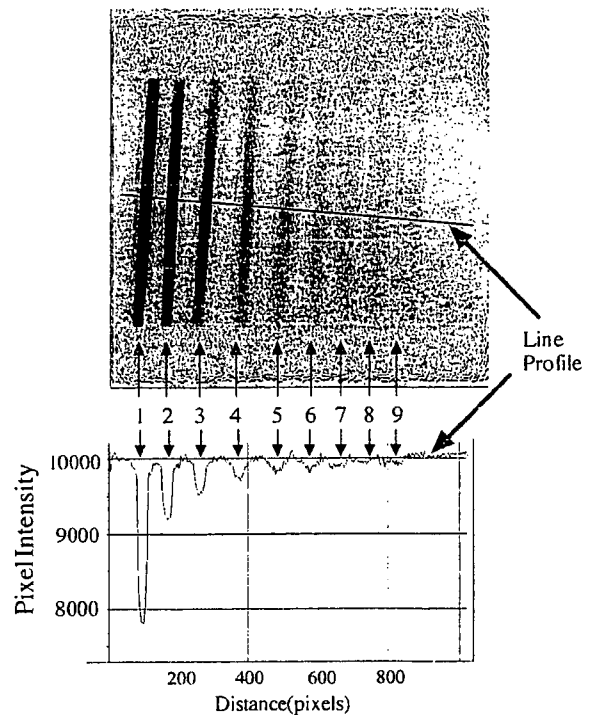


Fig. 2. *Top*: an angiograph image obtained from a phantom study that assessed X-ray absorption of 9 tubes containing 1) Iopamiron 370 (370 mg/cm^3 iodine; Nihon Schering), 2) 127 mg/cm^3 iodine (diluted contrast material with distilled water), 3) 64 mg/cm^3 iodine, 4) 32 mg/cm^3 iodine, 5) 12.8 mg/cm^3 iodine, 6) 6.4 mg/cm^3 iodine, 7) 1.28 mg/cm^3 iodine, 8) distilled water, and 9) air. A 5-mm-thick aluminum mask was overlaid to simulate attenuation by the animal body. Concentrations ranging from 32 to 127 mg/cm^3 of iodine simulate typical *in vivo* conditions for intra-arterial injection techniques. *Bottom*: computerized line profile (representing the solid line transecting all 9 tubes in *top*) was performed to show that changes in pixel intensity (i.e., brightness), which reflect the magnitude of absorption, are dependent on iodine concentration.

203.26 μm . This is a margin of error similar to that obtained for multiple measurements of the tungsten reference wire.

These results show that measurements of 200 μm in diameter can be made precisely for those vessels with an iodine concentration of >32 mg/cm^3 . Moreover, it seems reasonable to suppose that measurements of $\sim 100 \mu\text{m}$ in diameter can be made with the required precision for those vessels with an iodine concentration of 64 mg/cm^3 or greater.

Statistical analysis. All statistical analyses were conducted with Statview (version 5.01; SAS Institute). All results are presented as means \pm SE. Two-way ANOVA (repeated measures) was used to test whether propranolol significantly altered the dynamic pulmonary vasoconstriction response to acute hypoxia. One-way ANOVA (factorial) was used to test for differences in 1) vessel caliber during normoxia and acute hypoxia and 2) baseline values for normoxic rats (N-rats) compared with chronic hypoxic rats (CH-rats). Where statistical significance was reached, post hoc analyses were incorporated using the paired or unpaired *t*-test with the Dunnett's correction for multiple comparisons. A *P* value ≤ 0.05 was predetermined as the level of significance for all statistical analysis.

RESULTS

Baseline. Rats had an initial body weight of $\sim 285 \text{ g}$ before being placed into a normoxic (N-rats) or hypoxic chamber (CH-rats) for 4 wk. The gain in body weight of CH-rats ($\sim 20\%$

increase) was significantly lower ($P < 0.01$) than that for N-rats (~74% increase) over the 4-wk period. Chronic hypoxia induced PAH (see Table 1), as demonstrated by our observation that systolic RVP of CH-rats was ~120% higher than that of N-rats ($P < 0.01$). Chronic hypoxia did not significantly alter mean ABP (MABP) or HR.

Using SR, we were able to clearly visualize the blood flow of pulmonary microvessels ($>80 \mu\text{m}$) in the left lung of both N-rats and CH-rats. The typical branching pattern of the pulmonary circulation from the main axial artery of the left lobe to the fourth generation of branching (within the 9.5×9.5 mm imaging window) of a N-rat and a CH-rat is presented below (see Fig. 4).

The internal diameter of 52 vessels was measured in five N-rats: 24 vessels with a diameter between 100 and 200 μm , 15 vessels with a diameter between 200 and 300 μm , 7 vessels with a diameter between 300 and 500 μm , and 6 vessels with a diameter $>500 \mu\text{m}$. In comparison, CH-rats had comparatively fewer vessels (Fig. 3A); thus the internal diameter of 39 vessels was measured in five CH-rats: 15 vessels with a diameter between 100 and 200 μm , 10 vessels with a diameter between 200 and 300 μm , 7 vessels with a diameter between 300 and 500 μm , and 7 vessels with a diameter $>500 \mu\text{m}$.

The total number of vessel branches visible within each baseline image (i.e., 9.5×9.5 -mm imaging window) was counted. As illustrated in Fig. 3A, the number of opaque third- and fourth-generation vessels for CH-rats (9 ± 1 and 16 ± 2 vessels, respectively) was significantly fewer than the number for N-rats (14 ± 1 and 30 ± 2 vessels, respectively; $P < 0.05$). The numbers of first- and second-generation branches were not significantly different between N-rats and CH-rats (2 and 4 or 5, respectively).

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 (Fig. 3B). However, often more than one size category could be found within one branching generation. For example, the third generation of branching (135–290 μm) comprised vessels of the 100- to 200- μm and 200- to 300- μm categories (Fig. 3B). The internal diameter of the first-generation branch in CH-rats ($740 \pm 53 \mu\text{m}$) was significantly larger than that shown in N-rats ($511 \pm 57 \mu\text{m}$; $P < 0.05$), possibly because of the higher distending pressure (i.e., pulmonary arterial pressure) for CH-rats. Vessel caliber

Table 1. Systolic RVP, MABP, and HR data of anesthetized N-rats and CH-rats before and after propranolol administration

	Systolic RVP, mmHg	MABP, mmHg	HR, beats/min
<i>Saline</i>			
N-rats	24.3 \pm 1.0	103 \pm 7	338 \pm 13
CH-rats	53.1 \pm 2.0†	118 \pm 4	353 \pm 13
<i>Propranolol (2 mg/kg iv)</i>			
N-rats	27.3 \pm 1.4	105 \pm 6	319 \pm 4
CH-rats	54.2 \pm 1.7†	136 \pm 3*†	294 \pm 5*†

Values are means \pm SE. N-rats, normoxic rats ($n = 5$); CH-rats, chronic hypoxic rats ($n = 5$); RVP, right ventricular pressure; MABP, mean arterial blood pressure; HR, heart rate. *Significant difference between saline and propranolol ($P < 0.01$). †Significant difference between N-rats and CH-rats ($P < 0.01$).

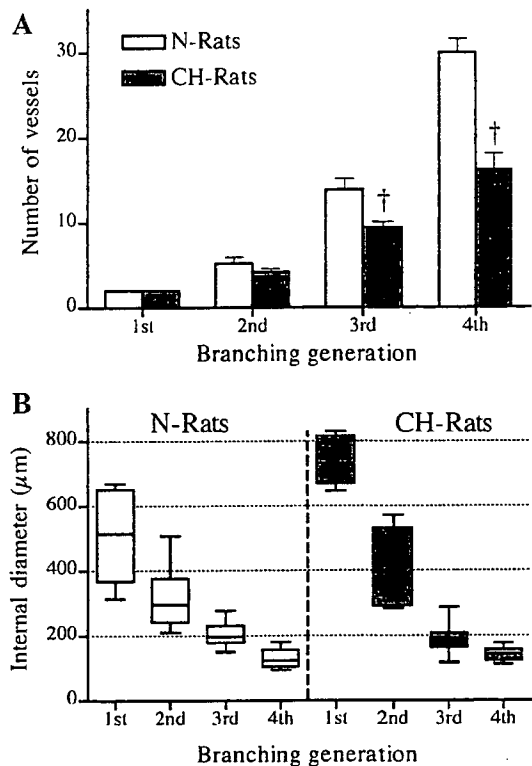


Fig. 3. Changes in the number of opaque vessels (means \pm SE) (A) and range of vessel sizes (box and whisker graph; B) at each of the first 4 branching generations of the pulmonary circulation in normoxic rats (N-rats; $n = 5$) and chronic hypoxic rats (CH-rats; $n = 5$). †Significant difference between N-rats and CH-rats ($P < 0.05$).

was not significantly different between N-rats and CH-rats for the second generation (210–570 μm), third generation (135–290 μm), and fourth generation of branching (90–180 μm) (Fig. 3B).

Responses to acute hypoxia. N-rats and CH-rats were exposed to 8% O_2 for 4 min. Acute hypoxia caused a significant decrease in the internal diameter of all vessels with an internal diameter $<500 \mu\text{m}$ (for N-rats) or $<300 \mu\text{m}$ (for CH-rats) (Figs. 4 and 5). In both groups of rats, the magnitude of constriction tended to increase as vessel caliber decreased, with the greatest degree of vasoconstriction occurring in those vessels with a diameter between 100 and 300 μm . These vessels were generally of the third to fourth generation of branching. There was no significant difference between N-rats and CH-rats regarding the magnitude of HPV for all vessel sizes.

The magnitudes of responses to hypoxia for systolic RVP, MABP, and HR are presented in Fig. 6. Acute hypoxia induced a significant 21% increase in systolic RVP ($P < 0.01$) in N-rats, which was not significantly different from that observed in CH-rats (27% increase above baseline). This increase in systolic RVP reflects the HPV seen with microangiography. Hypoxia decreased MABP in CH-rats, but significantly more so in N-rats (40% and 54% decrease, respectively). Hypoxia did not alter HR in either N-rats or CH-rats.

Responses to propranolol. N-rats and CH-rats were administered the β -receptor blocker propranolol (2 mg/kg iv). Propranolol did not significantly alter baseline pulmonary vessel

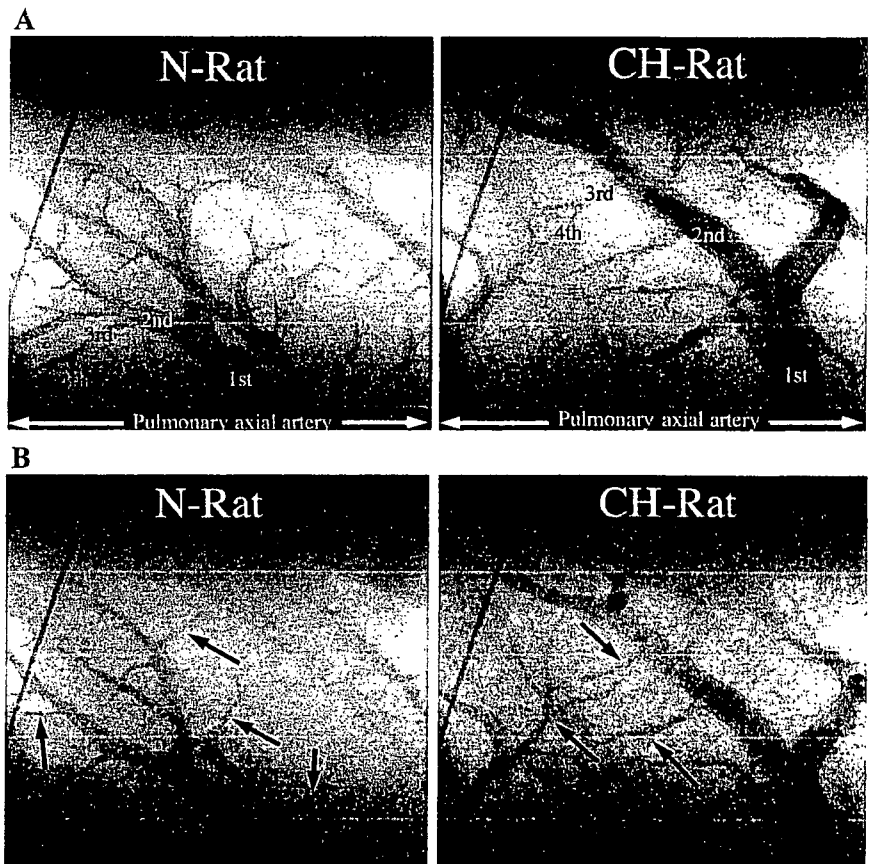


Fig. 4. Typical microangiogram images showing the branching pattern of small pulmonary arteries in N-rats ($n = 5$) and CH-rats ($n = 5$). Pulmonary branches to the 4th generation from the left main axial artery (not in the image) were visible. The tungsten wire in the top left of each image is a reference of 100 μm diameter. Images were recorded during air breathing (A) and after 4 min of acute hypoxia (8% O₂; B). Images for A and B were obtained from the same respective N-rat or CH-rat. The black arrows point to branches of the pulmonary vessels that have constricted in response to hypoxia.

caliber for any of the vessel size groups analyzed, in both N-rats and CH-rats. The lack of effect of propranolol on pulmonary vessel caliber for N-rats and CH-rats was reflected in the insignificant response of systolic RVP to propranolol (Table 1). In N-rats, propranolol did not significantly alter MABP or HR. In CH-rats, propranolol significantly increased MABP (15% increase) and caused a 17% decrease in HR ($P < 0.01$).

In N-rats, β -receptor blockade significantly exacerbated the magnitude of acute hypoxic vasoconstriction in 200- to 300- μm -sized pulmonary vessels (Fig. 5). However, the systolic RVP, MABP, and HR responses to acute hypoxia were unaltered (Fig. 6). In comparison, β -receptor blockade in CH-rats significantly accentuated the systolic RVP response to acute hypoxia ($P < 0.05$), reflecting extensive pulmonary vasocon-

striction in 200- to 300- μm -sized vessels and, unlike that observed for N-rats, even greater constriction in 100- to 200- μm -sized vessels (Fig. 5). The HPV response of vessels with a caliber $>300 \mu\text{m}$ was not altered by β -receptor blockade in N-rats or CH-rats.

DISCUSSION

This study has demonstrated the effectiveness of SR for visualizing the adverse anatomic and functional changes of the pulmonary circulation associated with the pathogenesis of pulmonary hypertension in a closed-chest rat model.

PAH. Although significant advances in the treatment of pulmonary disorders have been made in recent decades, the

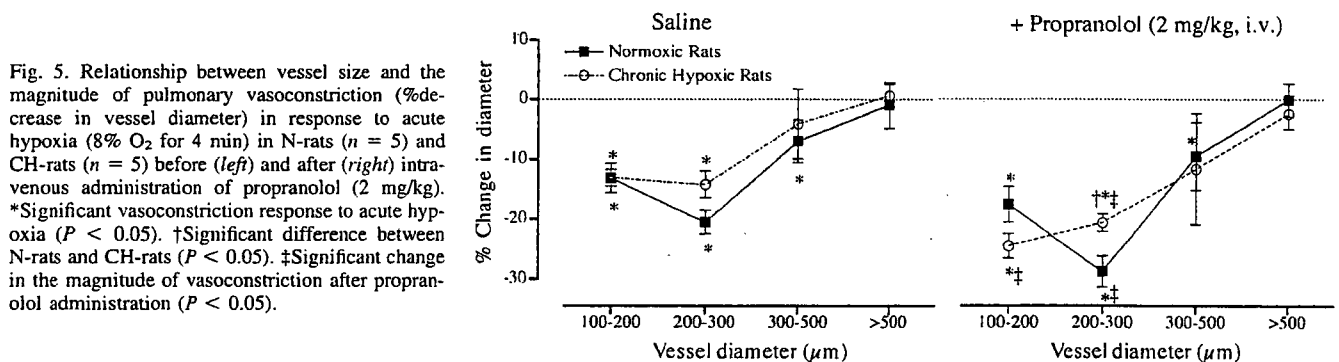


Fig. 5. Relationship between vessel size and the magnitude of pulmonary vasoconstriction (% decrease in vessel diameter) in response to acute hypoxia (8% O₂ for 4 min) in N-rats ($n = 5$) and CH-rats ($n = 5$) before (left) and after (right) intravenous administration of propranolol (2 mg/kg). *Significant vasoconstriction response to acute hypoxia ($P < 0.05$). †Significant difference between N-rats and CH-rats ($P < 0.05$). ‡Significant change in the magnitude of vasoconstriction after propranolol administration ($P < 0.05$).