

## Review

# Structural and functional adaptation to hypoxia in the rat brain

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### Summary

**Chronic exposure to a hypoxic environment leads to structural and functional adaptations in the rat brain. One significant adaptation is a decrease in intercapillary distances through a near doubling of the capillary density, which begins after about 1 week of hypoxic exposure and is completed by 3 weeks. Hypoxic angiogenesis is controlled by activation of downstream genes by Hypoxia Inducible Factor-1 and Angiopoietin-2. The processes that increase capillary density are reversible upon restoration of the ambient oxygen concentration. Capillary regression, which also occurs over a 3-week period, is accomplished**

**through activation of apoptosis. The implication from these observations is that the brain naturally functions in a low, but controlled, oxygen environment. Acute imbalances in oxygen delivery and metabolic demand are addressed through changes in blood flow; persistent imbalances activate mechanisms that adjust capillary density. The mechanisms that control these processes decline with age.**

Key words: hypoxia, rat brain, angiogenesis, capillary regression, apoptosis.

### Introduction

Prolonged exposure to low oxygen environments provokes well known systemic cardiovascular and respiratory adaptations (Lenfant and Sullivan, 1971), as listed in Table 1A, in order to maintain oxygen delivery to the brain (LaManna et al., 1992). The decreasing partial pressure of atmospheric oxygen with increasing altitude results in decreasing arterial hemoglobin saturation (hypoxemia) that is compensated for by increased cerebral blood flow.

Successful long-term adaptation is possible when hypoxemia is 'mild' (i.e. when arterial oxygen partial pressure,  $P_{aO_2}$ , is greater than ~6 kPa). These adaptations allow mammals to reside permanently at altitudes up to 4–5 km, and include increased ventilation, bicarbonate excretion, increased packed red cell volume (Hct) and weight loss, *inter alia*. For example, ventilatory rates more than double in rats exposed for a week or more to a pressure equivalent to half that at sea level (0.5 atm; 1 atm=1.013×10<sup>5</sup> Pa), approximating the atmospheric oxygen partial pressure at an altitude of 5500 m (Fig. 1). The increased ventilation results in arterial carbon dioxide partial pressures ( $P_{aCO_2}$ ) below 3 kPa (LaManna et al., 1992), which would result in severe respiratory alkalosis were it not for an increased renal bicarbonate excretion that maintains acid–base balance.

Body temperature also falls, at least transiently, suggesting decreased metabolism. The initial fall in temperature of a few °C and lasting for at least a few days has been well described (Mortola, 1993; Mortola et al., 1994; Wood, 1991; Wood and

Gonzales, 1996). Whether or not there is a longer term temperature depression has not been established. Fig. 2 shows the response of a single rat to continuous mild hypoxia (10% oxygen in nitrogen). After the initial transient fall there appears to be a residual depression of a few tenths of a °C in both the temperature recorded during the quiescent period during the day with the lights on, and in the active period during the night with the lights off. But, whether or not this small drop is associated with significant hypometabolism is unclear.

Taken together, these responses result in normal arterial oxygen delivery (ml O<sub>2</sub> g<sup>-1</sup> min<sup>-1</sup>). Nevertheless, these systemic adaptations cannot by themselves restore tissue oxygen status because the driving force of tissue oxygen transport is the gradient between capillary oxygen partial pressure ( $P_{cO_2}$ ) and tissue oxygen partial pressure ( $P_{tO_2}$ ). Thus, to restore  $P_{tO_2}$ , the reduced  $P_{cO_2}$  must be balanced by a decrease in intercapillary distance, and/or a decreased tissue oxygen consumption.

### Rat brain adaptations to mild hypoxia

The remainder of this review deals specifically with the rat model of chronic hypoxic exposure. The rat is a reasonable choice as a general mammalian model, and specifically as a human analog (Olson and Dempsey, 1978). Nevertheless, is important to keep firmly in mind that there are various species idiosyncrasies that have a significant impact on the

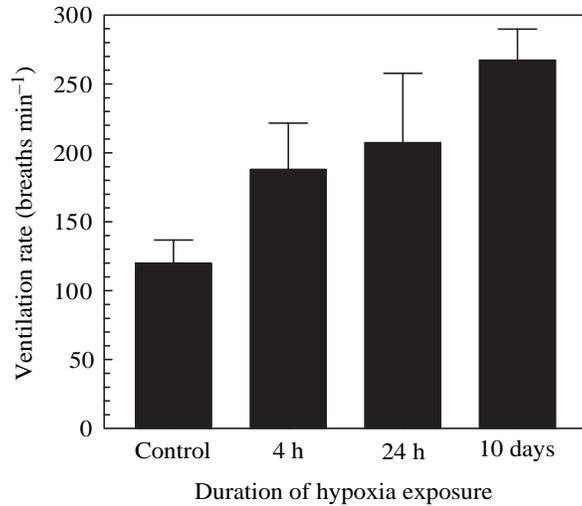


Fig. 1. Ventilation rate of rats (means  $\pm$  s.d.) placed in a plethysmograph (Buxco, Inc., Wilmington, NC, USA) after various periods of exposure to 10% oxygen (balance nitrogen).  $N=3$  rats per group (LaManna et al., unpublished observations).

interpretation of the mechanisms controlling hypoxic adaptation. For example, the hypoxic response will vary if the species is ruminant, or hibernates, or is found naturally at altitude. Less well appreciated are the differences introduced

Table 1. *Effects of hypoxia on animals*

(A) Major systemic adaptations to prolonged mild hypoxia

1. Increased ventilation
2. Core temperature fall
3. Right shift of hemoglobin dissociation curve
4. Bicarbonate ion secretion
5. Increased packed red cell volume (hematocrit)
6. Body mass loss

(B) Acute effects of hypoxia on CNS

1. Hemoglobin disoxygenation
2. Decreased tissue  $P_{O_2}$  (left shift of histogram)
3. Increased cerebral blood volume (vasodilation)
4. Faster capillary mean transit time
5. Increased cerebral blood flow
6. Increased cerebral metabolic rate for glucose (CMRglu)
7. EEG abnormalities

(C) Major CNS adaptations to prolonged mild hypoxia

1. Transient CBF increase
2. Vascular remodeling
3. Increased glucose transport across the blood–brain barrier and CMRglu
4. Increased glycolysis to maintain tissue acid–base balance
5. Decreased mitochondrial energy consumption
6. Restoration of tissue oxygen tension profiles

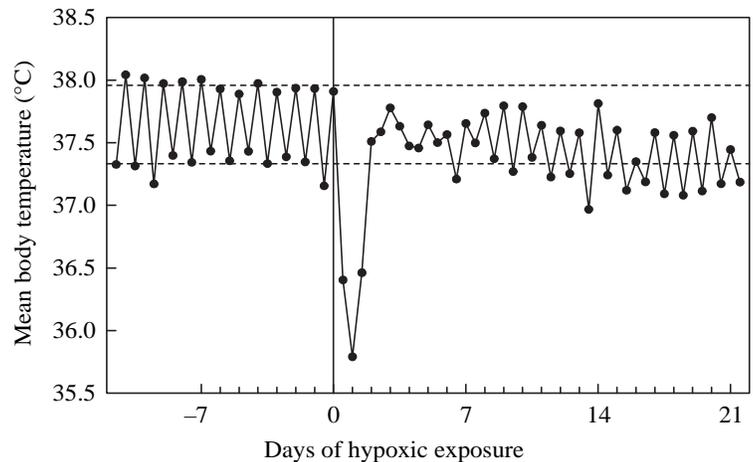


Fig. 2. Mean day (lower) and night time (upper) body temperature changes, recorded through an implantable sensor and transmitter system (Mini Mitter, Co. Inc., Bend, OR, USA), in a single rat exposed to hypobaric hypoxia initiated at Day 0. For each daily cycle, the higher temperature is recorded during the dark, behavioral active phase (night). After an initial drop and loss of diurnal rhythm, there is a recovery within 1 week and then a secondary minor down trend of about  $0.015^{\circ}\text{C day}^{-1}$  (LaManna et al., unpublished observations).

by the size of the animal, where issues of scaling are important. For example, the oxygen dissociation curve, energy metabolism and blood flow all scale with body mass, but blood volume, blood pressure and hematocrit do not (Schmidt-Nielsen, 1984).

*Acute brain effects of hypoxia*

As alluded to previously, in contrast to the systemic adaptations (see Table 1), the organ-specific adaptations are primarily found in the capillary bed and in the metabolic machinery of the tissue. In the mammalian central nervous system, the effects of hypoxia must be understood with respect to the unique features of cerebrovascular and metabolic physiology. In the mammalian brain, tissue oxygen tension  $P_{tO_2}$  varies spatially and temporally. Under resting, normoxic conditions, there is a distribution of brain  $P_{tO_2}$  that is significantly left-shifted compared to the more normal distribution found, e.g. in reptile brain (Fig. 3). That is, the mean  $P_{tO_2}$  is less than the venous partial pressure of oxygen ( $P_{vO_2}$ ), and there are many very low values (Sick et al., 1982). The low mean  $P_{tO_2}$  can theoretically only result if (1) the blood flow rate through the capillary is faster than the hemoglobin oxygen unloading time, which is unlikely since the capillary mean transit time in the rat is about 2 s; or (2) the blood flow to the tissue is limiting, either because it is at maximum or, because blood flow to many regions is controlled at a low resting perfusion rate. However, it cannot be due to the failure of blood flow or limitations in the capillary network, because with neuronal activation there are increases in blood flow and tissue oxygen tension. In fact, this transient hyperemic oxygenation is the physiological basis for the technique of functional magnetic resonance imaging (fMRI), although the

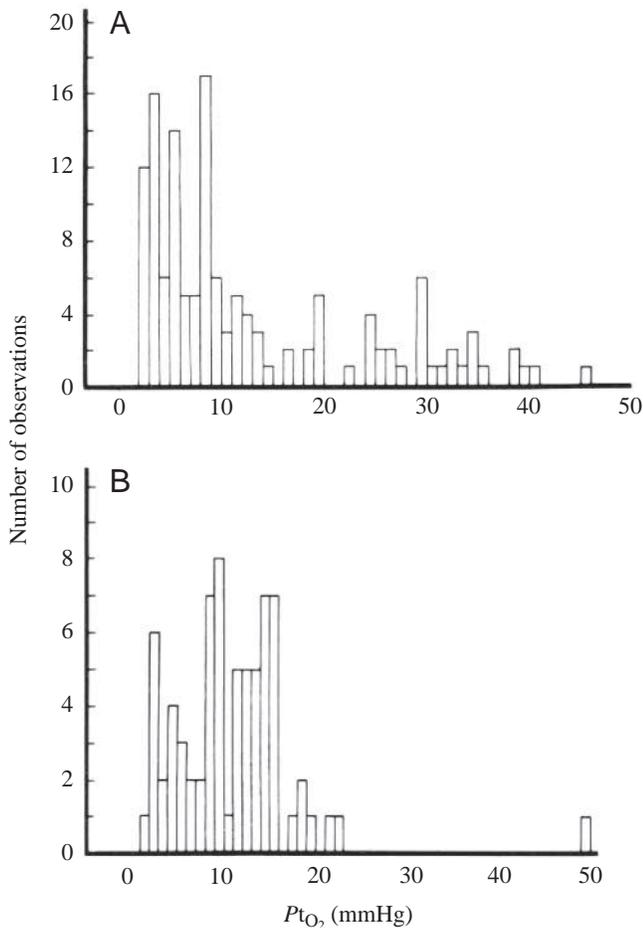


Fig. 3. (A) Histogram of  $O_2$  partial pressure in rat cerebral cortex ( $P_{tO_2}$ ) obtained by making multiple cortical penetrations (in 100  $\mu$ m steps) with an  $O_2$ -sensitive platinum microelectrode during normoxia. The histogram was constructed from 136 measurements taken from 13 rats. (B) Histogram of  $P_{tO_2}$  taken from telencephalon of the freshwater diving turtle (*P. scripta*). Histogram constructed from 71 samples from 11 turtles. The class interval in both A and B is 1 torr. Reprinted by permission from Sick et al. (1982).

mechanism for the hyperemia remains unknown. Thus, under normal control conditions the tissue oxygen is kept low (perhaps as an anti-oxygen toxicity mechanism), and this suggests that the mammalian central nervous system (CNS) can tolerate low oxygen environments, except of course when neuronal activity is increased. Neuronal activity is coupled to increased energy metabolism, and increased energy metabolism is linked to increased blood flow. It appears that even in normoxic environments, the brain subsists in a predominantly low-oxygen milieu, which is maintained physiologically. Thus, for the brain it appears that oxygen supply is regulated by the 'just sufficient' principle. That is, the oxygen tension is maintained at a level just sufficient for metabolic needs – no more, no less. The condition of hypoxia cannot be defined by ambient oxygen levels, but only occurs in the CNS when metabolic demand cannot be met by matched  $O_2$  delivery, either falling below a threshold at 'rest' in severe

hypoxia, or failing to increase during increased episodic activity. Thus, the mammalian brain might be considered to be hypoxia tolerant, but activity intolerant.

In the central nervous system, there are significant alterations in energy metabolism and the cerebrovasculature that act to preserve tissue oxygen and the energy supply needed to support optimal neuronal function (see Table 1B). Immediately with the onset of hypoxic exposure, cerebral blood flow (CBF) increases (despite decreased  $P_{aCO_2}$  which, in the absence of hypoxia, results in a vasoconstriction-induced fall in blood flow), and glucose consumption increases (Beck and Kriegstein, 1987), but  $P_{tO_2}$  still falls (Sick et al., 1982). The blood flow increase induced by hypoxia is at least partially due to central brainstem neuronal mechanisms (Golanov et al., 2001; Sun and Reis, 1994), but neuronal function, although altered, is not compromised (as long as  $P_{aO_2}$  remains above about 50 torr), and there are still hyperemic responses with neuronal stimulation (LaManna et al., 1984; Lindauer et al., 2003).

#### Chronic effects of hypoxia

Despite the acute hypoxic responses that appear to preserve neuronal function, there is obviously some residual hypoxic signal which, if maintained for any length of time, triggers structural changes that act to restore the baseline  $P_{tO_2}$ . In the case of ambient hypoxia, the physiological responses that increase the arterial oxygen content and minute delivery of oxygen cannot restore the tissue oxygen levels because these are determined, according to the Fick principle, primarily by the concentration gradient and the diffusion distance. The concentration gradient is mainly determined by the  $P_{aO_2}$  since tissue levels are very low already. Thus, the adaptive structural change in response to prolonged hypoxia is an increase in capillary density, which results in a decreased intercapillary distance and, therefore, a decreased diffusion distance. In the rat brain, the capillary density almost doubles, and the average intercapillary distance decreases from about 50 to about 40  $\mu$ m (Lauro and LaManna, 1997).

In the rat, by 3 weeks of adaptation, blood flow returns to the baseline range (LaManna et al., 1992); the initial hypoxic-induced increased flow returns to baseline by 5 days, concomitant with the increasing hematocrit, and consistent with the idea that CBF is controlled by oxygen content (Brown et al., 1985). Glucose consumption is only slightly elevated (about 15%) (Harik et al., 1995b), and tissue oxygen tension is restored (Chávez et al., 2000; Dunn et al., 2000). The rather small increase in glycolysis cannot substantively increase ATP production. It is more likely instead that the increase in glycolysis functions to balance the tissue acid-base disturbances, which are caused by hyperventilation induced decreased  $P_{aCO_2}$  (Lauro and LaManna, 1997). It is unknown whether brain oxygen consumption is depressed in hypoxic-adapted rats; oxygen consumption has been reported to be unchanged in humans at altitude (Moller et al., 2002), but rat brain cytochrome oxidase is decreased by about 15% (Caceda et al., 2001; Chávez et al., 1995; LaManna et al., 1996), and

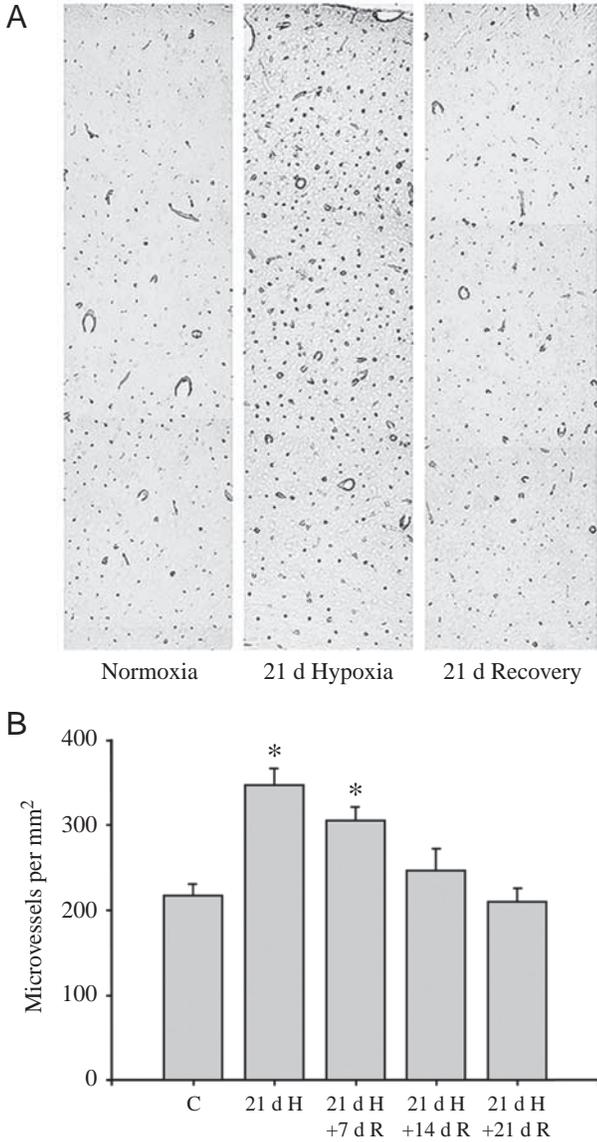


Fig. 4. Changes in cortical capillary density during prolonged hypoxia and deadaptation. (A) Composite photomicrograph of GLUT-1-stained sections spanning part of the parietal cortex at normoxia, 21 days (21 d) of hypoxia, and normoxic recovery for 21 days. (B) Capillary density analysis of GLUT-1-stained sections showing a significant increase at 21 days of hypoxia (21 d H). Subsequent normoxic recovery (7 d R, 14 d R, or 21 d R) caused reestablishment of prehypoxic capillary density. Values are means  $\pm$  S.D.;  $N=4$  rats in each group. \* $P<0.05$  compared with controls (C). Reprinted with permission from Pichiule and LaManna (2002).

neuronal mitochondrial density is lower (Stewart et al., 1997). The long term CNS structural and functional adjustments to prolonged hypoxia are listed in Table 1C.

**Molecular mechanisms of hypoxic adaptation**

The mechanisms responsible for matching capillary density to tissue oxygen levels are not unique to ambient hypoxic stimuli. Rather, these processes appear to be responsible for maintaining the balance between oxygen availability and neuronal metabolic demand. For example, this system probably underlies the capillary density changes observed with sensory or motor training (Black et al., 1987, 1991; Isaacs et al., 1992). Thus, there is a direct relationship between local capillary density, local blood flow and local metabolic rate.

The observed robust, hypoxia-induced angiogenesis was initially surprising, since conventional wisdom held the structural components of the mammalian brain as fixed and immutable except for pathological deterioration. There had been a few earlier observations and suggestions of hypoxia-induced capillary increases (Diemer and Henn, 1965; Miller, Jr and Hale, 1970; Opitz, 1951), but only more recently have these observations been confirmed and extended (Boero et al., 1999; Harik et al., 1995a; Mironov et al., 1994; Pichiule and LaManna, 2002).

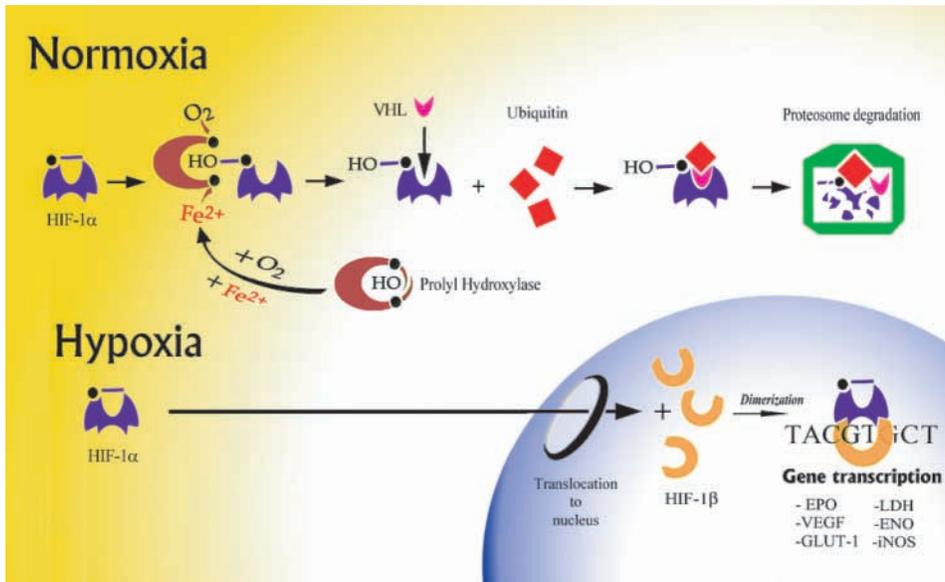


Fig. 5. This scheme represents the current concept for hypoxia-inducible factor-1 (HIF-1) pathways. In normoxic conditions, HIF-1 $\alpha$  is continuously produced and, in the presence of Fe<sup>2+</sup> and oxygen, is continuously degraded through the Von Hippel Lindau protein (VHL)/Ubiquitin/Proteasome pathway. When oxygen becomes limiting, prolyl hydroxylase is inhibited and HIF-1 $\alpha$  accumulates. After translocation and combination with the constitutive HIF-1 $\alpha$ , the transcription factor binds to hypoxic response element (HRE) sequences to upregulate a number of downstream genes such as erythropoietin (EPO), VEGF, lactate dehydrogenase (LDH), inducible nitric oxide synthase (iNOS), GLUT-1 and enolase-1 (ENO). (Drawing by Max Neal.)

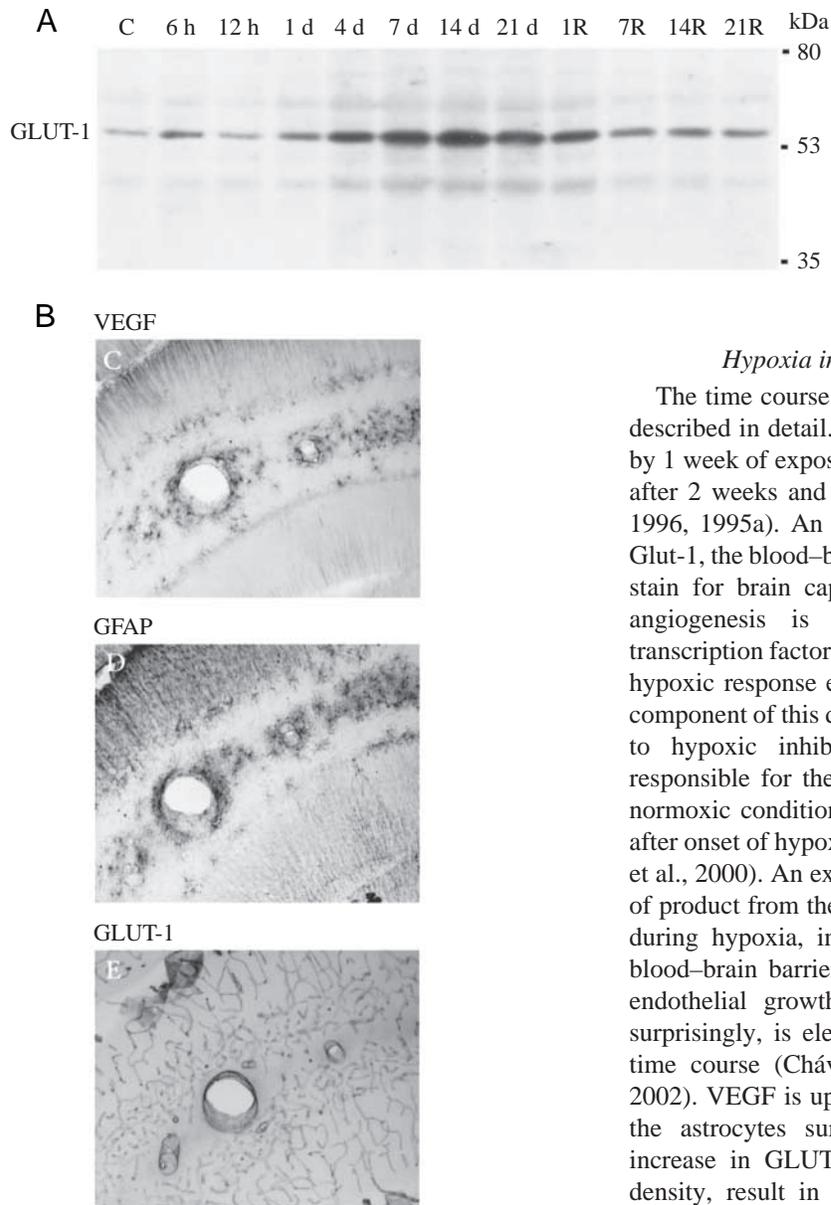


Fig. 6. (A) The glucose transporter at the blood–brain barrier, GLUT-1, is upregulated in rat brain in response to hypobaric hypoxia from 6 h (6h) to 21 days (21d), reaching a maximum between 1 and 2 weeks. After return to normoxia over 3 weeks (1R – 21R) there is a return towards control levels of the GLUT-1 protein. (B) Immunohistochemistry of serial sections from rat cerebellar cortex suggesting that VEGF is upregulated in cells that are also positive for the astrocyte specific marker glial fibrillary acidic protein (GFAP), rather than the capillary endothelial cells that are positive for the glucose transporter (GLUT-1) (LaManna et al., unpublished observations).

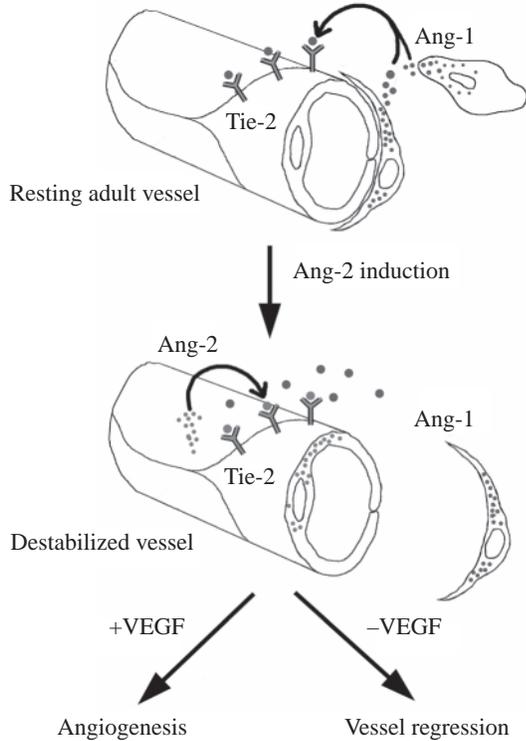
Capillary restructuring requires at least a week, and thus does not play a part in transient disturbances to the balance between oxygen delivery and energy demand. It might be concluded that blood flow alterations serve acute changes in oxygen delivery, while persistent changes are accounted for by

capillary density adjustments. There have been many mechanisms of oxygen sensing proposed, such as heme-containing proteins and oxygen-dependent potassium channels, (Lahiri, 2000; Lopez-Barneo et al., 2004; Wenger, 2000). This review will focus on the role of hypoxia inducible factor-1 as a tissue hypoxia sensor.

#### *Hypoxia inducible factor-1 and angiogenesis*

The time course of hypoxic-induced angiogenesis has been described in detail. Capillary density counts begin to increase by 1 week of exposure and the process is completed sometime after 2 weeks and before 3 weeks of exposure (Harik et al., 1996, 1995a). An example of this result is shown in Fig. 4. Glut-1, the blood–brain barrier glucose transporter, is a specific stain for brain capillaries. A likely candidate for initiating angiogenesis is Hypoxia-inducible factor-1 (HIF-1), a transcription factor that activates many downstream genes with hypoxic response elements (HRE) (Fig. 5). Briefly, the alpha component of this dimeric factor accumulates immediately due to hypoxic inhibition of prolyl hydroxylase, which is responsible for the continuous degradation of HIF-1 $\alpha$  under normoxic conditions. HIF-1 $\alpha$  is detected in the brain shortly after onset of hypoxia and persists for at least 2 weeks (Chávez et al., 2000). An example of the time course of the appearance of product from the induction of a downstream gene activated during hypoxia, in this case the glucose transporter at the blood–brain barrier (GLUT-1), is shown in Fig. 6. Vascular endothelial growth factor (VEGF) has an HRE and, not surprisingly, is elevated during hypoxia with an appropriate time course (Chávez et al., 2000; Pichiule and LaManna, 2002). VEGF is upregulated primarily in the glial end feet of the astrocytes surrounding the capillaries (Fig. 6B). The increase in GLUT-1 protein, and the increase in capillary density, result in an increase in the transport capacity for glucose transport across the blood–brain barrier. In the rat, this probably compensates for the increased glucose consumption in hypoxia and the fact that the plasma flow rate in the hypoxic adapted rats is half of the normoxic rate. The decreased plasma flow rate is a direct consequence of the normalization of the blood flow rate at the same time as the 50% increase in Hct. Only plasma glucose is available for blood–brain barrier transport in the rat because the rat erythrocyte lacks the GLUT-1 transporter (Dick et al., 1984).

The process of brain angiogenesis apparently also requires the cooperation of an additional, non-HIF-1 activated factor, angiopoietin-2 (Ang-2). Ang-2, which is not present under normoxic conditions, is induced after hypoxia and acts at an endothelial cell surface receptor tyrosine kinase, the Tie-2 receptor (Pichiule and LaManna, 2002). Normally Ang-1, generated from pericytes and other nearby cellular sources, occupies the Tie-2 receptor, maintaining structural integrity of the capillary. After induction of Ang-2 expression in



endothelial cells, Tie-2 receptor activity is blocked, pericytes pull away from the capillary and the microvessel is destabilized. In the presence of a growth factor such as VEGF, angiogenesis will be initiated. But, in the absence of growth factors, capillaries will undergo an apoptotic regression. The mechanism is shown in schematic form in Fig. 7.

Fig. 7. Scheme of the proposed relationship between VEGF and Ang-2 in controlling capillary density. The constitutive Ang-1, produced in pericytes and neighboring cells, normally maintains mechanical stability of the capillary through interaction with the Tie-2 receptor. Upregulated Ang-2, occurring during changes in oxygen availability, binds to the Tie-2 receptor without phosphorylation/activation, blocking Ang-1, releasing the pericyte and destabilizing the capillary. During hypoxic exposure, in the presence of VEGF, there is endothelial cell proliferation and angiogenesis. But, during reoxygenation after hypoxic adaptation in the absence of VEGF there is apoptosis and vessel regression. (Drawn by P. Pichiule, based on the hypothesis presented by Holash et al., 1999.)

*Deadaptation and aging*

The significance of the Ang-2 contribution to the control of capillary density can be appreciated by consideration of the process of deadaptation after return to normoxia (Fig. 4). When rats that have been previously exposed to hypoxia for three weeks are then reintroduced to a normoxic environment, there is a gradual loss of capillaries until the original capillary density is restored (Harik et al., 1996). This capillary regression is associated with increased Ang-2 and is accomplished through an apoptotic process (Pichiule and LaManna, 2002). Thus, it is apparent that Ang-2 is necessary for capillary restructuring (Fig. 7).

The upregulation of Ang-2 during hypoxia and during reoxygenation is due to induction of cyclooxygenase-2 enzyme activity in brain endothelial cells (Pichiule et al., 2004). This enzyme is responsible for metabolism of arachidonic acid to prostaglandin E2, which induces Ang-2 production.

The presence of physiological mechanisms for increasing and decreasing capillary density gives more support to the idea that there is a continuous matching of capillary density/structure and tissue oxygen balance. The identification of the mechanism of capillary regression is important because it may be the first example of physiological apoptosis in the adult mammal. Therefore, activation of apoptotic pathways after pathological stimuli such as ischemia could be from augmentation of intrinsic mechanisms as opposed to initiation of dormant processes.

Finally, we should note that if indeed there are mechanisms responsible for continuous maintenance of the capillary density in the brain, then there is the possibility that interference with these mechanisms could lead to pathological circumstances. In this regard, it is interesting to consider the finding that the responsiveness of HIF-1 to hypoxia, but not to cobalt chloride, wanes with age (Fig. 8) (Chavez and LaManna, 2003). It is possible that the lack of HIF-1 response results in the lack of the ability to adapt to hypoxia and the diminution in the ability to match neuronal activity and capillary density (Black et al., 1989), and thus the inability to maintain capillary density, results in lack of plasticity, with all the

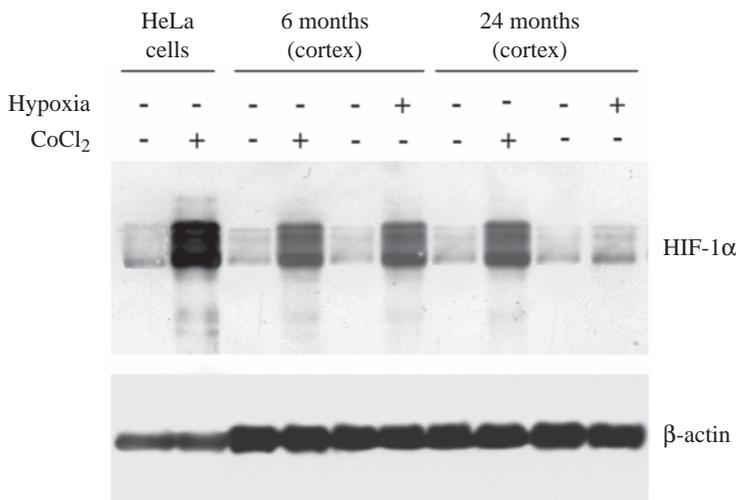


Fig. 8. HIF-1 $\alpha$  accumulates *in vivo* in response to both hypoxic hypoxia and cobalt chloride, administered intraperitoneally, in the 6 month rat brain cortex. At 24 months, however, there is greatly diminished accumulation of HIF-1 $\alpha$  under hypoxic conditions, but the response to cobalt chloride is still robust.  $\beta$ -actin was used as a loading control (Chavez and LaManna, 2003).

consequences that might be expected for learning, training and even neuronal survival.

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