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Acute oxygen sensing – inching ever closer to an elusive mechanism

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ABSTRACT: The carotid body has long been recognised as the body's primary acute oxygen sensor. For decades, this organ has been reluctant to reveal mechanisms underlying its sensory activity. In this issue of Cell Metabolism Fernandez-Aguera et al (2015) take us closer to a fuller understanding of this fundamental process.

MAIN TEXT: The existence of carotid body, a tiny structure (approx. 10mg in man) found at the bifurcation of the common carotid arteries, been acknowledged for more than two centuries. Only with the exquisite anatomical studies of de Castro, however, followed shortly thereafter by functional studies particularly by Heymans (father and son) in the early 20th century, we now know it functions as a sensory organ (see (Kumar & Prabhakar, 2012) for a review). It has an extremely high blood supply and is a polymodal sensor of arterial blood, responding to changes in pO_2, pH, pCO_2, glucose and other agents, by altering afferent nerve activity which in turn powerfully influences central control of ventilation and other sympathetically driven cardiovascular activities. It is a sensor of acute hypoxia - at levels that other tissues would fail to notice - that has generated most scientific interest. Furthermore the carotid body plays important roles in cardiorespiratory diseases such as hypertension and heart failure (Kumar & Prabhakar, 2012).

Within the carotid body, type I (glomus) cells cluster tightly in small groups closely apposed to both blood vessels and afferent sensory nerves. These transmitter-filled cells have long been considered the sensory elements of this organ. In the late 1980's a handful of groups independently developed preparations of isolated type I cells, allowing accessibility to multiple cell biological techniques. Rapid progress was subsequently made

towards understanding the cellular basis of O_2 sensing. Lopez-Barneo's group and collaborators in Valladolid demonstrated that these cells expressed K^+ channels which were rapidly and reversibly inhibited by acute hypoxia (Lopez-Barneo et al., 1988). For some time controversies around this topic simmered (Kumar & Prabhakar, 2012), but eventually distilled into what is accepted now as the membrane hypothesis for chemoreception (Prabhakar & Peers, 2014). Put simply, the hypothesis states that hypoxia (amongst other stimuli, as mentioned above) inhibits K^+ channels causing membrane depolarization and consequent voltage-gated Ca^{2+} entry, which is sufficient to trigger transmitter release and so excite afferent nerves, thereby exerting powerful cardiorespiratory effects. The key question since then has been "how?". How does a fall in pO_2 lead rapidly to K^+ channel inhibition? While numerous ideas have been proposed (Peers et al., 2010), a uniform agreement as to this fundamental process has not been forthcoming. However, mitochondria have long been implicated, since the suggestion made almost 45 years ago by Mills and Jobsis (Mills & Jobsis, 1972) that the carotid body may possess a cytochrome oxidase with unusual O_2 affinity. The work of Fernandez-Aguera et al, published in this issue (Fernandez-Aguera et al., 2015), places mitochondria firmly centre stage and, crucially, points to complex I of the electron transport chain as being a key element for oxygen sensing. It also brings to prominence once again a signalling role for reactive oxygen species (ROS).

Fundamental to this study, Fernandez-Aguera et al generated a knockout mouse which lacked the 49kD protein, Ndufs-2, specifically in catecholaminergic cells including type I cells (and adrenal chromaffin cells, also studied in this paper). Ndufs2 forms part of the ubiquinone binding site (where the classical inhibitor rotenone binds to inhibit ubiquinone reduction). Without Ndufs-2, the ability of complex I to perform its physiological role of catalyzing NADH oxidation, ubiquinone reduction and H^+ transport, is highly impaired. However, the cells were not markedly bioenergetically compromised, because of unusually high activity of succinate-dependent oxidative phosphorylation. Lopez-Barneo and colleagues had previously shown that rotenone, one of the many mitochondrial inhibitors which were known to mimic / occlude effects of hypoxia in the carotid body, was particularly effective, thus leading them to suggest a key role for complex I in O_2 sensing over 10 years ago (Ortega-Saenz et al., 2003).

Whilst the responses to most chemostimuli of type I cells from Ndufs-2 deficient mice remained intact, they showed an impressive lack of sensitivity to hypoxia, as assessed by

several different approaches, including intact hypoxic ventilatory responses, amperometric transmitter detection, $[Ca^{2+}]_i$ measurements and NAD(P)H autofluorescence. Key to the membrane hypothesis, the authors used patch-clamp recordings to demonstrate a lack of effect of hypoxia on K^+ channels in these cells. In fact, it appeared that K^+ channels in Ndufs-2 deficient mice were under tonic inhibition (and were depolarized as compared with control cells). Buffering of intracellular ROS relieved this inhibition, but not in control cells. Conversely, in control cells, oxidants mimicked and occluded hypoxic inhibition of K^+ channels, effects not observed in Ndufs-2 deficient cells. The implication from these observations was that hypoxia inhibits K^+ channels (the fundamental step in the membrane hypothesis) via a complex I dependent mechanism which involves a rise of ROS to modulate K^+ channels, possibly directly. This was further supported by fluorescence imaging of reversible hypoxic rises of ROS in mitochondria of control cells, whilst Ndufs-2 cells showed generally raised mitochondrial ROS levels but no obvious hypoxic response.

Emerging from these data is the overall proposal that hypoxia may slow type I cell electron transport and cause reduced quinone accumulation, leading to a highly reduced state in complex I. In this state (which may even reverse its activity) ROS production and concomitant NADH accumulation occurs rapidly and both may contribute to inhibition of K^+ channels. The process does not involve alterations in ATP levels, which are maintained via succinate / complex II.

This study will provoke excitement and debate within and beyond the field of chemoreception. It supports the once controversial idea that ROS levels increase rather than decrease during hypoxia (Guzy & Schumacker, 2006). It also places mitochondria as central to O_2 sensing, but not via reduced ATP formation. It does not conflict with the concept of a cytochrome oxidase with unusual O_2 affinity (Mills & Jobsis, 1972); data have emerged supporting this suggestion (Duchen & Biscoe, 1992;Buckler & Turner, 2013) but it remains to be determined exactly how hypoxia disrupts mitochondrial activity in such a specialised way. However, what we do know now, due to this multidisciplinary, comprehensive and compelling study (Fernandez-Aguera et al., 2015) is that complex I is essential in coupling pO_2 to K^+ channel regulation.

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