

Regulation of gene expression by HIF-1

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Abstract. Hypoxia-inducible factor 1 (HIF-1) is a critical mediator of physiological responses to acute and chronic hypoxia. First, HIF-1 is required for the development of the systems that mediate these responses, including the heart, blood and blood vessels. Mice with complete HIF-1 α deficiency manifest developmental defects that involve all three components of the circulatory system. Second, HIF-1 mediates changes in gene expression that underlie physiological responses to chronic hypoxia, such as increased erythropoiesis and angiogenesis. *Hif1a*^{+/-} mice, which are partially HIF-1 α deficient, manifest impaired hypoxia-induced pulmonary vascular remodelling. Smooth muscle cells from pulmonary arteries (PASMCs) of wild-type mice subjected to chronic hypoxia manifest hypertrophy, depolarization, increased [Ca²⁺]_i, and decreased voltage-gated K⁺ currents. These responses are impaired in PASMCs from *Hif1a*^{+/-} mice. Carotid bodies isolated from *Hif1a*^{+/-} mice are unresponsive to hypoxia despite normal histology and normal responses to cyanide stimulation. Rat PC12 cells share properties with O₂-sensing glomus cells of the carotid body, including hypoxia-inducible expression of tyrosine hydroxylase, the rate limiting enzyme for catecholamine biosynthesis. In PC12 cells subjected to intermittent hypoxia, Ca²⁺/calmodulin-dependent kinase activity leads to HIF-1 transcriptional activity and tyrosine hydroxylase mRNA expression. Thus, HIF-1 regulates both acute and chronic responses to continuous and intermittent hypoxia.

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The average adult consumes O₂ at a rate of approximately 250 ml per minute or about 360 litres of O₂ per day. A variety of biochemical reactions require O₂, most notably the process of oxidative phosphorylation, in which electrons are passed from NADH and FADH₂ to respiratory cytochromes in the inner mitochondrial membrane, and finally to O₂. The electromotive force that is generated during this process is used to catalyze the formation of ATP, which is utilized as the energy

source for most reactions that are required to maintain cellular viability. This consumption of O_2 is dependent upon the activity of the respiratory system, which mediates the intake of 5–6 litres of air per minute or about 8000 litres per day. Once delivered to the pulmonary alveolar air sacs, O_2 diffuses into red blood cells, in which it is bound to haemoglobin, and then transported via the cardiovascular system for delivery to every cell of the body. Through the combined efforts of the respiratory and circulatory systems, every one of the more than 10^{14} cells of a healthy adult obtains sufficient O_2 to maintain metabolic homeostasis. The mechanisms that maintain cellular and systemic homeostasis have been the subject of investigation by physiologists for centuries. However, it has only been within the last decade that a unifying molecular mechanism for the control of oxygen homeostasis within individual cells, in tissues and organs, and within the body as a whole, both during development and in postnatal life, has been elucidated.

Discovery of HIF-1 as a transcriptional regulator of the *EPO* gene

In vertebrates, erythrocytes are specialized for the transport of O_2 from the lungs to body tissue and red cell mass determines the blood O_2 -carrying capacity. Specialized cells in the kidney produce erythropoietin (EPO), which is secreted into the bloodstream and binds to receptors on bone marrow erythroid progenitor cells, activating a signal transduction pathway leading to cell survival. When O_2 delivery is reduced, increased levels of EPO are produced, resulting in a compensatory increase in red cell mass. A α -acting regulatory element was identified in the *EPO* gene that is required for hypoxia-induced gene transcription (Beck et al 1991, Pugh et al 1991, Semenza et al 1991). The hypoxia response element (HRE) was used as a molecular probe to identify the binding of a transcription factor, which was designated hypoxia-inducible factor 1 (HIF-1) because it was detected in nuclear extracts of cells exposed to hypoxia and undetectable in nuclear extracts prepared from cells that were cultured under non-hypoxic conditions (Semenza & Wang 1992). HIF-1 was purified by DNA affinity chromatography and shown to be a heterodimer of HIF-1 α and HIF-1 β subunits (Wang & Semenza 1995). Partial protein sequence analysis provided sufficient information to isolate complete cDNA sequences encoding both subunits (Wang et al 1995). HIF-1 α protein levels and transcriptional activity were found to be dramatically regulated by the cellular O_2 concentration (Jiang et al 1996, 1997). O_2 -dependent hydroxylation of proline and asparagine residues in HIF-1 α represent the mechanism for transducing changes in cellular oxygenation into changes in HIF-1 activity (Epstein et al 2001, Ivan et al 2001, Lando et al 2002, Yu et al 2001). Two additional proteins involved in the negative regulation of HIF-1 α protein stability are OS-9, which binds to both the prolyl hydroxylases and to HIF-1 α (Baek et al 2005), and ARD1, which acetylates lysine 532 of HIF-1 α (Jeong et al 2002). Mitochondrial reactive oxygen species

production may also contribute to inactivation of the HIF-1 α hydroxylases under hypoxic conditions (Chandel et al 2000).

HIF-1 is required for embryonic survival

Unlike the *EPO* gene, which is expressed only in a limited number of cell types, HIF-1 activity was induced under hypoxic conditions in all cell types tested (Wang & Semenza 1993), which suggested that HIF-1 played a more general role in oxygen homeostasis. Analysis of mice in which the gene encoding either HIF-1 α or HIF-1 β was inactivated by homologous recombination revealed that HIF-1 was required for embryonic survival (Carmeliet et al 1998, Iyer et al 1998, Maltepe et al 1997, Ryan et al 1998). The absence of HIF-1 activity results in lethality at midgestation with defective development of the heart, blood and vessels, i.e. all three components of the circulatory system.

HIF-1 is a critical regulator of vascularization

In the case of tissue vascularization, each cell insures that it receives adequate perfusion by hypoxia-induced expression of angiogenic growth factors, particularly vascular endothelial growth factor (VEGF) (Shweiki et al 1992), which activates endothelial cells leading to capillary sprouting. Human and rodent *VEGF* genes were shown to contain an HRE in their 5'-flanking region (Levy et al 1995, Liu et al 1995) that was activated by HIF-1 binding (Forsythe et al 1996). More recent gain-of-function and loss-of-function experiments have shown that HIF-1 controls the expression of many of the key angiogenic growth factors including VEGF, placental growth factor, platelet-derived growth factor B, angiopoietin 1 and angiopoietin 2 (Kelly et al 2003), which are produced by hypoxic cells in tissues and bind to receptors on vascular endothelial and smooth muscle cells. In addition, HIF-1 also controls cell-autonomous responses to hypoxia in vascular endothelial cells by regulating the expression of hundreds of genes (Manalo et al 2005). Loss-of-function and gain-of-function studies indicate that HIF-1 plays a critical role in vascularization both during development and in postnatal life (Carmeliet et al 1998, Iyer et al 1998, Kelly et al 2003, Ryan et al 1998).

Involvement of HIF-1 in pulmonary vascular remodelling in response to chronic hypoxia

HIF-1 also controls remodelling of pre-existing vessels in response to hypoxia. When humans and experimental animals are subjected to alveolar hypoxia as a result of chronic obstructive pulmonary disease or exposure to reduced ambient O₂, respectively, pulmonary arterioles undergo a remodelling process involving hyper-

trophy and hyperplasia of smooth muscle cells in the medial compartment of the vessel wall, which results in a reduction in luminal area, increased resistance to blood flow, and pulmonary hypertension. *Hif1a*^{+/-} mice, which are heterozygous for a null allele at the locus encoding HIF-1 α and thus partially HIF-1 α deficient, have impaired pulmonary arterial remodelling in response to chronic hypoxia (Yu et al 1999). Electrophysiological studies of pulmonary artery smooth muscle cells (PASMCs) isolated from pulmonary arteries of *Hif1a*^{+/-} mice and wild-type littermates revealed that the hypoxia-induced depolarization and reduction of K_v channel current that were observed in PASMCs from wild-type mice were markedly blunted in the heterozygotes (Shimoda et al 2001). Hypoxia induced hypertrophy of PASMCs isolated from wild-type mice but not their heterozygous littermates. Thus, HIF-1 mediates two of the classic pathological responses to chronic hypoxia: hypertrophy and depolarization of PASMCs.

HIF-1 is required for carotid body responses to acute and chronic hypoxia

Whereas the responses to hypoxia described above occur on a timescale of weeks, acute responses to hypoxia occur within seconds. The classic acute physiological responses to hypoxia are the increase in respiratory and heart rate that occur in response to the stimulation of brainstem centres by neural signals emanating from the carotid body, which is a small organ located at the bifurcation of the carotid artery that functions as the primary chemoreceptor for sensing arterial pO₂ (López-Barneo 2003, Prabhakar 2000). Exposure of *Hif1a*^{+/-} mice to acute hypoxia or hypercarbia was associated with increases in respiratory rate (RR), tidal volume, and minute ventilation that were similar to wild-type littermates (Kline et al 2002). However, exposure of wild-type mice to chronic hypobaric hypoxia (three days at 0.4 ATM) resulted in an augmented ventilatory response to a subsequent acute hypoxic exposure, whereas in *Hif1a*^{+/-} mice the acute ventilatory response was actually blunted following chronic hypoxia. The carotid body plays a critical role in ventilatory adaptation to chronic hypoxia. To analyse carotid body function, we performed the Dejours test (Dejours 1962). Exposure of wild-type mice to a brief hyperoxic challenge inhibited RR and minute neural respiration (RR \times integrated phrenic nerve activity), whereas this response was blunted in *Hif1a*^{+/-} mice, providing further evidence for a defect in the carotid body.

When carotid bodies from wild-type mice were exposed to 100% O₂ followed by 12% O₂ there was a dramatic increase in carotid sinus nerve activity. The response was absent in carotid bodies from *Hif1a*^{+/-} mice. However, these carotid bodies responded normally to cyanide administration. Furthermore, immunohistochemistry revealed that glomus cells were present in normal numbers, were of normal morphology and showed normal production of chromogranin A and tyrosine hydroxylase (Kline et al 2002). Thus in mice with only a partial deficiency of

HIF-1 α expression the ability of the carotid body to either sense or respond to hypoxia is specifically lost. In these mice, peripheral chemoreceptors have compensated for the loss of carotid body function, similar to the effect of carotid sinus nerve transection, which initially abolishes acute hypoxic ventilatory responses but subsequently leads to a reorganization of the chemoreflex pathway with recovery of the hypoxic ventilatory response (Martin-Body et al 1986). In support of this hypothesis, the ventilatory response to hypoxia was markedly impaired after vagotomy in wild-type mice but not in heterozygotes.

HIF-1 is induced by intermittent hypoxia

In addition to playing an important role in adaptation to chronic hypoxia, the carotid body is required for responses to intermittent hypoxia, which occurs during sleep-disordered breathing, a condition that affects >18 million people in the USA and results in systemic hypertension (Kiley et al 1995). To analyse molecular mechanisms underlying involvement of HIF-1 α in carotid body responses to intermittent hypoxia, we have utilized rat PC12 cells, which share many properties with glomus cells of the carotid body, including O₂-regulated neurotransmitter release (Kumar et al 1998) and expression of tyrosine hydroxylase, the rate limiting enzyme for catecholamine biosynthesis (Hui et al 2003). Cells were exposed to alternating cycles of 1.5% O₂ for 30s followed by 20% O₂ for 4min (Yuan et al 2004). HIF-1 α protein expression and HIF-1 transcriptional activity were induced by exposure of cells to 30, 60 or 120 cycles of intermittent hypoxia. Addition of the intracellular Ca²⁺ chelator BAPTA-AM or the Ca²⁺/calmodulin-dependent (CaM) kinase inhibitor KN93 blocked the induction of HIF-1 transcriptional activity in response to intermittent hypoxia. CaM kinase activity increased fivefold in cells subjected to intermittent hypoxia. KN93 blocked intermittent hypoxia-induced transcriptional activation mediated by HIF-1 α or its coactivator p300, which was phosphorylated by CaM kinase *in vitro*. HIF-1-regulated expression of TH mRNA, encoding tyrosine hydroxylase, was induced by intermittent hypoxia and this effect was blocked by KN93. In contrast, the induction of TH mRNA by continuous hypoxia was not blocked by KN93. HIF-1 transcriptional activity and TH mRNA expression were induced in non-hypoxic cells transfected with a plasmid encoding a constitutively active form of CaM kinase II (Yuan et al 2004). Taken together, these results indicate that intermittent hypoxia induces HIF-1 transcriptional activity and TH mRNA expression via a novel pathway involving CaM kinase, an enzyme that is activated by the increase in intracellular Ca²⁺ levels that occurs during depolarization. Thus, HIF-1 represents a bridge between the acute (depolarization and neurotransmission) and chronic (changes in gene and protein expression) responses to hypoxia.

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DISCUSSION

Duchen: Do you see the involvement of HIF-1 in carotid body oxygen sensing as an acute role, or an involvement in the regulation of a channel or some other protein? Do you think that HIF-1 has an acute role to play in oxygen sensing?

Semenza: We don't know what the mechanism is that accounts for the lack of response in the heterozygotes. The most likely explanation is that there are genes that are not being transcribed at sufficient levels to produce critical proteins such

as channels or regulators of channels. A more novel role would be if HIF-1 is somehow directly contributing to the response by virtue of the fact that it is being regulated by the oxygen concentration, so the presence or absence of the protein could be used as a signal for things other than transcription. We don't have any evidence for this, though.

Duchen: The actual oxygen sensor in this pathway is the prolyl hydroxylase. Can you manipulate this? It could mediate an oxygen sensing mechanism.

Semenza: The problem is that you are still going to be manipulating HIF-1. It won't answer the question of the mechanism; it will just say that HIF-1 is involved, and we know that already. In terms of the mechanism, unless it led us directly to a protein that was being hydroxylated—which is another possibility—looking at prolyl hydroxylase would tell us little.

Ratcliffe: Wasn't the HIF-1 α heterozygote phenotype initially held to be normal, with this phenotype becoming apparent later? The HIF-1 β phenotype was also held to be normal initially. What do we now know about this? We might expect that these effects would be seen.

Semenza: They might even be more severe, because HIF-2 α has also been implicated.

Ratcliffe: If it was less severe, then this would raise a question.

Semenza: I don't know of anyone who has done experiments with the HIF-1 β heterozygote.

Ratcliffe: A related question, then, is with your adenovirus delivery system you could induce a transcriptionally disabled HIF: have you done this yet, and if you have, are there any effects on gene expression?

Semenza: No, we haven't done this yet. We are hoping to isolate the glomus cells and use them. PC12 cells are a useful model, but they have their limitations.

López-Barneo: I have a comment on the role of HIF-1 α in acute oxygen sensing in the carotid body. We have done experiments not using the whole carotid body preparation but rather a slice preparation that in our hands mimics what is seen *in vivo* very well. We can see secretory responses to hypoxia that are almost the same as those in *in vivo* preparations. We don't see any change in the hypoxia sensitivity in the HIF-1 α heterozygote. We have tried to acutely inhibit prolyl hydroxylases by adding dimethylxalylglycine (an inhibitor of prolyl hydroxylases) to our slice, and we didn't see any effect on acute oxygen sensing. So, at least in our hands, prolyl hydroxylation doesn't seem to be involved in the acute oxygen sensitivity in the carotid body.

Prabhakar: When you measured the secretory activity by amperometry, I presume that you were measuring the catecholamine secretion. We do not know the role of catecholamines in the sensory excitation by hypoxia. In order to understand the role of transmitters, especially catecholamines in sensory transmission during hypoxia, in addition to secretory activity it is imperative that we measure the sinus nerve activity as an output.

López-Barneo: We are using the carotid body thin slice as a model to study oxygen sensitivity. I am not talking about the whole organ sensitivity: this depends on ATP, acetylcholine release from the terminals, and so on. I am talking about a well established model. I think this catecholamine release in response to hypoxia is a good indication of oxygen sensitivity, regardless of what the catecholamines are doing. We are looking at whether the glomus cell is still oxygen sensitive, and is able to depolarise and induce catecholamine release in a Ca^{2+} -dependent manner. In this preparation we don't see any difference between catecholamine release induced by hypoxia in the normal animal and in the HIF-1 α heterozygote.

Semenza: That is the value of the genetic models: they allow us to dissect the various components of the physiological response. They tell us that HIF-1 is not involved in this part of the response, but it must be involved in some other critical aspect of the response to hypoxia, because we see this dramatic effect at the level of the carotid sinus nerve transmission. There has to be another component of this response that is critical, which HIF-1 does control. Your data suggest that it does not control the secretory response. This is interesting, because we have to ask what controls that. Could it be HIF-2 α ? We are trying to understand at the molecular level how these different responses are controlled.

Duchen: Is there any ultrastructural change in the carotid bodies in these animals?

Prabhakar: We looked only at gross morphology at the light microscopic level and have not done electron microscopy. It's an important question.

Acker: It is well known that *in vivo* organ pO₂ distribution is ranging from 0–90 Torr. The mean pO₂ is about 20–30 Torr. Most of the cells live under low pO₂ conditions. There must be a quite different mechanism for inhibiting the HIF response under these low pO₂ conditions. However, cells are able to respond to a change in the whole pO₂ field. Most of the cells live under low oxygen conditions but they are not hypoxic. It is difficult to mirror this heterogeneity in tissue culture. Do you have indications that in the organ there is also a distribution of HIF responses?

Semenza: These are complicated questions. It is all relative: the normal oxygen concentration will be different for different cells. Likewise, the level of HIF-1 α expression at any particular pO₂ is not absolute: it differs from cell to cell. The hydroxylases are not operating under equilibrium conditions. The level of the expression of the enzymes determines the dose–response curve, shifting it in one direction or another. With all the components of the pathway, it seems that they can be decreased or increased and effects are seen in the response: nothing seems to be rate limiting in the pathway. For example, the expression of the hydroxylases may be controlled by many other factors.

Acker: The medulla of the kidney has very low pO₂ levels, but we don't see any HIF. The lung has very high pO₂ values: perhaps this is the reason why it is so sensitive to changes in pO₂. Another example is the liver, which has very drastic

mapping of periportal and perivenous distribution of HIF stabilization. There must be a complicated number of control mechanisms.

Chandel: There are some data to suggest that the prolyl hydroxylases (PHDs) come up during hypoxia. This would suggest that chronically, in tissues that are more hypoxic, HIF-1 α would be turned off.

Semenza: This raises a new issue: the feedback controls on the system. First of all, there is the complexity of each cell being at a different set point, and then there is the feedback regulation. This has been known for a long time: if someone is made anaemic by removal of large volumes of blood their EPO levels will go up and come back down before the haematocrit has changed at all. This is necessary so that they don't overshoot. If there are too many red blood cells this could be dangerous because of increased blood viscosity. There has to be feedback that is clearly oxygen independent, because it occurs before any change in the oxygen carrying capacity. The up-regulation of the PHD genes is also controlled by HIF-1, and this is one of the mechanisms by which this feedback occurs. Depending on the nature of that response, you could modulate the feedback. That is, the kinetics and the intensity of that feedback relationship can also be modulated. In every cell HIF-1 is going to be induced, but the level of expression of particular downstream genes is modulated by other transcription factors, which creates a whole new level of complexity.

Chandel: Could you comment on HIF-2 α ? Specifically, it is clear from the phenotypes that HIF-1 α and HIF-2 α have distinct functions, but there is not a list of distinct target genes. Why?

Semenza: HIF-1 α is expressed in all cell types, and coordinates response to hypoxia in all. HIF-2 α has a more specialized role, and appears to be much more cell-type specific. It is expressed in a restricted number of cell types and has a more specialized role. Part of the problem is that the experiments haven't been done in the correct cell types. If we did the experiment in a cell type where HIF-2 α has a critical physiological function then we might uncover those genes. Doing the experiment in tissue culture cells where it may not play an important role is unlikely to give the answer. The HIF-2 α knockout mice have not been pursued to the same degree. Joe Garcia is now doing nice work with them and is posing interesting hypotheses about HIF-2 α potentially being involved in the response to oxidative stress that are quite unexpected. I think that analysing physiological responses in the knockout mice will prove key.

Peet: Endothelial cells are one of the cell types where HIF-2 α is highly expressed. You had some nice results with endothelial cells. It looked as if many of the target genes you saw changing were similar with hypoxia and over-expression of HIF-1 α .

Semenza: That is how the experiment was set up: we were specifically looking for these. This was another case where there was an oversimplification: originally HIF-2 α was found in endothelial cells and was hypothesized to play a critical role there

whereas HIF-1 α was not thought to be active in endothelial cells. This is not correct. There are interesting data emerging about which target genes are regulated by both HIF-1 α and HIF-2 α and which target genes are distinctly regulated by HIF-1 α or HIF-2 α .

Duchen: What is the lifetime of HIF?

Semenza: In isolated, perfused, ventilated ferret lung preparations, when we ventilate the lungs with 21% oxygen after ventilating with 0% oxygen the protein is degraded with a half-life of less than one minute. I don't know of any protein that has a shorter half life.

Schumacker: How do you explain the intermittent hypoxia when you give 30 s of hypoxia and 20 min of normoxia, and you see a progressive increase in HIF after just 10–20 s?

Semenza: This is because hypoxia and re-oxygenation, rather than hypoxia, is the signal. We have shown that HIF-1 α transactivation is not being mediated through changes in the hydroxylation of HIF-1 α , but rather through the phosphorylation of p300 that is mediated by the CaM kinase pathway. Probably, the signal transduction pathways are being activated by reactive oxygen species that arise as a result of hypoxia and then reoxygenation in a repetitive fashion.

Prabhakar: We monitored O₂ profiles near cells during intermittent hypoxia, and found that they drop by about ~25 mmHg with each episode of hypoxia. With sustained hypoxia for 15 minutes, which is equivalent to the 120 cycles of intermittent hypoxia, pO₂ drops by about ~60 mmHg. Despite the modest drop in pO₂, intermittent hypoxia is more potent a stimulus in activating *c-fos*; whereas 15 min of sustained hypoxia caused a more substantial drop in pO₂, it was ineffective in evoking *c-fos*, suggesting that the effectiveness of intermittent hypoxia is due to something more than drop in pO₂, which we presume is reactive oxygen species (ROS).

Ward: This would predict that if you have a higher frequency of reoxygenation, you would increase the signal.

Prabhakar: If you change the duration of a single episode of hypoxia, it doesn't have an impact on the magnitude of *c-fos* activation, but if you increase the duration of reoxygenation phase, while keeping the duration of hypoxic episode constant, then you get a proportional increase in *c-fos* activation. It seems that the major stimulus is the reoxygenation, not the absolute fall in pO₂ during each episode of hypoxia. We published these observations at the beginning of last year (Yuan et al 2004).

Archer: I have a related question about redox signalling. We found a rat (the Fawn hooded rat; unpublished data) that has a mitochondrial defect leading to impaired hypoxic pulmonary vasoconstriction (HPV) and HIF activation which promotes development of pulmonary hypertension. This seems to be related to a failure to make radicals, and is associated with a loss of HPV in this animal. If this conference is trying to look for unifying themes, what do you think of the argument that

the same redox signal that is involved in acute oxygen sensing is involved in the remodelling responses? If this signal is removed, HIF activation may ensue.

Semenza: Another one of the complexities of this system is that under hypoxia it appears that there is actually ROS generation in the mitochondria, which provides a signal that is important for the response as well. I hope Paul Schumacker will tell us later how this impacts on the hydroxylation system in continuous hypoxia. There is some role for ROS in that system as well, which is much harder to understand for me than in intermittent hypoxia, where it is easy to understand how ROS are generated. Obviously, the mitochondria are the main consumers of oxygen in the cell, and that a signal from the mitochondria is also critical for the response to hypoxia shows how well the cell is integrating all these signals at the level of HIF-1 in terms of determining the output.

Ward: Going back to the intermittent bit, there was some work we did a long time ago looking at intermittent hypoxia in rats. We used right ventricular hypertrophy and polycythaemia as a model. One of the things we showed was that the summed length of hypoxia was important. It didn't matter whether we had 12 half-hour pulses through the day or one six hour period: the same level of response was observed. This is slightly different from what you are saying.

Semenza: When your units are hours, I view this as continuous hypoxia, as opposed to 30-second hypoxic exposure, which is what we use in our intermittent hypoxia experiments. Under the conditions that you described, HIF-1 α accumulates as a result of decreased hydroxylation during the hypoxic exposure, and the resulting effect on gene expression is cumulative.

Ward: Yes, I suppose from the point of view of the patient with sleep apnoea, the periods of hypoxia are around 45 s at the very most.

Prabhakar: The average duration of apnoea in adult human subjects averages ~12 s, and the range is between 7–30 s.

Ward: The question is whether the tissues actually see significant hypoxia under these conditions, considering the short period and rate of diffusion from the blood to the cells.

Prabhakar: I do not know whether other tissues see hypoxia, but I believe carotid bodies do see hypoxia because of their close proximity to the lungs, and the circulation time from lung to carotid body is ~6 s. I also believe that reoxygenation is more important than hypoxia. In the patient population there appear to be two patterns of sleep apnoea; one monomorphic pattern, wherein the number of apnoeic episodes is far more and associated with a modest drop in arterial O₂ saturation. The other is a polymorphic pattern, where the numbers of apnoeic episodes are relatively less but associated with a greater fall in O₂ saturations.

Semenza: The important point is that the carotid body senses and responds to arterial hypoxemia. The responses are mediated by the carotid body through activation of the sympathetic nervous system. This is why patients with sleep apnoea

who are subjected to intermittent hypoxia mainly get systemic hypertension and not pulmonary hypertension, because it is a systemic effect mediated via the carotid body whereas continuous hypoxia has a direct effect on the pulmonary vasculature.

Ward: You still get polycythaemia, which is presumably not mediated via the carotid body and sympathetic system.

Semenza: We have shown that intermittent hypoxia does induce EPO through HIF-1.

Reference

Yuan G, Adhikary G, McCormick AA, Holcroft JJ, Kumar GK, Prabhakar NR 2004 Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. *J Physiol* 557:773–783