A paradigm shift in oxygen sensing with a twist in the tale!

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Abstract

AMP-activated protein kinase (AMPK) is pivotal to metabolic homeostasis in eukaryotes, serving as a critical energy sensor. Increased AMPK activity during oxygen deprivation (hypoxia) protects against potentially catastrophic deficits in ATP supply. Whilst the nervous system circuitry for elaboration of the complex cardiorespiratory response to hypoxia has been understood in some detail for many decades, there is continued and considerable interest in the molecular machinery underpinning the mechanism(s) of oxygen sensing. In this issue of the *Biochemical Journal*, Evans et al. (2016) review their recent work, which points to a pivotal role for AMPK in the transduction of cellular hypoxic stress to integrated ventilatory behaviour, critical in the defence of whole-body oxygen homeostasis. Of great surprise, there is profound blunting of the hyperventilatory response to hypoxic stress in AMPK deficient mice, with resultant dysregulated breathing arising in spite of normal peripheral oxygen sensing and appropriate sensory input to the brain! Their pointedly provocative review challenges current dogma, and in doing so raises intriguing questions that probe fundamental aspects of our understanding of the mammalian ventilatory response to hypoxic stress. The engaging review by Evans et al. (2016) is an interesting read that is sure to encourage colourful debate.

Commentary

AMP-activated protein kinase (AMPK) is pivotal to energy homeostasis in eukaryotic cells (Carling and Viollet, 2015; Hardie *et al*., 2016). During metabolic stress, such as oxygen deprivation (hypoxia), AMPK activity is increased serving to drive catabolic signalling that helps to buffer potentially catastrophic deficits in ATP supply from hypoxia-dependent depression of mitochondrial oxidative phosphorylation. As such, AMPK functions as a crucial cellular 'energy-stat'. This fundamental gatekeeper role, coupled with observations of polymorphisms in the gene for AMPK-α1 in hypoxic-adapted high altitude natives, has led to speculation that AMPK is perhaps perfectly poised to coordinate integrative responses to oxygen deficiency.

The physiological response to whole-body hypoxic stress, observed for example at altitude, in pulmonary diseases, and in respiratory control disorders that commonly present during sleep, includes the archetypal reflex cardiorespiratory adjustments of increased ventilation (serving to better oxygenate the pulmonary blood), increased cardiac output (improving oxygen delivery to the systemic circuit), and systemic vasodilatation (enhancing local delivery and uptake of oxygen into peripheral tissues) that together protect against profound oxygen deficiency in the face of global hypoxic stress (Teppema and Dahan, 2010; Prabhakar and Semenza, 2012). Whilst the integral neural circuitry for elaboration of the cardiorespiratory response to hypoxia has been understood in some detail for many decades, there remains considerable interest and controversy in respect of the precise molecular mechanisms underpinning the peculiar particulars of oxygen sensing by chemoreceptors (Lopez-Barneo *et al*., 2016; Prabhakar and Peers, 2014). Beyond that, there is curiosity and debate too in the potential for hypoxic sensing in structures other than the carotid bodies—the primary blood oxygen sensors found bilaterally at the bifurcation of the common carotid arteries in the neck (Kumar and Prabhakar, 2012). In this issue of the *Biochemical Journal*, Evans *et al*. (2016) review their recent exciting

contribution to this important dynamic field. Their engaging and entertaining thesis posits a new perspective on pivotal pieces in the oxygen sensing puzzle.

The original work of Mahmoud *et al*. (2016) examined whole-body ventilatory responses to acute hypoxic challenge in wild-type mice, and mice engineered with conditional deletion of the *AMPK-α1* and/or *AMPK-α2* genes in catecholaminergic (tyrosine hydroxylase expressing) cells of the hypoxia-responsive respiratory network, which extend from the carotid body, the peripheral (traditionally-viewed) primary oxygen sensor, to the brainstem respiratory networks that shape the rhythm and pattern of breathing. Under baseline conditions breathing room air, ventilatory parameters were similar in wild-type and *AMPK* knockout mice. However, upon exposure to hypoxic gas challenge, *AMPK* knockout mice presented with a profound blunting of the classic hyperventilatory response, and instead were seen to express respiratory depression and instability culminating in protracted pauses in respiration termed apnoeas—ventilatory dysfunction that was proportional to the severity of the hypoxic stimulus. This aberrant ventilatory response to hypoxia was further exacerbated under anaesthesia, which removes volitional control of breathing and perhaps better reveals the extraordinary extent of inappropriate brainstem neural activation during whole-body hypoxic stress; exposure to severe hypoxia in anaesthetised *AMPK* deficient mice resulted in respiratory failure! Selectivity for the loss of oxygen sensing *per se* was illustrated by way of preserved ventilatory responsiveness to hypercapnia (elevated carbon dioxide), which restored regular breathing patterns and responses in *AMPK* knockout mice. On the face of it, the data strongly argue in favour of a pivotal role for AMPK in the transduction of hypoxic stress to an integrated ventilatory response, critical in the defence of whole organism oxygen homeostasis. The preservation of ventilatory drive during hypercapnic hypoxia in *AMPK* knockout mice is an important observation, in that it highlights that *AMPK* deletion is not associated with a generic disruption of neuronal activation or synaptic transmission in catecholaminergic neurons *per se*,

which are known to be CO_2/pH sensitive. Rather, there appears to be a specific failure within the neural network to transduce hypoxic stress, culminating in a dramatic unaroused global response to the insidious stimulus of oxygen lack. Consistent with this proposal, functional magnetic resonance imaging revealed an attenuated activation of caudal brainstem nuclei during hypoxia in *AMPK* knockout mice, with further corroboration of a restrained central neural response provided by evidence of decreased *cfos* activation during hypoxia in catecholaminergic cells in critical sites of the brainstem respiratory network, with no apparent change in the number and distribution of such cells and no obvious structural abnormalities. One would be forgiven for assuming that these observations point to a single unifying feature, namely aberrant oxygen sensing at the level of the peripheral oxygen sensor—the carotid body. The simplest basis for the authors' observations ought to have been that hypoxic-dependent excitation of carotid body afferent (sensory) input to the brainstem in *AMPK* knockout mice was profoundly blunted. In this way, a reduced sensory cue would expectedly translate to blunted and ineffective efferent (motor) responses that would be consistent with the atypical ventilatory responses to hypoxia in *AMPK* knockout mice. Unexpectedly, chemo-afferent responses to hypoxia were shown to be perfectly normal in *AMPK*-deficient carotid bodies. Thus, inactivation of central neural networks and grossly perturbed breathing responses during hypoxic challenge in *AMPK* knockout mice occur in spite of the normal increased chemo-afferent input from the carotid body during hypoxia, and not because of an absence of it! Of interest, the observation definitively, if surprisingly, excludes an obligatory role for AMPK in the mechanism of oxygen sensing by the dominant peripheral oxygen chemosensor. More than that however, the findings challenge fundamental aspects of our understanding of the mechanisms of CNS responsiveness to hypoxic stress, and in doing so potentially rock the foundations of our hitherto appreciation of the integrated ventilatory response to hypoxia.

The deliberately provocative, but always well-spirited review by Evans et al. (2016) expands on the authors' original research paper (Mahmoud *et al*., 2016) drawing from the historical literature (including comparative studies in amphibians), further informed by contemporary observations of their own and by those of others employing global or conditional knockout mice, to present a coherent argument that concludes that the hypoxic ventilatory response must be determined by the coordinated action of the carotid body and a hypoxia-responsive circuit within the brainstem. The authors delight in the revelation that this is not an entirely new concept, rather one that has been overshadowed by the intense interest (and evidence) in the dominant role of the carotid body in determining the hypoxic ventilatory response. Somewhat tongue-in-cheek, the authors propose the existence of (an elusive) nucleus or node of oxygen-sensitive neurons, or perhaps multi-nodal circuit that serves as true gatekeeper of the hypoxic ventilatory response, the authors emphasizing the pivotal role for AMPK ordinarily within this network in protecting against hypoventilation and apnoea (dysregulated breathing) during hypoxia by way of normal robust ventilatory activation.

The fly in the ointment of this fine work, lies not in those measurements made by the investigators, but rather in one arguably key parameter that is notably missing from the studies to date. Measurements of metabolism during hypoxia could prove especially important in unravelling the mystery of aberrant ventilatory responsiveness to hypoxia in *AMPK* knockout mice. Small rodents, particularly mice, express quite short-lived (minutes) ventilatory responses to hypoxia, adopting instead a useful hypometabolic strategy, electing to decrease considerably oxygen demand in the face of oxygen deprivation, different to the strategy adopted by larger animals including humans characterised by increased physiological work subserving persistent cardiorespiratory strategies aimed at improving oxygen supply (Mortola and Maskrey, 2011). Whilst one cannot readily challenge the authors' observation of a significant blunting of the peak hypoxic ventilatory response in *AMPK* knockout mice (with

presumed normal carotid body activation), it is tempting to consider that perhaps a component of the response thereafter relates to different metabolic (and hence ventilatory) strategies between wild-type and knockout mice in response to hypoxia. The striking behavioural response of *AMPK* knockout mice in hypoxia, adopting as the authors put it, a torpor-like state, illustrates that minimizing oxygen utilization is clearly a determined outcome in knockout animals upon exposure to hypoxia. The question that arises is whether this contributes to or is exclusively a consequence of the abnormal ventilatory phenotype. Did the profoundly abnormal ventilatory response to hypoxia trigger the behavioural phenotype or did a profoundly abnormal metabolic response to hypoxia contribute in some way to dysregulated breathing? Admittedly, it is more likely that the typical hypometabolic strategy of the mouse has consequence for hypoxic challenges of time domains much longer than that utilized by the authors in their study, but nevertheless assessment of metabolism, and the relationship of ventilation to metabolism during hypoxia would be a useful addition for consideration in future investigations.

Evans *et al*. (2016) also provide a short but informative review of the evidence supporting a potentially critical role for AMPK signalling in the physiology of oxygen delivery in the lungs and placenta by way of regulation of smooth muscle tone. The emerging evidence excitedly points to a primary role for AMPK in maintaining oxygen homeostasis in these tissues. Moreover, aberrant signalling might underpin pathophysiological traits in these critical gas exchange organs and as such an increased understanding of basic mechanisms in model systems might help reveal potential therapeutic strategies in disease states into the future.

There is a rich history of exploration of the familiar facets of the ventilatory response to hypoxia over various time domains of relevance to health and disease. Evans *et al*. (2016) provide a thought-provoking and thoroughly engaging review of their important contribution to this exciting and evolving field. Contemporary sophisticated experimental tools are revealing

nature's long-held secret strategies, but in doing so they are also providing us with new questions more enigmatic than the answers that we find!

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