Angiopoietin-2 and rat brain capillary remodeling during adaptation and deadaptation to prolonged mild hypoxia

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Pichiule, Paola, and Joseph C. LaManna. Angiopoietin-2 and rat brain capillary remodeling during adaptation and deadaptation to prolonged mild hypoxia. J Appl Physiol 93: 1131–1139, 2002.—Angiogenesis is a crucial component of rat brain adaptation to prolonged hypoxia, but it is not known whether this structural change is permanent or reversible on return to normoxia. Also, the intrinsic mechanisms controlling brain microvascular plasticity in response to oxygen availability remains unclear. Our results indicate that capillary density in the rat cerebral cortex increased by 60% after 3 wk of hypoxia and that it progressively decreased to prehypoxic values after 3 wk of normoxic recovery (deadaptation). Angiopoietin-2 (Ang2) expression in the capillary endothelium was induced between 6 h and 14 days of hypoxia but fell to control levels at 21 days of hypoxia. During deadaptation, Ang2 levels were elevated at 1–14 days but decreased to baseline at 21 days. In contrast, the constitutive expression of Ang1 and Tie2 was not affected during hypoxia or deadaptation. TUNEL-positive endothelial cells and caspase-3 activation were observed at 7 and 14 days of deadaptation. These data suggest that Ang2 might modulate both angiogenesis and vascular regression in the rat brain and that capillary regression occurring during deadaptation involves activation of apoptosis.

angiopoietins; brain capillary density; angiogenesis; vascular regression

The mammalian brain depends on a continuous supply of glucose and oxygen for normal function. Metabolic stress such as a decrease in oxygen availability activates compensatory mechanisms aimed at maintaining the balance between local oxygen delivery and tissue oxygen consumption. Immediate brain adaptive responses to hypoxia include a reversible increase in blood flow, whereas long-term responses involve a series of metabolic and vascular changes (20). It was previously reported that prolonged hypobaric hypoxia caused a significant increase in brain microvessel density in rats and mice (4, 21). This response will presumably result in shorter intercapillary diffusional distances and improvement of tissue oxygenation. Although some aspects of the mechanism controlling hypoxia-induced brain angiogenesis have been deduced, there have been very few, if any, studies about the reversibility of this structural change once hypoxic exposure is terminated. Moreover, the intrinsic mechanisms linking brain microvascular density to oxygen availability remain undefined.

A critical mediator of hypoxia-driven angiogenesis 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) (11). Accordingly, prolonged hypoxia has been reported to induce transient accumulation of the oxygen-regulated HIF-1α subunit and upregulation of VEGF expression in the rat brain (6).

Angiogenesis requires coordinated signaling events among a variety of endothelial cell growth factors and their receptors. Together with VEGF, the angiopoietin family is considered to exert a pivotal role in the formation of new functional and stable capillaries (5, 13). Of the four currently known angiopoietins (Ang1–4), the best characterized are Ang1 and Ang2 (17). Ang1 is an agonistic ligand that induces activation of Tie2, an endothelium-specific tyrosine kinase receptor, whereas Ang2, considered to be an endogenous antagonist, binds to this receptor but does not stimulate its phosphorylation (8, 23). In adult tissues, Ang1 appears to be widely expressed and Tie2 has been found to be constitutively phosphorylated in quiescent endothelium (23, 35). On the other hand, Ang2 in adults is primarily expressed at sites of vascular remodeling and in highly vascularized tumors (16, 23, 33, 37). The actions of the angiopoietins appear to be different from those of VEGF. Analysis of cultured endothelial cells has revealed that Ang1 did not elicit mitogenic responses, although it could stimulate endothelial cell survival, migration, and sprouting (8, 12, 27, 28). In vivo analysis by targeted inactivation of Ang1 and Tie2 expression has established the absolute requirement of this system in embryonic vascular sprouting, remodeling, recruitment of periendothelial supporting cells, and maintenance of vascular stability (9, 29, 31). It has been proposed that, in the presence of angiogenic stimulators such as VEGF, Ang2 facilitates angiogenesis by...
blocking the constitutively stabilizing function of Ang1. On the other hand, in the absence of VEGF, Ang2 expression may trigger endothelial cell apoptosis (16, 23). However, recent reports showed that Ang2 treatment under particular conditions could stimulate Tie2 autophosphorylation and promote endothelial cell survival (18, 32), suggesting that Ang2 plays a complex role in vascular remodeling.

Considering that the Ang-Tie2 system seems to both stimulate and inhibit angiogenesis in different contexts, this study attempted to determine whether this system is part of the molecular mechanism linking brain capillary density to oxygen availability in vivo. Thus we studied the expression of angiopoietins and Tie2 receptor in the brain during chronic hypoxia and deadadaptation. We also sought to establish the time course of the reversal of the increased capillary density on return to normoxia from chronic hypoxia and whether this event involves activation of an apoptotic mechanism.

**MATERIALS AND METHODS**

**Exposure to chronic hypoxia.** Male Wistar rats (2–3 mo) were exposed to hypoxia for periods up to 3 wk in hypobaric chambers maintained at a pressure of 380 Torr (0.5 atm, equivalent to 10% normobaric oxygen). Normoxic controls were kept outside the chambers but in the same location. When animals were kept for more than 1 day, chambers were opened for cage cleaning and food and water replenishment. A group of rats that were initially adapted for 3 wk of hypobaric hypoxia were allowed to recover at normoxia for up to 3 wk (deadadaptation).

**RT-PCR.** Total RNA was extracted from brain cortical samples using the RNAgents total RNA isolation system (Promega) according to the manufacturer's protocol. RT-PCR was performed with the Access RT-PCR system kit (Promega). Ang1, Ang2, and β-actin mRNAs were reverse transcribed and amplified using these specific primers: 5'-GGGACATCTGAGTGGAAAATTA-3' and 5'-TCTTCAATTTTTCCTCA-TTCTTA-3' for Ang1, 5'-AAAGAGTACAAAGAGGGCTTC-3' and 5'-TCCAGTAGTACCCTTGATC-3' for Ang2, and 5'-AACCCCTAAGGCAACCTGAAAAAGAG-3' and 5'-TCATGGAGATTG-CCTAAGGT-3' for β-actin. The reverse transcription reaction was initiated with 1 μl of total RNA for 45 min at 48°C and 2 min at 94°C. PCR cycling conditions consisted of 30 s at 94°C, 60 s at 60°C, and 120 s at 68°C for designated cycles, followed by 7 min at 68°C for one cycle. The PCR cycles selected were within the linear amplification range of the genes analyzed: 35 cycles for Ang1 and Ang2 and 20 cycles for β-actin. Reaction products were size fractionated on agarose gels, and bands were visualized by ethidium bromide staining. The resulting amplification fragments corresponded to the expected size (360, 415, and 265 bp for Ang1, Ang2, and β-actin, respectively), and the identity of each PCR product was confirmed by sequencing. Expression levels were quantified by the 1D Image Analysis software (Kodak) and normalized to β-actin levels to account for differences in sample loading.

**Immunoprecipitation and Western blot analysis.** Anesthetized animals were decapitated, and brains were retrieved and stored at −80°C for further processing. Cortex was dissected and homogenized in ice-cold lysis buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 100 mM Na3VO4) supplemented with protease inhibitors (1 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1.5 μg/ml pepstatin, and 0.1 mg/ml phenylmethylsulfonyl fluoride). The homogenate was centrifuged (10,000 g for 10 min, 4°C), and the supernatant (tissue lysate) was stored at −80°C. Protein content in the supernatant was determined by the Bradford protein assay (Bio-Rad) with BSA as a standard. Fifty micrograms (for Tie2 immunoblot) or 100 μg of protein (for VEGF immunoblot) were resolved on either 7.5 or 15% reducing SDS-PAGE gel and transferred electrophoretically to nitrocellulose membrane. Membrane was blocked with 10% non-fat dry milk in TBS containing 0.1% Tween 20 and probed overnight at 4°C with anti-Tie2 (1:600, Santa Cruz) or anti-VEGF (1:400, Santa Cruz). This was followed by incubation with an appropriate horseradish peroxidase-conjugated secondary antibody. Equivalent protein loading was demonstrated by probing the same blot with polyclonal anti-β-actin antibody (1:2,000, Santa Cruz). Ang1 and Ang2 protein expressions were analyzed by immunoprecipitation followed by immunoblotting. The tissue lysate (200 μg) was incubated with either goat anti-Ang1 or anti-Ang2 IgG (Santa Cruz) and rocked overnight at 4°C. Subsequently, 10 μl of protein G-agarose were added, and the sample was incubated for another 2 h at 4°C. The pellet was collected by centrifugation (1,000 g for 5 min), washed with PBS several times, and boiled for 3 min in Laemml sample buffer. Samples were separated by SDS-PAGE (7.5%; reducing conditions), and Western blot was performed with anti-Ang1 or anti-Ang2 antibodies (1:200, Santa Cruz). In all cases, detection was performed through enhanced chemiluminescence reaction (Amersham) and subsequent exposure of the membrane to Hyperfilm (Amersham).

**Isolation of brain microvessels.** Microvessels were obtained from rat brain cortex as described previously by Sympton et al. (30). Briefly, cortex mantle was homogenized in a glass-Teflon homogenizer in ice-cold microvascular buffer (MB) containing 15 mM HEPES, 147 mM NaCl, 4 mM KCl, 3 mM CaCl2, and 12 mM MgCl2, supplemented with proteases inhibitors (1 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1.5 μg/ml pepstatin, and 0.1 mg/ml phenylmethylsulfonyl fluoride). The homogenate was centrifuged at 900 g for 10 min (4°C), and the resulting pellet was resuspended in 17% dextran/MB and then centrifuged at 3,600 g for 15 min (4°C). Next, the vessel-containing pellet was resuspended in MB, filtered through 125-μm nylon mesh, and passed through a glass bead column (supported on 40-μm mesh). After elution from beads with BSA-free MB, microvessels were pelleted and washed several times. The quality of the microvessel preparation was routinely monitored by phase-contrast microscopy. Microvessels were resuspended in lysis buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 100 mM Na3VO4, supplemented with protease inhibitors) and used for Western blot analysis of active caspase-3 (1:400, Cell Signaling Tech).

**Immunohistochemistry and TUNEL staining.** Rats were deeply anesthetized and transcardially perfused with ice-cold PBS (pH 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed, postfixed in the same fixative for 24 h at 4°C, and embedded in paraffin. Coronal serial sections (6 μm) were deparaffinized, hydrated, and subjected to antigen retrieval at 90°C for 15 min using a target retrieval solution (Dako). Subsequently, sections were incubated with 10% normal horse serum for 2 h and incubated overnight with a goat polyclonal anti-Ang2 antibody (1:200, Santa Cruz). After several washings with PBS, sections were incubated for 2 h with Oregon green-conjugated IgG secondary antibody 1:500, Molecular Probes). The specificity of Ang2 staining was demonstrated with the appropriate blocking peptide (Santa Cruz). For double labeling with von Willebrand factor (vWF), sections were rinsed with PBS and incubated with appropriate...
10% normal serum for 2 h. After that, sections were incubated overnight with rabbit anti-human vWF (1:200, Dako) followed by incubation for 2 h with biotinylated anti-rabbit IgG secondary antibody (1:200, Vector) and developed with Cy3-conjugated streptavidin (1:500, Jackson ImmunoLab).

For active caspase-3 immunohistochemistry, sections were incubated with a 1:200 dilution of the primary antibody (Cell Signaling Tech) and then incubated with the Oregon green-conjugated secondary antibody. This antibody properly recognized the large fragment of activated caspase-3 (~17 kDa) by Western blot analysis of 5-day-old rat cerebellum. When required, active caspase-3-labeled sections were further processed for Ang2 staining with a Cy3- or Texas red-conjugated secondary antibody (1:500, Jackson ImmunoLab).

Terminal deoxynucleotidyl transferase-mediated UTP nick end labeling (TUNEL) was performed with the fluorescein apoptosis detection system according to the manufacturer’s instructions (Promega). For double labeling with TUNEL and immunohistochemistry, sections were first processed for vWF staining and then processed for TUNEL staining.

Microvessel density. Immunohistochemical staining for GLUT-1 was performed for assessing brain microvascular density. Three sections (6 μm thick) cut 150 μm apart corresponding approximately to plates 10–14 in a rat brain atlas (Paxinos and Watson) were stained using a goat polyclonal anti-GLUT-1 antibody (1:200, Santa Cruz) and a biotinylated secondary antibody (Vector Lab). Color detection was carried out with the use of avidin-biotin horseradish peroxidase solution and the diaminobenzidine peroxidase substrate kit (Vector Lab). Images spanning the full depth of the parietal cortex were digitized with a SPOT digital camera connected to a Nikon E600 Eclipse microscope with a ×20 objective. A computer-aided image analysis system (ImageProPlus) was used to determine the number of GLUT-1-positive capillary profiles per unit area that were <20 μm in diameter. Quantification of capillary density was performed in the following experimental groups: normoxia, 3 wk hypoxia, and 3 wk hypoxia with normoxic recovery for 7, 14, or 21 days (n = 4 per each experimental group).

Statistical analysis. Quantitative data are expressed as means ± SD. Statistical comparisons were performed by one-way ANOVA followed by Tukey’s correction with SPSS version 10.1. In all cases, P < 0.05 was considered significant.

RESULTS

Systemic physiological changes in response to hypoxia. Rats subjected to hypobaric hypoxia develop polycythemia. In this study, packed red cell volume was significantly elevated from 50 ± 3 (n = 38) to 64 ± 3 after 7 days of hypoxia (n = 33). It continued to be higher than controls at 14 (69 ± 3, n = 29) and 21 days of hypoxia (70 ± 3, n = 37). This hypoxia-induced polycythemia was gradually reversed with normoxic recovery. Hematocrit was still significantly increased at 1 wk of recovery (69 ± 3, n = 23); it decreased to 61 ± 3 at 2 wk (n = 16) and returned to prehypoxic values at 3 wk of normoxic recovery (52 ± 3, n = 10).

In addition, rats exposed to hypobaric hypoxia gained no body weight throughout the 3 wk of exposure (348 ± 7 g vs. 346 ± 17 g, n = 4). During the same period, the body weight of normoxic control rats significantly increased from 345 ± 13 to 433 ± 24 g (n = 4). However, when those rats that adapted to 3 wk of hypoxia were allowed to recover in normoxia for 3 wk, they gained weight so that the difference between the control (432 ± 21, n = 4) and experimental (458 ± 8.4, n = 4) groups was no longer significant.

Increased brain capillary density during chronic hypoxia and its reversibility during deadaptation. Cerebral capillaries were identified by GLUT-1 immunostaining and their density quantified by counting the number of positive capillaries per unit area. As shown in Fig. 1A, 3 wk of hypoxic exposure caused an

Fig. 1. Changes in cortical capillary density during prolonged hypoxia and deadaptation. A: composite photomicrograph of GLUT-1-stained sections spanning part of the parietal cortex at normoxia, 21 days (21d) of hypoxia, and normoxic recovery for 21 days. B: capillary density analysis of GLUT-1-stained sections showing a significant increase at 21 days of hypoxia (21dH). Subsequent normoxic recovery (7dR, 14dR, or 21dR) caused reestablishment of prehypoxic capillary density. Values are means ± SD; n = 4 rats in each group. *P < 0.05 compared with controls (C).
evident increase in GLUT-1-positive vascular profiles; this returned to control levels after 3 wk of normoxic recovery.

In agreement with previous reports (14, 21), quantification analysis showed that capillary density in the parietal cortex was significantly increased by ~60% after 3 wk of hypoxia compared with controls (347 ± 20 vs. 217 ± 14 microvessels/mm²). Normoxic recovery from chronic hypoxia was accompanied by cerebral vascular regression, as demonstrated by a progressive decrease of capillary density. At 1 wk of normoxic recovery, capillary density was still increased by 41% (305 ± 16 microvessels/mm²); however, by 2 and 3 wk of normoxic recovery, capillary density was no longer significantly different from the control level (Fig. 1B).

Ang1, Ang2, and Tie2 expression in the brain cortex during hypoxia and deadaptation. To determine whether angiopoietins are involved in the brain vascular remodeling process during chronic hypoxia and deadaptation, we analyzed mRNA and protein levels of Ang1 and Ang2 in the brain cortex during these conditions.

Analysis of Ang2 mRNA levels by RT-PCR showed basal levels of expression in the normoxic brain cortex that slightly increased at 6 h of hypoxia. However, Ang2 mRNA level significantly increased by ~52% at 12 h of hypoxia and continuously increased by ~140% at 14 days and then returned to prehypoxic levels between 14 and 21 days of hypoxia. Interestingly, Ang2 mRNA was also induced to about the same extent during normoxic recovery from hypoxia. Significant upregulation was observed at 7 and 14 days of deadaptation by 102 and 126%, respectively. Between 14
and 21 days of deadaptation, Ang2 mRNA levels returned to control values (Fig. 2, A and C). Conversely, Ang1 mRNA levels did not change during hypoxia or deadaptation (Fig. 2, A and B).

Immunoprecipitation followed by Western blot analysis showed no detectable levels of Ang2 in the normoxic samples; however, hypoxic exposure caused a strong induction of Ang2 protein levels. Induction of Ang2 protein was observed as early as 6 h after hypoxia induction, whereas mRNA levels were only slightly affected. Ang2 protein induction then continued for at least 14 days. Between 14 and 21 days, Ang2 protein levels fell to control levels, despite continued hypoxic exposure. During deadaptation, Ang2 protein induction was observed at 1–14 days of normoxic recovery, but it was no longer detectable by 21 days (Fig. 3A).

In contrast to Ang2, a strong constitutive Ang1 protein expression was observed, which remained unaffected during hypoxia and deadaptation (Fig. 3A). Similarly, Western blot analysis of brain cortex lysates showed no significant changes in Tie2 protein levels during hypoxia or during deadaptation (Fig. 3B). As previously reported, VEGF protein expression transiently increased in the brain cortex during hypobaric hypoxia (6) and remained at normoxic basal levels during deadaptation (Fig. 3C).

**Localization of Ang2 during hypoxia and deadaptation.** Next, we examined the cellular localization of Ang2 expression in the brain during hypoxia and deadaptation. Consistent with the data obtained by Western blot, immunohistochemical analysis showed no detectable Ang2 expression in the normoxic control brains (Fig. 4A). At 7 days of hypoxia, Ang2 expression was strongly induced throughout the cortex, and this expression was associated with microvessels (Fig. 4B). In addition, Ang2-positive vessels were detected at 7

![Image](http://jap.physiology.org/)

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**Fig. 4.** Immunohistochemistry of Ang2 during hypoxia and deadaptation. Ang2 expression (green) was not detected in normoxic control cortex (A) but was evident at 7 days of hypoxia (B) and at 7 days of normoxic recovery (C). Double immunostaining of the same sections for the von Willebrand factor (vWF, red) are shown in D–F. Note in the overlapped images that, at 7 days of hypoxia (H) and at 7 days of deadaptation (I), some vWF-positive endothelial cells express Ang2 (yellow), whereas others do not (red). Magnification = ×400 for all panels.
(Fig. 4C) and 14 days of deadaptation (not shown). Double immunostaining with vWF, an endothelial marker, showed Ang2 expression in brain endothelial cells. No positive Ang2 profiles were observed that did not also stain for vWF. It should be noted that only a subset of microvessels became positive for Ang2 both during hypoxia or deadaptation (Fig. 4, H and I). In agreement with Ang2 immunoblotting results, no Ang2-positive vessels were detected at 21 days of hypoxia or at 21 days of deadaptation (data not shown).

**Brain endothelial cell apoptosis during deadaptation.** We then performed TUNEL and active caspase-3 immunohistochemistry to ascertain whether vascular regression during normoxic recovery from prolonged hypoxia occurred through an apoptotic programmed cell death-type mechanism. No brain TUNEL-positive cells were observed at normoxia or at 1 or 3 wk of hypoxia (data not shown). Conversely, TUNEL staining consistently showed patchy clusters of positive nuclei lining the capillaries at 7 and 14 days of deadaptation (Fig. 5). At 3 wk of deadaptation, TUNEL-positive cells were rarely detected. To further characterize the origin of these TUNEL-positive nuclei, we performed double labeling with the vWF endothelial marker and demonstrated that most TUNEL-positive cells were endothelial cells (Fig. 5). However, some TUNEL-positive nuclei did not show colocalization with vWF, although they were in close association with vessels (Fig. 5I, arrow). It remains to be shown whether these cells might be pericytes undergoing cell death.

Detection of active caspase-3, a major mediator of apoptosis, during deadaptation was performed by immunohistochemistry and immunoblot analysis of isolated brain microvessels.

Immunoblot analysis showed that active caspase-3 (~17 kDa) was not detected in microvessel prepara-
tions from normoxic or hypoxic rats but only in microvessel samples from 7 and 14 days of deadaptation. At 21 days of deadaptation, active caspase-3 was no longer detected (Fig. 6A). For these experiments, whole tissue homogenates from newborn rat cerebellums were used as positive control. Immunohistochemistry only showed positive staining associated with capillaries at 7 and 14 days of deadaptation. No staining was detected in normoxic or 7-day hypoxia sections (not shown). In view of our results showing Ang2 expression during deadaptation, we performed double staining of Ang2 and active caspase-3 at 7 and 14 days of deadaptation. Our results showed colocalization of Ang2 and a cleaved caspase-3 signal in many capillaries (Fig. 6B).

DISCUSSION

The vascular network of the adult brain is relatively stable, and vascular endothelial cells exist in a differentiated, quiescent state and rarely proliferate (15). Microvascular remodeling occurs only in certain conditions, hypoxia and ischemia being strong inducers of brain angiogenesis (4, 19, 21, 34). In this study, we confirmed the previous finding that capillary density in the rat cerebral cortex was increased after 3 wk of hypobaric hypoxia (14, 21) and demonstrated that it decreased to prehypoxic values between 7 and 14 days of normoxic recovery. Moreover, we provide evidence that activation of programmed cell death plays a role in brain microvascular remodeling and that the Ang-Tie2 system is part of the cellular mechanism controlling this process.

In contrast to the large amount of information regarding the role of VEGF signaling in hypoxia-induced angiogenesis, much less is known about the regulation of the Ang-Tie2 system. It was reported that acute hypoxia (12–48 h) caused an increase of Ang2 mRNA expression in rat brain, whereas no changes or down-regulation of Ang1 and Tie2 mRNA were found (1, 24). In addition, induction of Ang1, Ang2, Tie1, and Tie2 expression after focal cerebral ischemia was previously reported (2, 22, 38). In this study, we found that prolonged mild hypoxia transiently induced Ang2 mRNA and protein expression in the brain cortex. Because upregulation of Ang2 protein occurred before mRNA levels were significantly affected, it is possible that in vivo regulation of Ang2 gene expression during hypoxia is a complex process involving an increase in mRNA levels as well as an increase in protein synthesis and/or stability. It was previously shown that HIF-1 regulates the expression of several angiogenic regulators such as VEGF (11). However, it is unknown whether this transcription factor also regulates Ang2 expression during hypoxia. Although glial and neuronal cells have been reported to express VEGF during hypoxia (25), in this study we demonstrated that Ang2 is expressed in brain endothelial cells. Thus hypoxia-
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Driven brain angiogenesis seems to require the coordinated interplay of various cell types expressing several angiogenic regulators (e.g., VEGF, Ang2). Similar to the HIF-1α and VEGF response (6), Ang2 induction subsided between 2 and 3 wk of hypoxia. This is compatible with previous speculation that the increase in brain microvascular density and other metabolic changes may restore normal tissue oxygen tension despite the persistent hypoxemia (6) and that a negative feedback mechanism decreases HIF-1, VEGF, and Ang2 expression to basal levels. Thus other angiogenic regulators might be necessary for the maintenance of increased capillary density as long as the hypoxic exposure persists. In contrast to Ang2, prolonged hypoxia did not affect the constitutive expression of Ang1 and Tie2 in the brain cortex. This is in agreement with previous reports showing that hypoxia induced Ang2 but not Ang1 or Tie2 expression in the rat brain and in cultured endothelial cells (24, 26).

Detailed expression analysis of Ang2 during the vascular remodeling process occurring in the ovary and in tumors showed association of Ang2 expression with vessel regression (16, 23, 37). Consistent with these findings, we provide evidence that during deadaptation, when capillary regression occurs, Ang2 mRNA and protein expression are dramatically induced whereas Ang1 and VEGF expression remain at basal levels. Ang2 induction subsided by 3 wk of deadaptation when vascular density was at its prehypoxic value. In addition, our results suggest that capillary regression occurring during deadaptation involves activation of programmed cell death. Evidence of endothelial cells undergoing apoptosis was found by TUNEL histochemical analysis and immunodetection of active caspase-3.

The mechanisms regulating the initiation and progression of vascular apoptosis during deadaptation are not known. Our results showing colocalization of cleaved caspase-3 and Ang2 in the brain endothelium during deadaptation suggest that Ang2 might mediate apoptotic death of vascular cells. This may occur through the interference of the Ang1-Tie2 survival signaling (16) and may be facilitated by the low basal levels of the survival factor VEGF.

Although our study did not explore the signaling pathways involved in Ang2 induction and initiation of vascular apoptosis, some potential mechanisms can be proposed. In our rat model of prolonged hypoxia and subsequent normoxic recovery, normal brain microvascular density was restored before hematocrit decreased to prehypoxic values. Thus it is possible that the persistent polycythemia and increased brain microvascular density during the initial phase of deadaptation produced a transient relative hypoxic condition in the tissue that might be the signal triggering Ang2 expression and vascular regression during deadaptation. Cortical tissue oxygen tension has not been measured under hypoxic conditions in hypoxia-adapted rats. However, cortical oxygen tension has been measured under normoxic conditions in rats previously adapted to 3 wk of hypobaric hypoxia, where it was shown to be increased compared with nonadapted rats (10). These results imply that oxygen delivery capacity is greatly increased after adaptation and that this might be responsible for a relative tissue hyperoxia on exposure to normoxia. Apoptosis in the adult brain has been described as generally associated with pathological conditions such as ischemia, traumatic injury, and chronic neurodegenerative diseases (3, 7, 36). Here we describe the physiological activation of an apoptotic response in the adult rat brain during recovery from chronic hypoxia as part of an intrinsic mechanism aimed to couple brain vascular density to tissue oxygen availability in the physiological range.

In summary, these results implicate Ang2 in the process of adult brain physiological vascular remodeling involving both angiogenesis and vascular regression. Because Ang2 is present in the brain during both adaptation and deadaptation to hypoxia, the fate of the endothelial cell, whether to undergo angiogenesis or apoptosis, must be determined by an additional angiogenic factor or factors.

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REFERENCES

12. Fujikawa K, de AS, I, Jain SK, Presman E, Christensen RA, and Varticovski L. Role of PI 3-kinase in angiopoietin-1


