Oxygen Sensing in the Brain – *Invited Article*

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Abstract Carotid body arterial chemoreceptors are essential for a normal hypoxic ventilatory response (HVR) and ventilatory acclimatization to hypoxia (VAH). However, recent results show that O_2-sensing in the brain is involved in these responses also. O_2-sensing in the rostral ventrolateral medulla, the posterior hypothalamus, the pre-Bötzinger complex and the nucleus tractus solitarius contribute to the acute HVR. Chronic hypoxia causes plasticity in the brain that contributes to VAH and represents another time domain of central O_2-sensing. The cellular and molecular mechanisms of acute O_2-sensing in the brain remain to be determined but they appear to involve O_2-sensitive ion channels and heme oxygenase-2, which acts by a different mechanism than has been described for the carotid body. It is not known if plasticity in such mechanisms of acute central O_2-sensitivity contributes to VAH. However, O_2-sensitive changes in gene expression in the brain do contribute to VAH and demonstrate another mechanism of O_2-sensing that is important for ventilatory control. This time domain of O_2-sensing in the brain involves gene expression under the control of hypoxia inducible factor-1α (HIF-1α) and potentially several HIF-1α targets, such as erythropoietin, endothelin-1, heme oxygenase and tyrosine hydroxylase.

Keywords Central chemoreceptor · Heme oxygenase · Hypoxia inducible factor-1 · Pre-Bötzinger complex · Rostral ventrolateral medulla

1 Background

The effect of hypoxia to stimulate breathing and the cardiovascular system has been well known for more than a century (reviewed by Neubauer 2004). In the 1920s and 1930s, Heymans and his colleagues did critical experiments showing the importance of carotid body chemoreceptors for the hypoxic ventilatory response (HVR)

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and this work was rewarded with the Nobel Prize for Physiology or Medicine in 1938. However, it was known that hypoxia continued to stimulate ventilation and the cardiovascular system in the absence of carotid body chemoreceptors and this lead Comroe to propose that other arterial chemoreceptors or sites in the central nervous system (CNS) were sensitive to hypoxia. Subsequently, Comroe showed the importance of the aortic body chemoreceptors for hypoxic responses, which established the idea that arterial chemoreceptors are the dominant O_2-sensitive chemoreceptors for cardiopulmonary control. Indeed, when the carotid and aortic body chemoreceptors are eliminated, hypoventilation ensues and the acute HVR is essentially obliterated. Arterial chemoreceptors are also necessary for other time domains of the HVR, such as ventilatory acclimatization to hypoxia (VAH), which is the time-dependent increase in ventilation and O_2-sensitivity that occurs with sustained hypoxia.

Modern studies of different time domains of the HVR have implicated the CNS as an important site of O_2-senstivity also. These include developmental studies of the biphasic HVR in neonates (i.e. hyperventilation followed by ventilatory decline during sustained hypoxia) and studies of respiratory central pattern generators that exhibit intrinsic chemosensitivity. However, the seminal observation of central O_2-sensitivity in the modern era was arguably that of Sun et al. (1992) that showed focal hypoxia produced by cyanide in the medulla excites sympathoexcitatory neurons. This work was quickly recognized by the community of scientists studying respiratory control and has lead to considerable advances in our understanding of the sites, mechanisms and physiological importance of O_2-sensing in the brain.

While the depressing effects of hypoxia on CNS neurons may function as sensing mechanisms instead of representing failing metabolism or energetics, they will not be considered further in this article. Similarly, the activation of inhibitory networks by hypoxia that contribute to ventilatory decline will not be considered because that story is complicated by developmental changes as this phenomenon is observed most prominently in neonatal animals (cf. Neubauer and Sunderram 2004).

2 Sites of Central O_2-Chemosensitivity

Several methods have been used to demonstrate nuclei in the CNS that are directly activated by hypoxia and this was recently reviewed by Neubauer and Sunderram (2004). These include mapping c-fos expression in animals exposed to hypoxia with and without arterial chemoreceptor denervation, neural recordings in a region with focal hypoxia from cyanide microinjections, and neural recordings from cultured cells isolated from putative O_2-sensitive regions. The effects of focal hypoxia from cyanide is reversible, dose dependent and is not replicated by hypercapnia or acidosis. Evidence for O_2-sensitivity is published for the C1 sympathoexcitatory region of the rostral ventrolateral medulla, the posterior hypothalamus, the pre-Bötzinger complex and the nucleus tractus solitarius.

Neurons in the C1 region of the brainstem were among the first to be described as excited by hypoxia and having cardiovascular or respiratory effects, as mentioned above (Sun et al. 1992). Neurons with intrinsic O_2-sensitivity in the posterior hypothalamus also increase sympathetic activity, heart rate and blood pressure and it is interesting that these project to the C1 sympathoexcitatory region (Neubauer and Sunderram 2004). However, experiments using focal hypoxia have demonstrated intrinsic O_2-sensitivity in C1 neurons. The pre-Bötzinger complex is adjacent to the C1 region and also exhibits O_2-sensitivity, which suggests common O_2-sensing mechanisms for both sites. Focal hypoxia in the pre-Bötzinger complex induces gasping, which is a form of autoresuscitation and thought to be physiologically significant O_2-sensitivity in the central rhythm generator for respiration (Solomon and Edelman 2003). O_2-sensitivity in the nucleus tractus solitarius is interesting because this is the site of the primary synapse from carotid body chemoreceptors. This suggests possible interactions between central and peripheral O_2-sensitivity.

3 Mechanisms of Central O_2-Chemosensitivity

Studies of the carotid bodies have revealed several O_2-sensitive ion channels that could play a role in the CNS also (reviewed by Neubauer and Sunderram 2004). Hypoxic inhibition of potassium (K^+) channels contributes to depolarization in glomus cells in the carotid body. For example, voltage-independent leak K^+ currents (TASK-1) channels contribute to O_2-sensing in carotid bodies and are present in the CNS in O_2-sensitive regions. Large-conductance calcium-activated K^+ channels are also inhibited by hypoxia in glomus cells. Finally, hypoxia could decrease K^+ channel conductance by decreasing ATP levels, which could affect ATP-activated K^+ channels, or by producing neuromodulators to act on G-protein coupled inward-rectifying K^+ channels.

Other ion channels proposed to contribute to O_2-sensitivity in carotid bodies include calcium (Ca^{++}) and sodium (Na^+) channels, which can increase conductance in hypoxia (reviewed by Neubauer 2004). L-type Ca^{++} currents are increased by hypoxia in glomus cells and brainstem neurons, and there is indirect evidence for hypoxic activation of Ca^{++} channels in dissociated neurons from the RVLM. Hypoxia generally decreases Na^+ conductivity, presumably to protect neurons during decreased energy supply. However, hypoxia increases the persistent Na^+ current in glomus cells (Donnelly 2008) and this channel is important for the pacemaker activity in the pre-Bötzinger complex (Del Negro et al. 2001). Hence, O_2-sensitivity of persistent Na^+ currents might explain the O_2-sensitivity of the pre-Bötzinger complex described above.

Beside ion channels, there are two forms of heme oxygenase (HO) that could function as an O_2-sensor in the CNS. HO depends on O_2 to catalyze heme into CO, biliverdin and iron, all of which can affect ion channels (reviewed by Powell and Fu 2008). HO-2 is expressed constitutively in O_2-sensitive regions of the RVLM and pre-Bötzinger complex while HO-1 is only observed in these regions

after exposure to chronic hypoxia. Hence, HO-2 is a more likely O_2-sensor in the CNS for acute hypoxia. It is interesting that HO is necessary for hypoxic excitation of neurons in the RVLM while it is inhibitory in the carotid body (Neubauer and Sunderram 2004).

A novel mechanism of O_2-sensitivity described for the nucleus tractus solitarius (NTS) involves of S-nitrosothiols (SNOs), which are released from hemoglobin when it is deoxygenated (Lipton et al. 2001). SNOs are complexes of NO bound to the sulfhydryl groups in cysteine. The specific SNO molecule that stimulates ventilation in the NTS appears to be a metabolite of S-nitrosoglutathione (GSNO) that is cleaved by γ-glutamyl transpeptidase (γ-GT). GSNO could be formed when nitric oxide synthase is activated in neurons in the NTS, where it is known to occur. It is suggested that γ-GT is present in neurons but not the vasculature to explain the positive effect of GSNO in the NTS on ventilation versus in its lack of effect in the vasculature, where SNOs transfer NO from hemoglobin and cause vasodilation.

4 Sensitivity to Chronic Hypoxia: O_2-Sensitive Gene Expression in the Brain

Chronic sustained hypoxia increases (a) ventilation in hypoxia, (b) ventilation when normoxia is restored, and (c) ventilatory sensitivity to hypoxia, as demonstrated by an increased slope of the isocapnic HVR (Powell et al. 1998). These changes are collectively called ventilatory acclimatization to hypoxia (VAH), which involves both increased O_2-sensitivity of arterial chemoreceptors (Powell 2007) and plasticity in the CNS (Powell et al. 2000). Hence, the brain has multiple time domains of O_2-sensitivity that can elicit different ventilatory responses to acute versus chronic hypoxia.

The physiological mechanisms of the CNS component of VAH are not known but they may include plasticity in (a) the CNS mechanisms of acute O_2-sensing, (b) the CNS integration of peripheral O_2-sensitive reflexes (e.g. carotid body chemoreflex), and/or (c) the CNS integration of other non-chemoreflex ventilatory control pathways such as reflexes from pulmonary vagal chemoreceptors, or respiratory rhythm generators. VAH does not occur in animals without functional carotid bodies (Dempsey and Forster 1982) so generally it has been assumed that central O_2-sensitivity is not important for VAH. However, modern experimental designs used to study central O_2-sensitivity have not been applied systematically in chronically hypoxic preparations to rigorously test the hypothesis that VAH involves enhancement of CNS mechanisms of acute O_2-sensing. Similar comments apply to the possibilities for plasticity in CNS integration of non-chemoreflex pathways or central pattern generation of the respiratory rhythm. However, there is strong experimental evidence for enhanced CNS integration of arterial chemoreceptor afferent input. In an experimental preparation that isolates the reflex effects of the CNS, the phrenic nerve response to fixed levels of carotid sinus nerve stimulation is increased after chronic hypoxia (Dwinell and Powell 1999).

Any of these mechanisms for the CNS component of VAH are likely to involve changes in gene expression. Therefore, O_2-sensitive gene expression represents another mechanism of O_2-sensing in the brain that can affect cardiopulmonary function. Hypoxia increases immediate early gene expression in the CNS, which in turn produce transcription factors for other genes (Banasiak et al. 2000). Hypoxia also induces transcription factors such as nuclear factor-κB and activator protein-1 but their effects on cardiopulmonary responses to hypoxia are largely unknown (reviewed by Powell and Fu 2008). To date, the most important regulator of O_2-sensitive gene expression in the brain shown to have a role in ventilatory control is hypoxia inducible factor-1 (HIF-1). HIF-1α, which is the oxygen regulated part of the functional HIF-1 heterodimer, increases in respiratory centers of the CNS after as little as one hour and HIF-1α expression in the brain is necessary for normal VAH (reviewed by Powell and Fu 2008). Hence, O_2-sensing by HIF-1α could be involved in VAH by increasing the expression of several genes with products known to modulate the hypoxic ventilatory response. These are considered next.

HIF-1 was originally discovered as an important transcription factor for erythropoietin (EPO), which is released from the kidneys in response to hypoxia and increases the oxygen carrying capacity of blood by stimulating erythropoiesis in bone marrow (Semenza 1999). However, EPO and its receptor (EPO-R) have also been localized to neurons and glia in the CNS, including the pre-Bötzinger complex and nucleus tractus solitarius (Soliz et al. 2005), where it must have different effects. Transgenic mice that overexpress human EPO in the brain can sustain a virtually normal HVR following carotid body denervation, which ablates the HVR in wild type mice, and they show enhanced VAH compared to wild type mice (Soliz et al. 2005). Soluble EPO receptor (sEPO-R) may be the primary mediator for EPO effects on VAH in the brain. Chronic hypoxia downregulates sEPO-R in the brain and intracerebroventricular infusion of sEPO-R decreases EPO and reverses VAH in chronically hypoxic mice (Soliz et al. 2007). Experiments have not been done yet to determine if EPO and its receptor are involved directly in the acute mechanisms of central O_2-sensitivity or modulation of other pathways, e.g. arterial chemoreflexes or respiratory rhythm generators.

Endothelin- (ET-1) is a vasoactive peptide that is regulated by HIF-1 in hypoxia. ET-1 is widely distributed in the brain, where it plays an important role in stress responses, and in other O_2-sensitive tissues such as the carotid body and pulmonary artery (reviewed by Powell and Fu 2008). ET-1 causes enhanced O_2-sensitivity in the carotid bodies with chronic hypoxia (Chen et al. 2002) but it remains to be determined if ET-1 affects acute O_2-sensitivity in the brain or other ventilatory control pathways.

Heme oxygenase-1 (HO-1) is also induced by HIF-1 (reviewed by Powell and Fu 2008). Chronic hypoxia increases HO-1 in the rostral ventrolateral medulla and this has been hypothesized to contribute to ventilatory acclimatization to hypoxia (Mazza et al. 2001). However as discussed above, HO-1 is not expressed in the ventrolateral medulla of normoxic control animals, in contrast to HO-2. It remains to be determined if increased HO-1 with chronic hypoxia acts by the same putative

mechanism for HO-2 in acute O_2-sensing in the CNS, and adds to it, or if it even has an effect on ventilation.

Dopamine is another factor that can modulate the HVR by central mechanisms and these effects change during exposure chronic hypoxia (Huey et al. 2003). The rate limiting enzyme for dopamine synthesis is tyrosine hydroxylase, which is generally thought to be under control of HIF-1 (reviewed by Powell and Fu 2008). HIF-1α is increased by hypoxia selectively in cardiorespiratory centers of the brainstem and is co-localized with tyrosine hydroxylase in selected catecholaminergic cell groups in the brainstem, e.g. A1C1 and A2C2 (Pascual et al. 2001). Note these are the same cell groups that were observed to have increased catecholamines in mice that over-express EPO in the brain (Soliz et al. 2005). The precise roles of tyrosine hydroxylase and EPO in such changes with chronic hypoxia remain to be determined.

Summarizing, there are several potential neurochemical mechanisms whereby O_2-sensitive changes in gene expression in the brain, mediated by HIF-1, could contribute to plasticity in the acute hypoxic ventilatory response. However, no direct relationships have been demonstrated yet between acute mechanisms of O_2-sensing in the brain and this longer time domain of O_2-sensing.

5 Lessons from Central CO_2-Sensing in the Brain

The importance of central CO_2 chemoreceptors has been recognized much longer than O_2-sensing in the brain, so it may be instructive to compare and contrast the two phenomena. For CO_2, Guyenet points out a distinction between (a) *chemosensitivity* of CNS neurons and (b) central *chemoreceptors* (Guyenet et al. 2008). This is similar to the distinction that Dawes and Comroe (1954) drew in their review of peripheral chemoreflexes from the heart and lungs for (a) *chemoreflexes*, i.e. "reflexes initiated by a chemical substance whether they act upon true chemoreceptors, other types of sensory receptors or upon nerve endings themselves," versus (b) *chemoreceptors*, i.e. "sensory nerve endings which normally respond to changes in their natural chemical environment in health and disease." While many different experimental methods and preparations have revealed multiple sites and mechanisms of CO_2-sensitivity, Guyenet and colleagues (2008) argue that the evidence for multiple CO_2 chemoreceptors is not as strong. Applying this analysis to the problem of O_2-sensing in the brain, leads to the conclusion that there are multiple sites of O_2-sensitivity in the brain but central O_2-chemoreceptors have not been identified to date.

For central CO_2-sensitivity, there are also competing theories for (a) *distributed chemoreception* versus (b) *specialized chemoreceptors*. Again, Guyenet and colleagues (2008) argue that the evidence for distributed chemoreception is not as strong as that for specialized chemoreceptors that are primarily (if not exclusively) in the retrotrapezoid nucleus. However, there are several studies using whole animal awake or anesthetized preparations showing physiological ventilatory responses to

focal hypercapnia or acidosis in sites of the brainstem besides the retrotrapezoid nucleus (e.g. Coates et al. 1993). Further study will be necessary to determine if such distributed responses can be explained by universal properties of H^+/CO_2-sensitivity in neurons that just happen to be in a respiratory pathway or if some neurons in these pathways are true chemoreceptor. The same questions can be asked and need to be answered for O_2-sensing in the brain.

Finally, there are more teleological questions about central CO_2-sensitivity (Guyenet et al. 2008): Why are there so many CO_2-sensitive molecules in CNS neurons and why are there so many CO_2-sensitive sites in the brain? Of course, these questions are impossible to answer experimentally. However, it would increase our understanding of ventilatory control if physiologically significant roles could be described for multiple mechanisms of chemosenstivity or multiple chemoreceptor sites. Multiple CO_2-sensing mechanisms are hypothesized to increase the range and sensitivity of responsiveness (Jiang et al. 2005) and there is evidence that different central CO_2 chemoreceptor sites have different effects depending on sleep state (Nattie and Li 2002). Similar hypotheses should be explored for O_2-sensing in the brain and cardiorespiratory reflexes.

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