Hypoxia in the central nervous system

Joseph C. LaManna

Department of Anatomy, Case Western Reserve University, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-4930 U.S.A.

Abstract

The brain, as a very high energy consumer, is completely reliant on molecular oxygen but because oxygen is dangerous due to toxicity [1], there are mechanisms which allow the brain to exist under low oxygen conditions when ‘idling’ but increase oxygen delivery when activated. This situation means that the brain can respond naturally to mild hypoxia with acute and chronic adaptive mechanisms. These mechanisms involve systemic and central metabolic and vascular processes that are mediated by hypoxia-inducible factor (HIF)-1. HIF-1-mediated cerebral angiogenesis is completed within 3 weeks of exposure onset and is reversible over the same time frame if normoxia is restored. Hypoxic acclimatizing responses may be significantly impaired with aging and metabolic or vascular disease.

Introduction

The mammalian brain depends completely on continuous delivery of oxygen to maintain its function. Because mammals populate varied environmental niches they are exposed to variable ambient oxygen levels. Therefore it should not be surprising that there are numerous intrinsic and extrinsic mechanisms that maintain a suitable level of oxygen availability to the neurons and other cells of the central nervous system. Hypoxia is any decrease in the availability of oxygen. When used alone, the term refers to a decrease in the $PO_2$ (partial pressure of oxygen).
of oxygen) in the ambient air from the mean sea level value of about 21 kPa (dry gas). In normal physiology, the $P_{O_2}$ in the pulmonary vein is about 14 kPa due to the contribution of water vapour and carbon dioxide. Most of the oxygen is carried by haemoglobin and at these partial pressures, haemoglobin is fully saturated. Even if ambient oxygen decreases a little, haemoglobin remains fairly well-saturated and the tissue oxygen availability remains the same. When the arterial $P_{O_2}$ falls below 12 kPa, which can occur through decreases in the $F_iO_2$ (fraction inspired oxygen), decreased barometric pressure such as increasing altitude, or through lung pathology (pulmonary hypoxia), then a condition of hypoxaemia (lower blood oxygen) occurs. A decrease in the oxygen availability to the tissue such as that which occurs in uncompensated hypoxaemia, anaemia (anaemic hypoxia, i.e. low haemoglobin and thus lower oxygen-carrying capacity), carbon monoxide (toxic hypoxia), or blood flow restriction (ischaemic hypoxia), all result in activation of local tissue response mechanisms.

For acute hypoxic exposures, if blood oxygen is below 12 kPa, but above 6 kPa, then the hypoxia is mild and can be compensated for by normal physiological processes, and usually does not lead to any tissue damage. Thus there are no problems for humans up to about 3000 m. In the range of 4–6 kPa, the hypoxia is moderate and is partially compensated, but there is good chance of permanent damage. Below 4 kPa, hypoxia is severe and loss of consciousness and neuronal degeneration ensues.

If mild hypoxia persists, long-term compensatory responses change the scenario. These acclimatizing adaptations may take days to weeks to become established, but then allow habitation at moderate hypoxia and brief periods of severe hypoxia with far less damage than before acclimatization. Thus, for example, a climber without supplementary oxygen might reach the peak at Mount Everest (elevation 8850 m), which is at an approximate pressure of one third of an atmosphere (about 34 kPa), with an estimated pulmonary alveolar $P_{O_2}$ of 4.7 kPa, and an estimated arterial $P_{O_2}$ of only 2.8 kPa.

Chronic exposure to the hypoxia of altitude over generations leads to selection for adaptations that increase suitability to the high-altitude environment. These adaptations allow human populations to inhabit areas above 4000 m in the Andes, in the Himalayas and in Ethiopia. Similarly, there are many other mammalian species that are adapted to even higher altitudes. Interestingly, the adaptations of these different species and human populations are varied.

As implied above, hypoxia itself is not necessarily a pathological condition. Indeed, the very reactivity that makes oxygen useful for energy-producing reactions bestows a potential for toxicity that must be accounted for by the organism. One of these protective strategies is to limit the exposure of the tissue to oxygen except for the amount needed to drive activity-induced metabolism. Thus the mammalian brain usually exists in a low tissue oxygen milieu. Local tissue mechanisms ensure that the oxygen environment is controlled, not maximized. These physiological considerations underlie the fMRI (functional
magnetic resonance imaging) phenomenon known as BOLD (blood oxygen level dependent) response to a focal activation task, that is proving to be a valuable tool for understanding brain function and has promise for pathological diagnosis and treatment monitoring.

This chapter concerns itself with the brain responses to mild hypoxia, that is, to physiological hypoxia. This is the range of hypoxia that can be compensated for with physiological mechanisms. More severe hypoxia leads to pathology and cell death due to failure of these compensation mechanisms and subsequent energy depletion.

**Brain oxygen**

The pathway for oxygen can be summarized by the diagram in Figure 1 [2]. There are three main levels at which adaptation to prolonged mild hypoxia can occur: the lung, the circulation and at the tissue level itself, in this case the brain parenchyma. The changes in the lung include an increase in diffusing capacity [3,4] and an increase in minute ventilation [5] (driven by carotid body oxygen-sensing cells through brain stem nuclei and phrenic output). Also there is pulmonary vasoconstriction and pulmonary hypertension (which lead to right heart hypertrophy), and vascular remodelling [6]. The circulatory contribution
includes increased cardiac output, increased haemoglobin concentration and a right-shifted haemoglobin saturation curve. In the tissue, adaptations include increased capillary surface area, decreased energy demand and increased energy production efficiency.

The systemic mechanisms are fairly well understood. The lung, heart and kidney participate in the maintenance of oxygen delivery, defined as the product of blood oxygen content and blood flow, to the brain parenchyma through the level of the end arteriole.

Other systemic responses to prolonged mild hypoxia include decreased core temperature, bicarbonate ion excretion by the kidney to balance the blood alkalosis due to hyperventilation-induced carbon dioxide loss and body mass loss.

**The effect of acute hypoxia in brain**

The brain tissue parenchyma exists in a low oxygen environment. Quantitative measurements of brain tissue PO$_2$ by polarographic microelectrodes have shown that the distribution of oxygen tensions in brain is asymmetrical, shifted towards the lower concentrations, and best described with a log normal fit [7]. An example of the PO$_2$ distribution histogram is shown in Figure 2(A). Plotting these data as a cumulative occurrence function, as shown in Figure 2(B), makes it easier to observe that more than two-thirds of the values are below 2 kPa, and 90% are below the venous PO$_2$ partial pressure of 5 kPa. Decreases in inspired oxygen produce decreases in tissue PO$_2$. The response to, in this case eucapnic, hypoxia is shown in Figure 3. The capillary blood haemoglobin saturation falls, and with it the tissue PO$_2$. This decrease occurs despite an increased blood volume and faster capillary mean transit times as a result of vasodilation and increased cerebral blood flow [8]. The blood flow increase in mild hypoxia is at least partially mediated via oxygen-sensing neurons in the rostral ventrolateral medulla [9].

The failure of the acute central and systemic hypoxic responses to preserve tissue oxygen tension is a consequence of the limitations of oxygen diffusion from the capillaries to the tissue. Oxygen diffusion follows Fick’s law which means that the flux at any distance from the red cell in the capillary is dependent on the concentration gradient for oxygen. Because tissue PO$_2$ is small, the oxygen flux rate is essentially dependent on the capillary PO$_2$. Thus, even though oxygen delivery in terms of molecules of oxygen per min at the end arteriole can be preserved, tissue oxygen tension must fall because arterial PO$_2$ has fallen.

Two responses to hypoxia are activated that resist this fall. With hypoxia there is an increase in ventilation rate. This is driven by the carotid body chemosensor pathway through brain stem nuclei. Ventilation rate increases acutely, then is maintained. An example is shown in Figure 4. Hyperventilation results in a decreased PCO$_2$ (partial pressure of carbon dioxide) which allows the arterial oxygen tension to rise equivalently. This results in an increase of about...
10% in the oxygen-driving force at the arterial end of the capillary. The carotid body responds to $P_{O_2}$ [5], or at least, the minute ventilation continues to be inversely proportional to the $P_{O_2}$.

The other adaptation has a slower onset occurring only after weeks of exposure [10]. If mild hypoxic exposure is maintained, angiogenesis is initiated which results in an increased capillary branching and density which results in decreased intercapillary distances and thus decreased diffusion distances, restoring tissue oxygen tensions to baseline.

Figure 2. Cerebral cortical tissue oxygen distribution
(A) Histogram distribution of tissue oxygen tension measurements made from the cerebral cortices of 13 anaesthetized rats. The data have been replotted from [7]. A log normal distribution is indicated by the line. (B) The same data plotted as a cumulative distribution showing the $P_{O_2}$ for the 50 and 90 percentiles.
Figure 3. Hypoxia-induced acute changes in blood volume, haemoglobin saturation and tissue oxygen tension
The response to forced eucapnic ventilation with 10% oxygen on the oxygen tension, measured by an oxygen-sensitive platinum polarographic microelectrode; blood volume and tissue capillary haemoglobin saturation, measured by in vivo reflectance spectrophotometry; and the surface electrocorticogram, measured by the Ag-AgCl reference on the polarographic electrode, in anaesthetized rat cortex. This figure is from previously unpublished work collected during the studies reported in [43].

Figure 4. Blood oxygen saturation (black) and ventilation rate (blue) measured non-invasively in a restrained rat placed in a 10% oxygen environment
Chronic exposure

A primary player in hypoxic angiogenesis is hypoxia-inducible factor (HIF)-1α [11]. HIF-1 is a heterodimeric transcription factor with a constitutive HIF-1β, identical with ARNT (aryl hydrocarbon receptor nuclear translocator), and an inducible component HIF-1α. The levels of HIF-1α increase with hypoxia due to the inhibition of the oxygen requiring PHD (prolyl hydroxylase) in the breakdown pathway, and can thus serve as an oxygen sensor [12]. HIF-1 is a transcription factor that activates over 40 known genes that have an HRE (hypoxic-response element) in their promoter region. VEGF (vascular endothelial growth factor) is one such molecule that is upregulated by HIF-1 and initiates capillary angiogenesis.

Angiogenesis begins within the first week of hypoxic exposure and continues for at least another week. By 3 weeks the remodelling is complete. The result is an increase in capillary density. An example is shown in Figure 5.

HIF-1α remains responsive to tissue $PO_2$, it does not adapt. The HIF-1 signal is thus maintained until the tissue $PO_2$ is restored by angiogenesis.

Competent angiogenesis also requires participation by a second, non-HIF-1-dependent mechanism that involves up-regulation of COX-2 (cyclo-oxygenase-2) and subsequent up-regulation of angiopoietin-2 via PGE2 (prostaglandin E2) receptor activation [13,14]. The mechanism of up-regulation of COX-2 in hypoxia is not yet known.

Systemically, HIF-1 is responsible for hypoxic erythropoiesis. HIF-1 accumulation due to hypoxia in the kidney results in up-regulation of EPO (erythropoietin) that results in an increase, over days to a week, in packed red cell volume, that contributes to the oxygen-carrying capacity of the arterial blood [15]. In rats exposed to an equivalent altitude of 5500 m, where the $PO_2$ is about half that at sea level, the packed red cell volume is increased to 70% or more compared with less than 50% at sea level. As the oxygen-carrying capacity increases, the cerebral blood flow falls back to pre-exposure levels. This

![Mouse brain cortex](image)

Figure 5. A section from the cortex of a mouse exposed for 3 weeks to hypobaric hypoxia compared with a littermate kept in normoxic conditions

The hypoxic cortex shows a higher density of capillaries (stained immunohistologically for GLUT1, which is a specific marker for brain capillaries, at the blood–brain barrier).
occurs before brain tissue oxygen tension has been restored. Thus the sensor for this mechanism must be responsive to arterial oxygen content, not PO₂ in the blood or tissue [16].

Almost all of the enzymes involved in glycolysis have an HRE and are up-regulated in prolonged mild hypoxia [17]. The cerebral metabolic rate for glucose is elevated in both acute [8,18] and chronic hypoxia [19]. The increase of about 10–20% in CMRglu (cerebral metabolic rate for glucose) would contribute a relatively small amount to energy needs of the cells. Glycolysis produces almost twenty times less ATP per glucose molecule than oxidative phosphorylation, so a 20% increase would be negligible. Nevertheless, the increase in glycolysis appears to balance the tissue pH which would otherwise tend to become alkalotic due to the decrease in arterial PCO₂ [20]. Systemic alkalosis is balanced by secretion of bicarbonate in the kidney, but in the central nervous system alkalosis is offset by increased glycolytic ATP production. In oxidative phosphorylation, a proton is consumed when ATP is synthesized. In glycolysis, there is no net production or consumption of protons. When ATP is hydrolysed in energy-requiring reactions, a proton is produced. Thus utilization of ATP produced from oxidative phosphorylation is pH neutral and consumption of ATP from glycolysis is acid-producing [21]. Since glycolysis provides the substrate for oxidative phosphorylation, there are always some protons being produced, but an increase in glycolysis without a corresponding increase in oxidative phosphorylation will increase acid production, and this is demonstrated by an increase in brain lactate concentration.

Not only does the glycolytic rate increase, but the transport of glucose at the blood–brain barrier is also increased [22]. The GLUT1 (glucose transporter 1), responsible for carrier-mediated facilitated diffusion at the blood–brain barrier is also up-regulated by HIF-1 [11]. In the rat, the increase in glucose transporter and the increase in capillary density result in a 3-fold increase in glucose flux rate capacity in hypoxic-adapted rats. The large disparity in the increased transport capacity compared with the increased CMRglu can be explained by the net reduction by half of the arterial glucose delivery. In species such as the rat (but not humans) that lack GLUT1 in circulating red blood cells, the source of glucose available for transport is limited to the glucose in the plasma. After hypoxic adaptation, the rate of whole blood flow through the tissue re-normalizes, but this means that the plasma flow rate is half the pre-exposure rate. The increased transport capacity compensates for the decreased plasma flow rate, supports the increased resting CMRglu, and allows for transient increases in energy demand due to focal neuronal activation.

Other changes in the tissue include a decrease in the volume density of neuronal mitochondria [23] and cytochrome oxidase activity [24,25] that probably reflect an overall decrease in resting CMRO₂ (cerebral metabolic rate for oxygen) of about 15%, although this has not been measured directly. The tendency for hypoxia to decrease brain activity and metabolism may be related to the idea of central respiratory depression [26]. An overall decrease in body
temperature [27] may reflect decreased metabolism as an acclimatization strategy that includes a drop in body mass.

**Intrinsic brain tissue oxygen sensors**

The HIF-1 pathway clearly plays a role as an oxygen sensor in the brain. HIF-1 accumulates in hypoxia due to inhibition of PHD activity which requires oxygen. On the other hand, hypoxia is not the only agent resulting in HIF-1 accumulation. For example, non-hypoxic HIF-1 accumulation can occur due to growth factors such as IGF-1 (insulin-like growth factor 1), iron chelation, cobalt chloride and alteration of substrate availability. Likewise, overproduction or stimulation of PHD will prevent HIF-1 accumulation even in moderate hypoxia.

Other sensor candidates would be oxygen-dependent enzymes, such as tryptophan and tyrosine hydroxylases [28], with affinity constants in the range of physiological tissue oxygen tensions, i.e. less than 2 kPa. But monoamine metabolism shows only a transient decrease in activity that reverses after 36 h.

There are also iron-containing molecules that might act as sensors because they bind oxygen in the physiological range. For example, neuroglobin, a haem protein, is up-regulated by sustained hypoxia and may play a protective role [29,30]. The effector mechanisms of these iron-containing proteins have not as yet been investigated [31].

**Species differences**

Hypoxia-induced angiogenesis has not been studied in many different species. It is important to establish the fundamental principles of hypoxic mammalian brain physiology and distinguish mechanisms that are species specific. Hypoxic HIF-1 accumulation appears to be universal in all cells that have a competent metabolic apparatus, so a great deal of the response should be common. On the other hand, there are significant differences among vertebrates and even in special mammalian cases, such as diving mammals, with respect to strategies in dealing with hypoxia [32,33].

Environmental niche is certain to have a major influence on hypoxic response. Diving mammals are just one example of this. Burrowing animals which spend more time in high carbon dioxide environments, ungulates which have different dietary patterns, and animals that have existed at altitude for countless generations each will exhibit variations [34,35]. High-altitude animals have evolved a left-shifted haemoglobin saturation curve which facilitates haemoglobin loading in the lung as an adaptive trait, the opposite of low-land animals which respond to prolonged hypoxia with a right-shift to facilitate oxygen unloading in the tissues [36]. Studies on the capillary density in these species should be of interest.

Another important consideration, often overlooked, is the relationship of body mass to the pertinent physiological variables. For example, in addition to
well-known scaling of brain blood flow and brain metabolism, the oxygen dissociation curve apparently also scales with body mass [37], the smaller animals have a more right-shifted \( p_{50} \) (oxygen partial pressure producing 50% saturation).

Rats have similar physiological responses to hypoxia as humans for a number of variables [38], but the rat haemoglobin \( p_{50} \) is right-shifted compared with humans. Also unknown is the effect of GLUT1 which is quite prominent in human erythrocytes but absent in small mammals such as rats and mice.

**Effects of re-oxygenation**

As interesting as the hypoxia-induced angiogenesis is, what happens on return to normoxia is even more intriguing. Angiopoietin-2 is again found to be elevated, but HIF-1 and its downstream growth factors are not [13]. Under these conditions of re-oxygenation, a rarefaction of capillaries occurs through a process of physiological apoptosis. Thus it appears that the COX-2-mediated pathway is activated during re-remodelling to either increase or decrease capillary density, depending on the presence or absence of other factors [39]. The process of angiogenesis in the brain requires a remodelling signal because the capillaries are usually so tightly connected mechanically. In the case of hypoxia-induced angiogenesis, this involves angiopoietin-2, from COX-2 and PGE2, to prepare the vessels for remodelling. In the case of re-oxygenation, the remodelling signal is activated, but the growth factors from the HIF-1 system are missing. This has the effect of initiating removal of the microvessels through physiological apoptosis.

The remodelling of the cerebral capillary vasculature in response to changes in tissue oxygen implies a dynamic process of continual matching of local energy demand and energy supply in the tissue, with a response time of days to weeks. Appreciation of this process is still rudimentary. It is likely that these mechanisms participate in the modification of capillary density and neuronal metabolism seen in rats raised in complex sensory environments or in the motor training that accompanies prolonged exercise [40,41]. But the sensors, signals and effectors remain mostly unknown.

**Effects of age**

Finally, it should be noted that most of the research on brain hypoxia involves healthy young subjects. There is, however, known age sensitivities to hypoxia that could reveal profound implications for dynamic angiogenesis and be most useful for understanding the acclimatization process. At least in the case of older rats, HIF-1 fails to accumulate in mild hypoxia [42]. This does not occur through failure of synthesis since HIF-1 mRNA is functional, and HIF-1 accumulation does occur in response to iron chelators or cobalt chloride treatment. It appears that there is a primary deficit in the oxygen-sensing function, most
likely through overproduction of PHD. These older rats should have a much more difficult time acclimatizing to hypoxia, learning motor tasks or surviving metabolic stresses such as cerebral ischaemia.

**Conclusions**

Mild, prolonged hypoxia evokes systemic and central nervous system mechanisms that result in successful acclimatization. In mammals, the central nervous system response includes increased glucose metabolism, decreased oxidative capacity and microvascular remodelling by capillary angiogenesis which results in decreased diffusion distances for oxygen from erythrocytes to mitochondria. The changes induced by hypoxia are reversible upon return to a normal oxygen environment. The robust response indicates that the brain vasculature is not fixed but dynamically adjusted to the supply-and-demand conditions of the functional tissue units. The transcription factor, HIF-1, plays a major role in orchestrating the metabolic and vascular responses to hypoxia, but clearly does not act alone. Failure or inadequacy of this complex acclimatization process may underlie many of the pathological outcomes of metabolic and vascular diseases, especially in the aging population. It will be important to determine the specific characteristics of the hypoxic acclimatization process in humans and to investigate strategies for interventions that could promote health and recovery.

**Summary**

- The brain normally exists in a low oxygen environment.
- Brain tissue oxygen tension is spatially and temporally heterogeneous.
- There are systemic and central mechanisms that help compensate for acute brain hypoxia; (i) an increase in lung ventilation mediated by carotid body sensors that respond to arterial PO2; (ii) an increase in cerebral blood flow mediated by brain stem sensors that respond to arterial oxygen content; and (iii) an increased brain parenchymal capillary density mediated by local sensors that respond to tissue PO2.
- Angiogenesis occurs when hypoxia persists for a few weeks. These adaptive changes are reversible. These changes are impaired in aging.

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**References**


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