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Original Article

Vascular Changes in the Rat Brain during Chronic Hypoxia in the Presence and Absence of Hypercapnia

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Changes in brain vascularity in adult rats during adaptation to chronic normobaric hypoxia with or without elevated CO_2 were morphometrically investigated. Immunohistochemistry with anti-rat endothelial cell antigen (RECA-1) antibody was carried out for the vascular analysis. After the rats were subjected to hypoxia for 2 to 8 weeks (wks) (10% O_2 in N_2), the total area of blood vessels was measured in 6 brain regions. After 2 wks of hypoxia, the blood vessel area was found to be significantly increased in the frontal cortex, striatum, hippocampus, thalamus, cerebellum, and medulla oblongata, by 44%, 96%, 65%, 50%, 102% and 97%, respectively. The ratio of large vessels with an area $>500\ \mu m^2$ was also increased in all brain regions. Hypoxic adaptation in brain vascularity did not change during 8 wks of hypoxia, and the hypoxia-induced levels measured in the vasculature returned to control levels 2 wks after the termination of hypoxia in areas of the brain other than the cortex and thalamus. In addition, hypoxia-induced changes in terms of the total vascular area and vessel size distribution were significantly inhibited by the elevation in CO_2, whereas chronic hypercapnia without hypoxia had no effect on brain vascularity. These findings suggested that adaptations in brain vascularity in response to hypoxia are rapidly induced, and there are regional differences in the reversibility of such vascular changes. Carbon dioxide is a potent suppressor of hypoxia-induced vascular changes, and may play an important role in vascular remodeling during the process of adaptation to chronic hypoxia.

Key words: hypoxic adaptation, brain vascularity, anti-rat endothelial cell antigen, carbon dioxide

Prolonged hypoxia is experienced frequently by climbers in high mountainous areas and those who live at high altitudes. Information about the effects of hypoxia is physiologically relevant to understanding the adaptive changes that occur in humans in response to exposure to high altitudes. The mammalian brain depends on a continuous supply of glucose and oxygen in order to function normally. Thus, brain tissue is extremely vulnerable to hypoxia; decreases in oxygen availability are known to activate compensatory mechanisms aimed at maintaining the balance between local oxygen delivery and tissue oxygen consumption. Chronic hypobaric hypoxia

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has been found to lead to the dilation and elongation of brain capillaries in mice, which acts to alleviate any O_2 deficit [1]. This remodeling of capillary diameter and length during chronic hypoxia leads to a significant increase in O_2 conductance to neural tissues. Patt *et al.* [2] reported finding an adaptation of brain circulation in rats after 130 days of normobaric hypoxia; the observed effects included the angiogenesis and dilation of microvessels, with regional differences in the pattern of vascular changes. In rats, vascular dilation has been found to occur predominantly in the carotid body after 12 weeks (wks) of normobaric hypoxia [3]. Pichiule *et al.* [4] found that capillary density in the rat cerebral cortex increased by 60% after 3 wks of hypobaric hypoxia, and that capillary density progressively decreased to the pre-hypoxic level after 3 wks of normoxic recovery. These findings indicate that chronic hypobaric and normobaric hypoxia induce reversible angiogenesis in the brain. However, little is known about the time-course of the vascular changes, including the angiogenesis and dilation of vessels that occur during adaptation and deadaptation to chronic hypoxia in each brain region. The mechanisms of induction of these vascular changes also remain unclear.

Arterial hypoxia dilates cerebral blood vessels, consequently increasing both cerebral blood flow (CBF) and blood volume (CBV) [5]. In contrast, hyperventilation due to hypoxia reduces the arterial partial pressure of carbon dioxide ($PaCO_2$), and this has a powerful vasoconstrictor effect on cerebral blood vessels [6]. Not only the arterial pressure of oxygen (PaO_2) but also $PaCO_2$ appears to be an important factor in these changes in vascular size. Previous studies have suggested that CO_2 is involved in vascular adaptation to chronic hypoxia in the lung [7] and the carotid body [3, 8]. In a study of rats, hypoxic pulmonary vascular remodeling was inhibited by chronic hypercapnia [7]. Moreover, Kusakabe *et al.* [3, 8] demonstrated that the vasculature in the carotid body of hypoxic rats is enlarged in comparison with that of normoxic control rats, and the extent of vascular enlargement is suppressed by elevated CO_2 concentration in a hypoxic environment. These findings indicated that CO_2 affects hypoxic vascular remodeling. However, in most studies of brain vascular changes due to long-term hypoxic exposure, the levels of CO_2 have not been examined.

In the present study, we morphometrically studied the time course of vascular changes within 6 selected rat brain regions during long-term normobaric hypoxia and normoxic recovery. The blood vessels in the brain were immunostained with anti-rat endothelial cell antigen (RECA-1) antibody, which is strictly specific for rat endothelial cells [9]. The area of each blood vessel was measured, and these values were used to calculate the total area of the blood vessels using image analysis software. We also investigated whether CO_2 is involved in brain vascular remodeling during adaptation to hypoxia.

Materials and Methods

Animals and hypoxic exposure. Male Wistar rats weighing 350 to 400 g were used. The hypoxic protocol has been described in detail elsewhere [10]. Briefly, rats were placed in a home cage ($27 \times 18 \times 15$ cm) inside an airtight acrylic chamber ($50 \times 50 \times 60$ cm) with 2 holes. One hole, located at the top of a sidewall of the chamber, was connected to a multi-flowmeter (MODEL-1203, KOFLOC, Tokyo, Japan), and was used to deliver a hypoxic gas mixture into the chamber. The flow of air, N_2, and CO_2 was regulated by the multi-flowmeter (total, 10 L/min), and the O_2 and CO_2 levels within the box were monitored with a gas analyzer (Respina 1H26, NEC San-ei, Tokyo, Japan). The second hole was located at the bottom of the opposite wall of the chamber, and was used to flush out the gas mixture. The temperature within the chamber was maintained at 25 °C, and the chamber was opened for 10 min every 3 days for animal care. Brain vascularity was analyzed in 3 hypoxic experiments examining the following: vascular changes during adaptation and deadaptation, and the effects of CO_2. In the adaptation experiment, the rats were exposed to normobaric hypoxia (10% O_2 in N_2) for 2 wks, 4 wks, or 8 wks ($n = 6$ per each time point), with food and tap water provided *ad libitum*. According to our previous experiment, the mean value of PaO_2 was 36.2 ± 0.6 mmHg under the hypoxic condition [10]. Control animals were housed under the same physical conditions, but under conditions of normoxia ($n = 6$). In the deadaptation experiment, after 8 wks of hypoxic exposure, rats were exposed to normoxic conditions for 2, 4, or 8 wks ($n = 6$ each). In the CO_2 experiment, rats were exposed to one of 4 types of hypoxic or normoxic conditions for 4 wks: hypocapnic hypoxia, normocapnic hypoxia, hypercapnic hypoxia, and hypercapnic normoxia ($n = 6$ per each group). In the hypocapnic hypoxia group, the hypoxic protocol was identical to that of the adaptation

experiment. Due to hypoxia-induced hyperventilation, CO_2 was added to the gas mixture at a concentration (3–4%) sufficient to maintain the $PaCO_2$ close to the level measured during the control period of the normocapnic hypoxia group [10]. In the hypercapnic hypoxia group, the chamber was ventilated with an elevated CO_2 gas mixture (10% O_2 + 6.5–7% CO_2 in N_2). To analyze arterial blood gas, heparin-filled polyethylene catheters were inserted into the right femoral artery in another set of CO_2 experimental animals (n = 5 per each group). Blood samples (0.08 ml) were drawn during the control period and 20 min after the initiation of hypoxic exposure, and the blood was analyzed for PaO_2, $PaCO_2$, and pH by a gas analyzer (ABL-30, Radiometer, Denmark). All animal experiments were performed in accordance with the guidelines described in the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Histological examination. Rats were anesthetized with sodium pentobarbital (50 mg/kg) and perfused transcardially with an 18-gauge cannula connected to a reservoir of perfusate at a height of 1.5 m above the animals. Perfusion was performed with 0.01 M heparinized phosphate-buffered saline (PBS, pH 7.4) followed by a fixative consisting of 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4, PB) for 20 min. The brains were removed, postfixed in the same fixative for 24 h, cut into several blocks, cryoprotected in 30% sucrose at 4 °C, and embedded in OCT compound at −70 °C. Immunohistochemical staining for RECA-1 was performed to analyze brain vascularity. Brain sections (thickness, 25 μm) were cut using a freezing microtome, incubated with 0.3% Triton X-100 in 0.01 M PBS for 1 h, and then placed in 0.15% H_2O_2 in 0.01 M PBS for 1 h to quench endogenous peroxidase activity. After washing the sections with 0.01 M PBS, nonspecific reactions were blocked with 1% bovine serum albumin for 30 min. The sections were then incubated with anti-RECA-1 (1:400, mouse monoclonal antibody; Monosan, Uden, The Netherlands) overnight at 4 °C. After 5 rinses with PBS, the sections were incubated with a biotinylated secondary antibody for 30 min at room temperature. The sections were then incubated with an avidin-biotin-peroxidase complex (ABC) for 1 h at room temperature, according to the manufacturer's recommendations (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA). Finally, the sections were reacted with 3,3'-diaminobezidine (Sigma, Chemical Co., St. Louis, MO, USA) and H_2O_2. All sections were dehydrated and mounted on silan-coated slides.

Morphometric analysis. For each animal, sections of the frontal cortex, striatum, hippocampus, thalamus, cerebellum, and medulla oblongata were selected and digitized with a digital camera connected to an Olympus microscope (Olympus Optical Co., Tokyo, Japan) with a × 10 objective. Each region was analyzed on the following sections: 0.7 mm anterior to the bregma (frontal cortex and striatum), 3.8 mm posterior to the bregma (hippocampus and thalamus), 10.3 mm posterior to the bregma (medulla oblongata and cerebellum) [11]. The area of blood vessels stained with RECA-1 within these selected regions was measured and analyzed using National Institutes of Health (NIH) imaging software (ver. 1.6). The values were expressed as the total area of blood vessels per mm^2 brain tissue (mm^2/mm^2) and the distribution of blood vessel size (%). The blood vessels assessed in this study included arteries, arterioles, capillaries, and venules. A researcher with no prior knowledge of the experimental groups performed the morphometric measurements. Data are presented as mean ± standard deviation (SD). The quantitative data were analyzed by one-way analysis of variance (ANOVA) followed by Scheffé's test. A probability level of < 0.05 was considered to indicate statistical significance.

Results

Blood vessels in the brain were clearly stained and identified by RECA-1 immunohistochemistry (Fig. 1). After 2 wks of hypoxia, the vessels were enlarged and elongated, with many ramifications (Fig. 1). Vascular changes were quantified by measuring the area of vessels per mm^2 brain tissue. The time-course of vascular changes during hypoxic adaptation and normoxic recovery is shown in (Fig. 2). After 2 wks of hypoxia, brain vascularity was significantly increased, compared with that of the control ($P < 0.01$–0.05), by 44%, 96%, 65%, 50%, 102%, and 97% in the cortex, striatum, hippocampus, thalamus, cerebellum, and medulla oblongata, respectively. Brain vascularity remained significantly increased at 8 wks of hypoxia. In the cerebellum, due to the high SD, there were no statistically significant differences after 4 or 8 wks hypoxia, as compared with normal controls. In all brain regions, at 2 wks of hypoxia, the ratio of large vessels (cross-sectional area $> 500\ \mu m^2$) had increased, whereas that of small

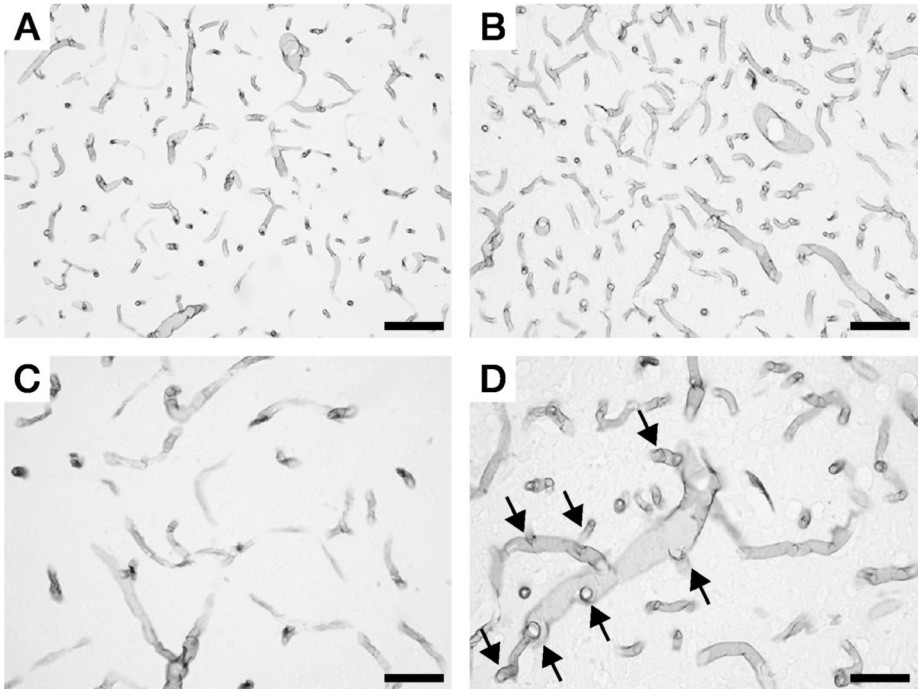

Fig. 1 Rat endothelial cell antigen (RECA-1) immunostaining of the striatum after hypoxia. **A** and **B**, normoxic control; **C** and **D**, 2 wks of exposure to hypoxia. Elongation and dilation of blood vessels were induced by 2 wks of exposure to hypoxia. Note that many ramifications are visible in the extended vessels (arrows). Bars = 100 μm in **A** and **C**, 50 μm in **B** and **D**.

vessels (cross-sectional area $< 150\ \mu m^2$) had decreased, and the ratio of large vessels remained elevated at 8 wks of hypoxia (data not shown). In all brain regions except for the cortex and thalamus, the chronic hypoxia-induced increase in the total blood vessel area was gradually reversed during normoxic recovery, with vascularity returning to the control level after 1 or 2 wks (Fig. 2). In the cortex and thalamus, after 8 wks of normoxic recovery, the total vascular area was still elevated, by 34% and 56%, respectively. After 8 wks of normoxic recovery, the distribution of blood vessels returned to the control level in the striatum, hippocampus, cerebellum, and medulla oblongata, whereas in the cortex and thalamus, the hypoxic changes in vessel size distribution were found to persist (data not shown).

Table 1 shows the mean values of PaO_2, $PaCO_2$, and pH in the control and 3 hypoxic groups (hypo-, normo, and hypercapnic hypoxia). Hypoxia induced a significant decrease in PaO_2 in the hypoxia group; however, the reduction in PaO_2 in the normo- and hypercapnic hypoxia groups was attenuated compared with that of the hypocapnic hypoxia group. As regards the levels of $PaCO_2$, hypocapnia induced a significant decrease, and hypercapnia induced a significant increase, whereas normocapnia produced no significant changes compared with the controls. With respect to pH, hypocapnia induced a significant increase, and hypercapnia induced a significant decrease, whereas normocapnia produced no significant changes compared with the controls. The effect of the CO_2 concentration on hypoxia-induced vascular changes is shown in Fig 3. In all brain regions except for in the cortex and thalamus, the increase in brain vascularity induced by chronic hypoxia was significantly suppressed by CO_2 augmentation.

With elevated CO_2, there were no significant differences among the control, normocapnic hypoxia, and hypercapnic hypoxia groups in the striatum, hippocampus, cerebellum, or medulla oblongata. In the cortex, elevated CO_2 inhibited hypoxia-induced increases in the vascular area, but there were still statistical differences in the normocapnic hypoxia and hypercapnic hypoxia groups compared with the normoxic controls ($P < 0.05$). In the thalamus, elevated CO_2 had no effect on hypoxic vascular changes. Hypercapnic normoxia did not induce changes in vascular area compared with that of the normal controls. The increase in the ratio of large

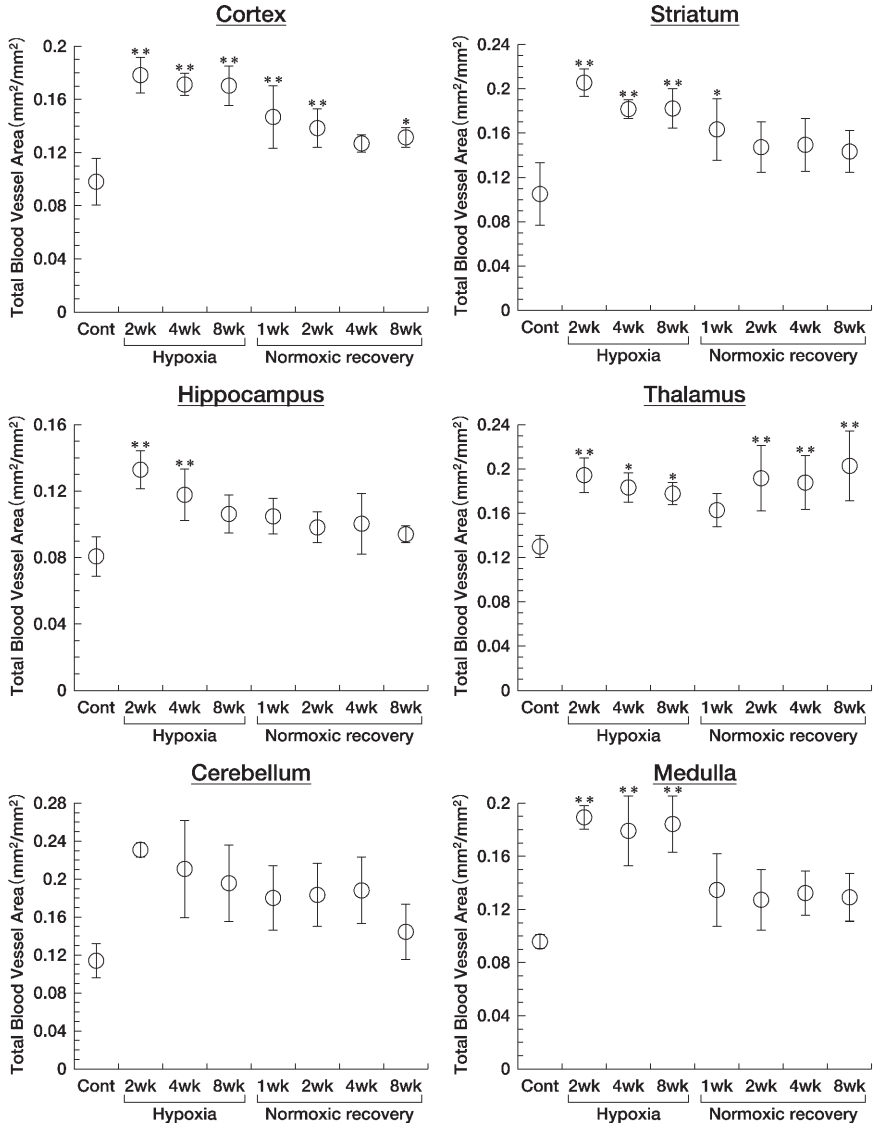

Fig. 2 Time-course of changes in total blood vessel area in each brain region during hypoxia and normoxic recovery. Brain vascularity was significantly increased ($P < 0.01$–0.05), compared with that of the controls, in all brain regions after 2 wks hypoxia. Total blood vessel area remained significantly elevated at 8 wks of hypoxia. In almost all brain regions, the increase in vessel area induced by chronic hypoxia gradually decreased, reaching the control level after 1 or 2 wks of normoxic recovery. However, the blood vessel area remained elevated in the cortex and thalamus, despite 8 wks of normoxic recovery. Values are mean ± SD. *$P < 0.05$, **$P < 0.01$ vs. normoxic controls.

Table 1 Arterial blood gas in hypoxia

	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH
Control	96.9 ± 6.2†	34.7 ± 1.9†	7.44 ± 0.03
Hypocapnic hypoxia	36.4 ± 1.5*	21.4 ± 2.8*	7.58 ± 0.05*
Normocapnic hypoxia	49.6 ± 3.7*†	35.6 ± 2.2†	7.42 ± 0.02
Hypercapnic hypoxia	52.7 ± 2.1*†	52.4 ± 5.7*†	7.31 ± 0.03*†

*< 0.01 compared with control, †< 0.01 compared with hypocapnic hypoxia.

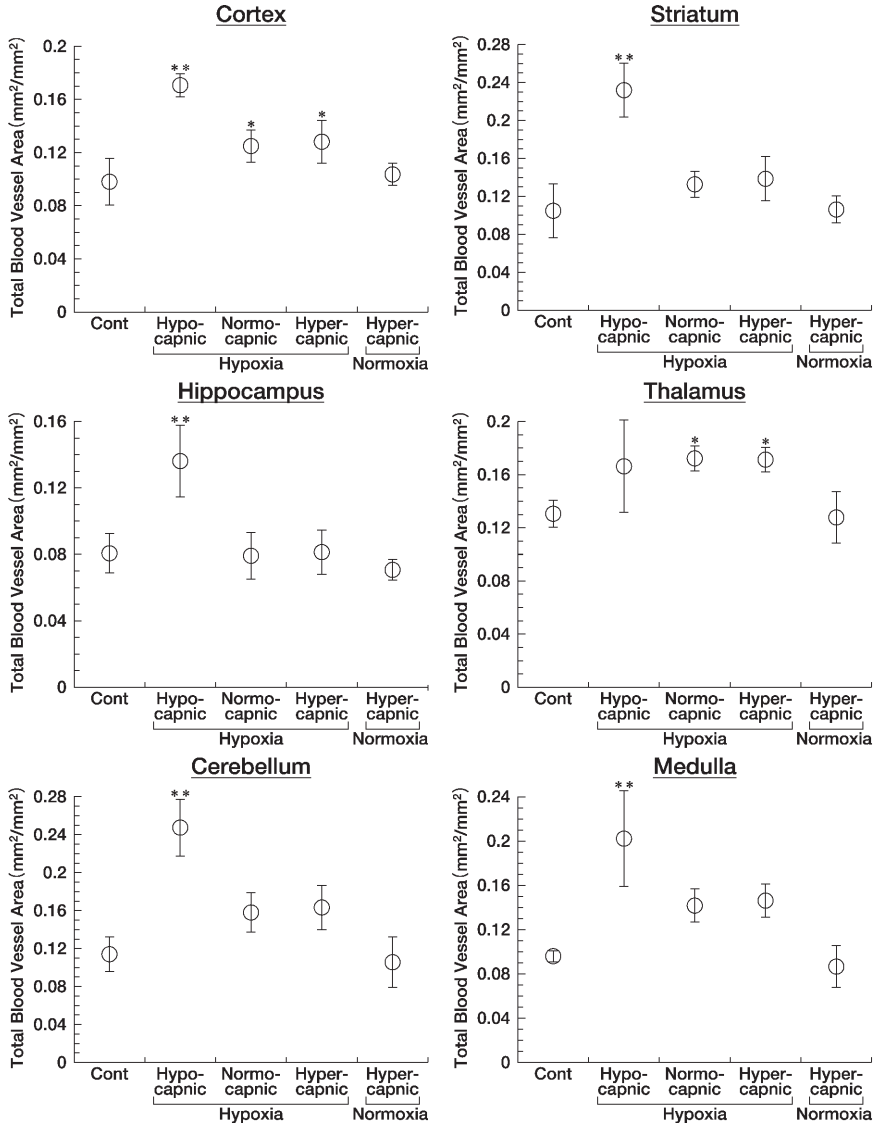

Fig. 3 Effects of carbon dioxide (CO_2) on hypoxia-induced remodeling in the brain. Rats were exposed to 4 wks of hypoxia with or without CO_2 elevation. Elevations in CO_2 significantly inhibited hypoxia-induced vascular remodeling. However, the suppressive effect of CO_2 was much less pronounced in the cortex and thalamus than in other regions of the brain. Hypercapnia without hypoxia did not induce vascular changes in the brain. Values are mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$ vs. normoxic controls.

vessels induced by hypocapnic hypoxia was also suppressed by elevated CO_2, and hypercapnia without hypoxia did not significantly affect vascular size distribution in any of the brain regions examined (data not shown).

Discussion

As previously reported, the vessels of the rat brain were clearly stained and distinguished by immunohistochemistry using anti-RECA-1 antibody [12]. RECA-1 has been recognized as a general endothelial marker that is more specific to endothelial cells than are other endothelial cell markers such as von Willebrand Factor [9]. Moreover, Silverman *et al*. demonstrated the ability of RECA-1 to identify even the immature blood vessel endothelium [13]. Therefore, using RECA-1, we were able to investigate all vessels, including angiogenetic vessels, in the brain. Both angiogenesis and the dilation

of vessels have been shown to occur during chronic hypoxia, by decreasing the intercapillary distance as an adaptation to low concentrations of O_2 [1]. Therefore, in the present study, the data were expressed as the total area of blood vessels in order to assess angiogenesis and the dilation of vessels under hypoxic conditions. Previous studies have shown that an increase in vascular density of the rat cortex can be induced by chronic hypobaric [1, 4, 14] and normobaric hypoxia [2, 15]. Vascular changes in response to chronic hypoxia have previously been analyzed using immunohistochemistry for the type 1 glucose transporter (GLUT-1), which is a major glucose transporter in cerebral microvessels [16], showing that capillary density in the cortex increased after 3 wks of hypobaric hypoxia (0.5 atm) [4, 14]. Studies have also shown that the increase in the vascular density of the cortex induced by 3 wks of hypoxia can be reversed by 3 wks of normoxic recovery. In the present study, 2 to 8 wks of hypoxia induced cortical vascular changes to a degree similar to that seen in previous studies. We found that the total area of blood vessels significantly increased in all brain regions after 2 wks of normobaric hypoxia (10 % O_2), and that this change persisted at almost the same level at 8 wks of hypoxia.

Immediate brain adaptive responses to hypoxia include an increase in blood flow, whereas long-term responses involve the remodeling of the cerebral blood vessels. A critical mediator of hypoxia-driven vascular remodeling is the transcription factor hypoxia-inducible factor-1 (HIF-1), which is responsible for the transcriptional activation of several proangiogenic growth factors such as vascular endothelial growth factor (VEGF) [17]. Chávez *et al.* reported that HIF and VEGF were induced 6 h after the onset of hypoxia and the levels of these factors did not decrease for 14 days; however, the levels had returned to normal after the passage of 21 days, despite exposure to continuous hypoxia [18]. The results of that previous report might account for the completion of hypoxia-driven vascular remodeling within 2 wks observed in the present study. The significant reduction in the ratio of small vessels and the increase in the ratio of large vessels occurred within a similar time frame during exposure to hypoxia. This finding suggests that the increase in vascularity in response to chronic hypoxia is primarily the result of the enlargement of preexisting vessels. In the present study, the increase in the total area of blood vessels induced by hypoxia was for the most part reversible, a finding which is consistent with those of previous studies [4, 13]. However, hypoxic changes in terms of both vascular size and size distribution persisted in the cortex and thalamus at 8 wks of normoxic recovery. This finding is consistent with the previously observed persistence of hypoxia-induced elevations in GLUT-1 density in the rat cortex, in spite of 3 wks of normoxic recovery [14]. The previous findings, taken together with those of the present study, indicate that the effects of hypoxia on vascular systems persist for a long period of time. Several researchers reported regional differences in response to hypoxia [2, 19, 20]. For example, Patt *et al.* suggested that the pattern of the microcirculatory changes in the brain is not homogenous throughout all regions of the brain [2]. In addition, brain stem neurons are known to depolarize faster than cortical neurons during hypoxia [19]. Magnetic resonance study has shown that the hippocampal CA1 region exhibits a reduced response to hypoxia relative to that of the cortex and basal ganglia [20]. Although the mechanisms underlying the regional differences in response during hypoxic adaptation and deadaptation remain unclear, the sensitivity of the brain to hypoxia might differ between brain regions. In the present study, the vascular areas were found to be approximately 8–10% in the normoxic controls. However, previous studies have reported that the CBV of rats was in the range of 1–4% [21–23]. Because blood volume (mm^3/mm^3) is mathematically identical to our measurement of area/area, a criticism of the present study might be that the vascular areas considered here were much greater than those of other studies. In this study, an overestimation of the values of the vascular areas due to the use of thick brain sections (25 μm) could not be excluded. Therefore, the value of the total area of blood vessels per mm^2 brain tissue (mm^2/mm^2) in this study should be recognized as an anatomical rather than as a physiological measure. In this context, it is also important to consider the modification of vascular area by fixation, since paraformaldehyde induces tissue contraction. Further analysis must therefore be carried out in order to avoid the artifacts of fixation.

Hypoxic exposure induces hypocapnia due to hyperventilation. The addition of CO_2 to hypoxic exposure (3–4% in the case of normocapnic hypoxia, and 6.5–7% in the case of hypercapnic hypoxia) maintained the rats in the normocapnic or hypercapnic condition. Hypercapnia induces cerebral vasodilation and increases both CBF and CBV, whereas hypocapnia induces cerebral vasoconstriction and leads to decreases in both CBF and CBV [22–

24]. Thus, carbon dioxide is a potent vasodilator of cerebral blood vessels [6, 25]. Unexpectedly, in the present study, normo- and hypercapnic hypoxia clearly inhibited what would have otherwise been a hypoxia-induced increase in vascular area. The expected changes in vessel size distribution induced by hypoxia were also suppressed by CO_2 elevation. Long-term normoxic hypercapnia was found to have no effect on brain vasculature, indicating that elevated CO_2 itself does not cause vascular remodeling under conditions of normoxia. These results suggest that normocapnia and hypercapnia prevent the compensatory vascular response to chronic hypoxia, including angiogenesis and vascular dilation. Although the precise mechanism of the observed inhibition of hypoxia-induced vascular changes by CO_2 remains unclear at present, several researchers have reported the influence of CO_2 on hypoxic adaptation [7, 8, 26]. Hypercapnia (4–10% CO_2) prevented a hypoxia-induced hypertrophic response [26] and vascular enlargement [8] in studies of responses to chronic hypoxia in the carotid body. Furthermore, Ooi *et al.* [7] reported that chronic hypercapnia (10% CO_2) inhibits the hypoxia-induced proliferation of pulmonary vascular smooth muscle cells, right ventricular hypertrophy, and the proliferation of red blood cells. Taken together, these findings suggest that elevated CO_2 exerts a general inhibitory effect on hypoxia-induced protein synthesis and cell proliferation [27]. Moreover, Kusakabe *et al.* [8] demonstrated that, in the carotid body during chronic hypoxic exposure, the $PaCO_2$ level affected the peptidergic innervation that regulates vascular tone [28, 29]. Moreover, the findings of that study indicated that the alteration of peptidergic innervation in the carotid body after chronic exposure to hypercapnic hypoxia is a mechanism of hypoxic adaptation. In addition, in the present study, the increase in CO_2 attenuated the decrease in PaO_2 20 min after the onset of hypoxia. Imray *et al.* [30] also reported that supplementation with 3% CO_2 increased cerebral oxygenation at a high altitude in humans, and they suggested several mechanisms for the improvements in tissue oxygenation. Hypercapnia increases ventilation and cardiac output, and shifts the oxyhaemoglobin dissociation curve to the right, resulting in the improvement of oxygen delivery to the tissues [30]. Thus, $PaCO_2$ as well as PaO_2 have profound effects on oxygen delivery to the brain; therefore, the possibility cannot be excluded that hypercapnia attenuates the hypoxic stimulation of the vasculature by improving oxygenation. However, these data were obtained from acute hypoxic experiments, and the effects of CO_2 on tissue oxygenation during chronic hypoxia remain to be examined.

In summary, we demonstrated that a significant increase in blood vessel area, including an increase in the ratio of large vessels, occurs in the rat brain after 2 wks of normobaric hypoxia, and these effects were found to persist at 8 wks of hypoxia. This vascular change was not reversed in any region of the brain studied, despite 8 wks of normoxic recovery. Furthermore, elevated CO_2 inhibited vascular responses to chronic hypoxia. These results suggest that vascular adaptation to hypoxia in the brain occurs rapidly, and that these vascular changes persist for a long time in several brain regions following the termination of hypoxia. Hypercapnia in cases of hypoxic exposure significantly inhibits the otherwise expected hypoxia-induced vascular changes, suggesting that CO_2 levels are involved in adaptive mechanisms to chronic hypoxia. Increasing numbers of people travel to high altitudes for both recreational and professional purposes. Hypoxic adaptation induces tolerance to ischemic brain injury, although its mechanisms are not yet fully understood ([31], as a review). Thus, information regarding the effects of hypoxia is not only physiologically but also clinically relevant to our understanding of the adaptive changes that occur in response to hypoxia. The findings of the present study will provide useful information for future clinical investigations of hypoxia.

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