JOURNAL CLUB

Lessons from neuronal HIF1a: Understanding its role in ventilatory acclimatization to hypoxia

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Oxygen is required for life. This fact is made painfully clear by the ongoing COVID-19 pandemic, which can result in a rapid drop in blood O_2 leading to organ failure and death (Tay *et al.* 2020). In the absence of disease, humans have evolved the ability to sense decreases in O_2 and adapt to hypoxia. At the whole animal level, increases in ventilation during hypoxia are initiated by peripheral chemoreceptors located in the carotid and aortic bodies, which contain cells that can sense changes in arteriole O_2. Following onset of hypoxia, afferent input from the carotid bodies is sent to brainstem respiratory centers to increase ventilation, thereby improving delivery of O_2 to the blood. This response is known as the hypoxic ventilatory response (HVR).

During prolonged challenges such as chronic sustained hypoxia, longer lasting adaptations are needed to maintain O_2 homeostasis. In pathological and non-pathological conditions (e.g. relocation to a higher altitude), the body adapts to hypoxia by increasing both the depth (tidal volume) and frequency of breathing in a process known as ventilatory acclimation to hypoxia (VAH). Interestingly, after returning to normoxic environments, those individuals who have experienced VAH typically show a more vigorous HVR to future bouts of hypoxia compared to non-acclimated individuals, thus indicating that VAH somehow sensitizes the body to hypoxia.

These adaptations probably occur at the cellular level and are mediated by hypoxia inducible factor 1-alpha (HIF1a), an O_2 sensitive transcription factor. The discovery of HIF1a and its role in how cells sense and adapt to O_2 availability by William Kaelin, Jr, Sir Peter Ratcliffe and Gregg Semenza earned them the 2019 Nobel Prize in Physiology or Medicine. Previous work showed that HIF1a in peripheral chemoreceptors is requisite for VAH. Although brainstem respiratory centers are sensitive to hypoxia and contribute to VAH, it was unclear whether HIF1a was involved in the CNS contribution to VAH.

To better understand potential contributions of HIF1a to central hypoxia adaptations, in this issue of the *The Journal of Physiology*, Moya *et al.* (2020) report examining the VAH response in two mouse models: (1) mice deficient in HIF1a throughout the CNS and (2) mice lacking HIF1a only in the nucleus tractus solitarius (NTS), the CNS relay centre for peripheral chemotransduction to the rest of the respiratory circuit. To conditionally delete HIF1a only from the CNS (CNS-HIF1a$^{-/-}$), Moya *et al.* (2020) crossed R1ag#5 mice that express cre recombinase under the control of a calcium/calmodulin-dependent protein kinase IIa promotor (CaMKIIa-Cre) with HIF1a$^{f/f}$ mice.

Using a reporter mouse, they confirmed that expression of cre was restricted to the CNS and absent from peripheral chemoreceptors. They further validated their model using quantitative RT-PCR to show that HIF1a was effectively eliminated from the brain. Adult male CNS-HIF1a$^{-/-}$ mice and littermate controls were acclimated to 7 days of chronic sustained hypoxia (P_{IO_2} = 70 Torr) alongside respective room air controls. Following the end of acclimatization, they compared the HVR between the chronic hypoxia and normoxic controls for both genotypes. As expected, the magnitude of HVR, as quantified by inspired ventilation, was higher in the chronic hypoxia group compared to the normoxia group. However, the magnitude of HVR was similar between genotypes exposed to chronic hypoxia condition.

A caveat to this experiment is that mice may compensate for loss of HIF1a during development. To address this possibility and at the same time focus on the brainstem region probably contributing to VAH, they injected an adeno-associated virus (AAV) to express cre-recombinase bilaterally in the NTS of adult HIF1a$^{f/f}$ mice to conditionally delete HIF1a only in the NTS (NTS-HIF1a$^{-/-}$). A virus that does not express cre-recombinase was used as a control. Five days after viral injections, animals were exposed to chronic hypoxia or normoxia control conditions. As expected, the HVR of the normoxia cohort was similar between genotypes. By contrast, NTS-HIF1a$^{-/-}$ mice exposed to chronic hypoxia showed a diminished HVR (primarily an inspiratory volume deficit) compared to control treated mice. Normalizing ventilation to CO_2 production in the normoxic condition, both genotypes showed similar trends when breathing 10% or 21% O_2, suggesting that blunted VAH is not secondary to a metabolic deficit. Injection sites were targeted to the caudal and medial portion of the NTS, and, unexpectedly, AAV preferentially infected glutamatergic neurons (vesicular GABA transporter immunoreactive) that were also activated (cFos expression) by chronic hypoxia.

These results show for the first time that expression of HIF1a in the NTS contributes to VAH. Specifically, deletion of HIF1a from glutamatergic NTS neurons disrupted VAH following exposure to chronic hypoxia. Interestingly, only glutamatergic neurons were infected. Because glutamatergic NTS neurons were also activated by hypoxia (cFos+), this finding suggests that AAV2, the serotype used in the study by Moya *et al.* (2020), preferentially infects cells in an activity-dependent manner. It is also possible that gene expression correlates with neural activity; thus, cre-recombinase may be expressed at higher levels in more active cells. This possibility is supported by evidence showing AAV2 vectors more readily integrate into actively expressed genes (Nakai *et al.* 2003). It should be noted that the study by Moya *et al.* (2020) cannot exclude the possible involvement of GABAergic NTS neurons in the VAH. Because the NTS contains both glutamatergic and GABAergic neurons, further investigation is needed to understand the role of HIF1a in gabaergic neurons and whether it contributes to the VAH response.

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There is considerable debate regarding the importance of central *vs.* peripheral nervous system contributions to the HVR and subsequent VAH (see *Journal of Physiology* CrossTalk Debate 38). These results support the possibility of a direct role of the central nervous system in the adaptive responses to hypoxia via HIF1a signalling in glutamatergic NTS neurons. However, because the NTS is at the interface between the central and peripheral nervous systems, it remains possible that NTS adaptive responses to hypoxia are facilitated by or requires excitatory peripheral input.

It is also possible that glia or glial-like cells serve as a common substrate for both central and peripheral hypoxia responses. For example, the primary O_2 sensing element of the carotid body comprises type I glomus cells. However, these are enveloped by type II glia-like cells that also respond to hypoxia (directly or indirectly by paracrine signalling from type I cells) by releasing signalling molecules including ATP to further modulate type I glomus cell activity (Leonard *et al.* 2018). Similarly, a subset of brainstem astrocytes in respiratory centers including the NTS are activated by afferent nerve stimulation or directly by hypoxia and modulate neural activity by paracrine ATP release or altered glutamate uptake (Accorsi-Mendonça *et al.* 2019). It is important to note that not all CNS astrocytes function similarly because astrocytes in the brainstem can widely vary in morphology, protein expression and CO_2/pH sensitivity (see *Journal of Physiology* CrossTalk Debate 38). These results suggest that subsets of astrocytes could modulate NTS function including adaptive responses to hypoxia. Moreover, as discussed by Moya *et al.* (2020), the serotype used in their study demonstrated a restricted area of action and is targeted to infect mainly neurons and not other CNS cells such as astrocytes. Therefore, we cannot disregard the contribution that other cells, such as glia, might have on VAH and this further supports the notion that more work is needed to determine region specific physiological roles of glia and glial-like cells. In particular, an understanding of unique genetic markers of functionally discrete astrocyte subsets is required to address this issue.

In sum, the study by Moya *et al.* (2020) broadens our understanding of the contribution of neuronal HIF1a and its contribution to facilitating VAH in the CNS. This work is clinically relevant because it identifies HIF1a in glutamatergic neurons as being an important mediator of adaptation to chronic hypoxia. Furthermore, based on evidence that people living under high altitude chronic hypoxia conditions may be less sensitive to the severe impact of COVID-19 (Arias-Reyes *et al.* 2020), understanding the adaptive response to chronic hypoxia may provide insight into treatments for COVID-19.

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Additional information

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