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Editorial

Oxygen sensing

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Molecular O_2 is an essential substrate for energy production. Hence, an adequate O_2 supply is critical for mammalian cell survival. Many physiological and pathophysiological situations lead to hypoxia or reduced O_2 supply to tissues. Maintenance of homeostasis under hypoxia depends on 'sensing' hypoxia and failure to sense O_2 can cause severe organ damage and even death. O_2 sensing can be 'innate' or 'adaptive.' While physiological responses to 'innate O_2 sensing' occur rapidly within few seconds to minutes, 'adaptive' O_2 sensing is slow in onset often taking a few hours. This editorial provides brief history of discovery, current theories and translational perspectives of 'innate' and 'acquired' O_2 sensing.

INNATE O_2 SENSING

As early as 1868, Pflüger, a German physiologist noted hypoxia leads to hyperventilation.[1] Pflüger's finding spurred interest in identifying structures responding and translating systemic hypoxia to stimulation of breathing. In 1920's Fernando de Castro at the Cajal Institute, Spain and Corneille Heymans in Belgium independently discovered carotid bodies (CB) as sensory receptors for monitoring arterial blood O_2 levels. CB chemoreflex evokes stimulation of breathing by hypoxemia.[2-9] 1938 Nobel Prize in Physiology and Medicine was awarded to Cornielle Heymans. However, the Nobel Citation duly acknowledged the seminal contributions of De Castro in discovering the sensory nature of the CB.[10]

Unlike other mammalian tissues, even a modest decrease in arterial PO_2 (from ~100–80 mmHg) is sufficient for activating CB and the response is rapid occurring within seconds after the onset of hypoxia.[11] Moreover, the increased CB activity is non-adapting and maintained during the entire period of hypoxia.[12] The remarkable sensitivity, the speed with which it responds, with little or no adaptation constitute important characteristics of 'innate' O_2 sensing by the CB. Considerable evidence suggests that glomus cells are the primary O_2-sensing cells of the CB.[11]

Current theories of 'innate' hypoxic sensing – Because the response is rapid, innate O_2 sensing by the CB utilises existing rather than *de novo* synthesis of proteins. In 1960s, Lloyd *et al.*[13] reported brief (1–2 breaths) inhalation of carbon monoxide (CO) inhibits hypoxia-evoked hyperventilation in humans. Because CO binds to haemoglobin more effectively than O_2, it was proposed a heme protein(s) might be involved in 'innate O_2 sensing' by the CB.[13]

Remarkably, CO, once used as a tool to understand O_2 sensing by the CB, is now shown to be produced in glomus cells by heme-containing enzyme heme oxygenase (HO)-2.[14] HO-2 is an O_2-sensitive enzyme and hypoxia progressively decreases CO production in the CB.[15] HO-2 binds to O_2 with low affinity with an apparent Km of 65 ± 5 mmHg (about 80 μM), thereby enabling HO-2 to transduce changes in O_2 to changes in CO production. CO inhibits CB sensory nerve

excitation by hypoxia.[14] These findings led to the suggestion that low sensory nerve activity during normoxia is due to high CO levels inhibiting CB sensory nerve activity and that hypoxia, by reducing CO production, relieves the inhibition and thereby increase the CB sensory nerve activity.[14]

Recent studies examined how CO inhibits CB sensory nerve activity under normoxia.[15-17] Glomus cells express cystathionine gamma-lyase (CSE), which produces hydrogen sulphide (H_2S).[17] H_2S, like hypoxia, stimulate the CB sensory nerve activity in rats, mice, rabbits and cats.[18] During normoxia, H_2S levels are low and hypoxia increases H_2S levels in a stimulus-dependent manner.[17] In a recent study, Yuan *et al.*[15] showed that high CO levels during normoxia inhibit H_2S production through protein kinase G (PKG)-dependent phosphorylation of Ser^{377} of CSE. Reduced CO generation during hypoxia relieves the inhibition of CSE, leading to increased H_2S generation in the CB.[15] CSE inhibitors and CSE knockout mice exhibit impaired glomus cell and sensory nerve and breathing response to hypoxia.[17] These findings suggest that low sensory discharge during normoxia is due to inhibition of H_2S production by high levels of CO generated by HO-2 and that the increased sensory nerve activity by hypoxia is due to relieving inhibition of H_2S synthesis by CO [Figure 1].

TRANSLATIONAL SIGNIFICANCE OF INNATE O_2 SENSING BY THE CB

CB sensory nerve activity is transmitted to brainstem neurons producing reflex stimulation of breathing and sympathetic nerve activity. Heightened CB chemo reflex has been implicated in several diseases associated with autonomic dysfunction.

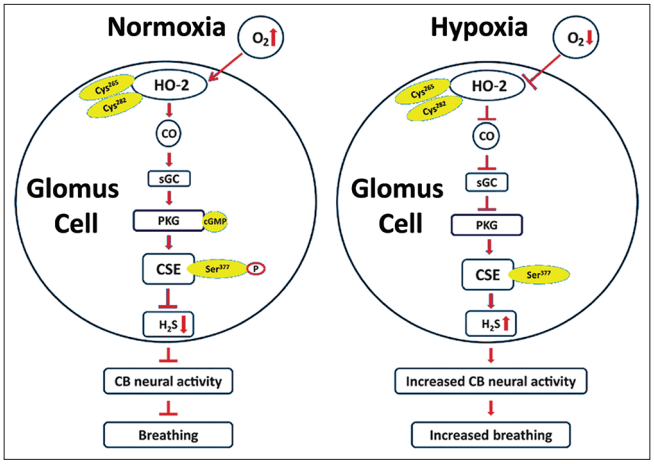

Figure 1: Schematic presentation of the signalling pathways associated with innate O_2 sensing by the carotid body (CB) glomus cells involving interplay between three gases—O_2, CO and H_2S and their impact on CB neural activity and breathing. Cys^{265} and Cys^{282} are located in the heme regulatory motif of heme oxygenase (HO)-2. Ser^{377} is the target residue in the putative PKG recognition sequence in cystathionine gamma-lyase (CSE) (Adapted from Yuan *et al.*, *Science Signalling*, 2015, 8:373).

Obstructive sleep apnoea (OSA)

OSA is a widespread respiratory disorder affecting 20–30% of men and 10–15% of women.[19,20] Intermittent hypoxia (IH) is a hallmark manifestation of OSA. HO-2 knockout mice exhibit OSA, which could be prevented with either genetic or pharmacological blockade of H_2S synthesis by CSE.[21,22] IH-treated rodents exhibit enhanced CB sensitivity to hypoxia, elevated H_2S and reduced CO levels in the glomus tissue.[22] IH-evoked CB hypersensitivity to hypoxia, increased sympathetic nerve activity and hypertension[22] are prevented with pharmacological blockade of H_2S synthesis.[22]

Neurogenic hypertension

CB chemo reflex is augmented in humans with essential hypertension.[23] Spontaneous hypertensive rats, a rodent model of neurogenic hypertension, manifest CB hypersensitivity to hypoxia and elevated H_2S levels in the CB.[16] Pharmacological blockade of H_2S synthesis lowers BP in SH rats[16] indicating pharmacological blockade of H_2S synthesis might be of therapeutic value in normalising BP in neurogenic hypertension.

Congestive cardiac failure (CCF)

Patients with CCF exhibit elevated sympathetic nerve activity, which contributes to progression of CCF and mortality.[24,25] Enhanced CB chemo reflex is an important contributor to increased sympathetic nerve activity in experimental models of CCF.[26] H_2S synthesis inhibitor normalises CB hypersensitivity to hypoxia and prevents the increased sympathetic nerve activity in experimental models of CCF.[26]

ADAPTIVE O_2 SENSING

Whereas certain cells such as glomus cells of the CB exhibit 'innate' O_2 sensing, every mammalian cell responds to chronic hypoxia that is, 'adaptive' O_2 sensing. As early as 1890, it was recognised hypoxia associated with high altitude increases red cell production, facilitating the O_2-carrying capacity of blood.[27] Subsequent studies showed hypoxia increases erythropoietin, which stimulates RBC production. In early 1990s, Dr. Gregg L. Semenza at the Johns Hopkins University School of Medicine was investigating the molecular basis of oxygen-regulated human erythropoietin (EPO) gene expression. He identified a nuclear factor, known as hypoxia-inducible factor 1 (HIF-1), which binds to hypoxic response element (HRE) sequence in DNA initiating transcriptional activation of the EPO gene.[28] HIF-1 complex is a heterodimer composed of an O_2-regulated

HIF-1α subunit[29,30] and constitutively expressed HIF-1β subunit. In presence of O_2, HIF-1α is rapidly degraded and accumulated in cells only under hypoxia. How does O_2 regulate HIF-1α protein? Studies by Drs. Kaelin at Harvard and Sir Peter Ratcliffe at Oxford showed von-Hippel-Lindau (VHL) physically interacts with HIF-1α protein, leading to its degradation in normoxia.[31,32] How do O_2 levels regulate VHL and HIF-1α protein interaction? Ratcliffe's group showed that prolyl hydroxylase domain proteins (PHD 1, 2 and 3) catalyse the hydroxylation of proline residues in HIF-1α.[33] PHDs are highly sensitive to O_2 with an apparent Km of ~170 mmHg. Low affinity for O_2 suggests that even a small reduction in O_2 can inhibit PHDs, leading to reduced prolyl hydroxylation and less binding to VHL, thereby resulting accumulation of HIF-1α protein. The increased HIF-1α protein diamerzes with HIF-1β and the HIF-1 complex enters the nucleus and binds to HREs on DNA to drive gene transcription [Figure 2]. Following the initial discovery of HIF-1, two related proteins were identified including HIF-2α (also known as endothelial PAS domain protein [Epas]-1) and HIF-3α. HIF-1 is expressed in all mammalian tissues and cell types, whereas HIF-2α expression is restricted to specific cell types, including developing blood vessels and lungs. VHL and PHD signalling is utilised for O_2-dependent regulation of both HIF-1α and HIF-2α and these signalling pathways are evolutionarily conserved. The fundamental discoveries by Drs. Semenza, Ratcliffe and Kaelin established the molecular basis of 'adaptive' O_2 sensing and were recognised with Nobel Prize in Physiology and Medicine in 2019.

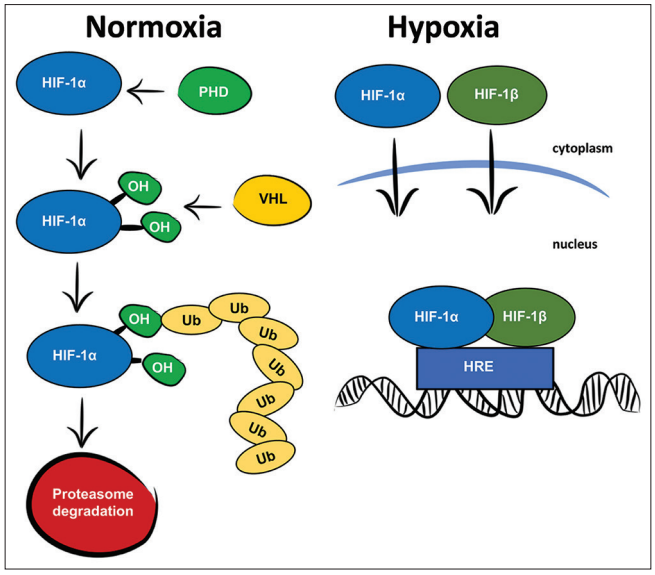

Figure 2: Hypoxia-inducible (HIF) signalling in adaptive O_2 sensing. Oxygen-dependent regulation of HIF-1α. In normoxic conditions, prolyl hydroxylase proteins (PHD) hydroxylate prolines (OH) residues in the oxygen-dependent degradation domain of the HIF-1α protein. The hydroxylated prolines are recognised by the von Hippel-Lindau protein (VHL) and ubiquitinated (Ub) for degradation by the proteasome. In hypoxic conditions, PHDs are inhibited resulting in accumulation of HIF-1α protein and HIF-1β is dimerised and translocated to the nucleus, binds to the hypoxia-responsive element (HRE) in DNA and activate transcription of genes regulated by hypoxia (Adapted from Cerychova and Gabriela, *Frontiers in Endocrinology*, 2018, 9:460).

HIF-1 mediates transcriptional regulation of hundreds of genes in response to hypoxia, including genes encoding proteins that control angiogenesis (i.e., O_2 delivery) as well as genes encoding enzymes and transporters that control energy metabolism (i.e., O_2 utilisation).

SIGNIFICANCE OF ADAPTIVE O_2 SENSING

Hypoxia associated with high altitude sojourn leads to a series of adaptations, including ventilatory acclimatisation to hypoxia (VAH), characterised by a progressive increase in baseline ventilation. Failure to hyperventilate at high altitudes leads to severe hypoxemia and pulmonary and brain oedema. VAH is severely impaired in mice partially deficient in HIF-1α.[34] Although Tibetan and Andean populations reside at high altitudes for thousands of years, they adapted differently to hypoxia. Tibetans developed increased ventilatory capacity, whereas Andeans have higher haemoglobin concentrations than lowlanders.[35] Absence of polycythemia in Tibetans suggests that they adapted more effectively to hypoxia. Independent studies from different research groups reported a remarkable correlation between genetic variation of HIF signalling pathways and physiological adaptation in Tibetan and Andean populations. Non-elevated haemoglobin phenotype in Tibetans was associated with selected haplotypes at the EGLN1 and PPARA loci, which are HIF-1 target genes that encode prolyl hydroxylase PHD2 and peroxisome proliferator-associated receptor, respectively.[36] Genome-wide analysis of Tibetans revealed a high correlation between the presence of single nucleotide polymorphisms at the EPAS1 locus (which encodes HIF-2α) and non-elevated haemoglobin concentrations in this population.[37,38] These studies provide compelling evidence that HIF signalling plays a major role in evolutionary and physiological adaptations to chronic hypoxia. Perhaps, the most important significance of HIFs lies in cancer biology. Tumours are hypoxic and exhibit elevated HIF proteins. HIF activation promotes angiogenesis in tumours facilitating metastasis. Therefore, blockade of HIF signalling inhibiting angiogenesis has the potential to block metastasis. Indeed, recent studies have shown that targeted pharmacological blockade of HIF-2α reduces tumour size in certain kidney cancers[39] and these drugs are in clinical trials. Thus, the adaptive O_2 sensing associated with discovery of HIF signalling has enormous physiological and pathophysiological significance.

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