Edinburgh Research Explorer

The emerging role of AMPK in the regulation of breathing and oxygen supply

Citation for published version:
Evans, AM, Mahmoud, AD, Moral-Sanz, J & Hartmann, S 2016, 'The emerging role of AMPK in the regulation of breathing and oxygen supply', Biochemical Journal, vol. 473, no. 17, pp. 2561-72.
https://doi.org/10.1042/BCJ20160002

Digital Object Identifier (DOI):
10.1042/BCJ20160002

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Biochemical Journal

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Regulation of breathing is critical to our capacity to accommodate deficits in oxygen availability and demand during, for example, sleep and ascent to altitude. It is generally accepted that a fall in arterial oxygen increases afferent discharge from the carotid bodies to the brainstem and thus delivers increased ventilatory drive, which restores oxygen supply and protects against hypventilation and apnoea. However, the precise molecular mechanisms involved remain unclear. We recently identified as critical to this process the AMP-activated protein kinase (AMPK), which is key to the cell-autonomous regulation of metabolic homoeostasis. This observation is significant for many reasons, not least because recent studies suggest that the gene for the AMPK-α1 catalytic subunit has been subjected to natural selection in high-altitude populations. It would appear, therefore, that evolutionary pressures have led to AMPK being utilized to regulate oxygen delivery and thus energy supply to the body in the short, medium and longer term. Contrary to current consensus, however, our findings suggest that AMPK regulates ventilation at the level of the caudal brainstem, even when afferent input responses from the carotid body are normal. We therefore hypothesize that AMPK integrates local hypoxic stress at defined loci within the brainstem respiratory network with an index of peripheral hypoxic status, namely afferent chemosensory inputs. Allied to this, AMPK is critical to the control of hypoxic pulmonary vasoconstriction and thus ventilation–perfusion matching at the lungs and may also determine oxygen supply to the foetus by, for example, modulating utero-placental blood flow.

Key words: AMP-activated protein kinase (AMPK), apnoea, Ca\(^{2+}\)-calmodulin-activated kinase-β (CaMKK-β), hypoxia, liver kinase B1 (LKB1), pulmonary, ventilation.

INTRODUCTION

Regulated oxygen supply is key to the maintenance of oxidative phosphorylation and thus cellular energy status in mammals, not least because of the limited capacity for cellular oxygen storage relative to the extensive reserves of other substrates. It was proposed, therefore, that natural selection may have employed AMP-activated protein kinase (AMPK) to co-ordinate system-level adjustments of whole-body function in response to oxygen deficits in animals [1]. Consistent with this view, recent studies on high-altitude Andean populations have shown that the gene for the AMPK-α1 subunit (PRKAA1) has been influenced by natural selection through single nucleotide polymorphisms [2]. Confirmation of a role for AMPK in oxygen delivery has now been provided by conclusive experimental evidence that, in addition to its well-recognized capacity as a regulator of cell-autonomous pathways of energy supply [3], AMPK is essential to the regulation of breathing during hypoxia and thus oxygen and energy distribution to the body [4].

FRAGMENTS FROM THE LIBRARIES OF BABYLON

The AMP-activated protein kinase

AMPK is a cellular energy sensor that acts to maintain energy homoeostasis. It exists as heterotrimers comprising one each of the two β and three γ regulatory subunits, which together may form at least 12 different heterotrimeric subunit combinations [5,6]. In this respect it is important to note that evidence is now emerging to suggest that different subunit combinations may be selected by a given cell type, that each combination may exhibit different sensitivities to activation by AMP and ADP and thus metabolic stresses, and that each may selectively phosphorylate and regulate a different spectrum of target proteins [8].

AMPK activities are exquisitely coupled to mitochondrial metabolism through changes in the cellular AMP/ATP and ADP/ATP ratios (Figure 1). There are four nucleotide-binding sites (CBS repeats) on the γ subunit, of which only sites designated 1, 3 and 4 may ever be occupied [8]. Binding of AMP to the γ subunit causes a 10-fold increase in AMPK activity by allosteric activation, with further activation of up to 100-fold generated by binding of either AMP or ADP through their promotion of phosphorylation and inhibition of dephosphorylation at Thr\(^{172}\) on the α subunit; each of these effects is opposed by ATP [9,10]. Thr\(^{172}\) is primarily phosphorylated by the tumour-suppressor kinase liver kinase B1 (LKB1), which appears to be constitutively active but phosphorylates AMPK more rapidly when AM(D)P is bound to the γ subunit [11]. There is also an alternative Ca\(^{2+}\)-dependent activation mechanism, the calmodulin-dependent protein kinase Ca\(^{2+}\)-calmodulin-activated kinase γ (CaMKK-γ), which phosphorylates Thr\(^{172}\) and thus activates AMPK in an AMP-independent manner [5,6,12]. Contrary to previous proposals [13], however, there is little...
evidence to support the view that AMPK is directly activated by reactive oxygen species (ROS) [14,15]. Once activated the classical action of AMPK is to phosphorylate targets that switch off non-essential anabolic processes that consume ATP and on catabolic pathways that generate ATP [12], thereby promoting displacement of ATP by AMP, and to a lesser extent by ADP, from three sites on the AMPK γ subunit. Binding of AMP or ADP to the γ subunit may promote phosphorylation by LKB1 and at the same time (2) inhibit dephosphorylation by PP2C. (3) AMP, but not ADP, binding also promotes further allosteric activation of AMPK. These three mechanisms deliver AMPK activation in response to metabolic stresses. In addition, AMPK can be activated in a Ca2+ -dependent manner through CaM KK-γ, which phosphorylates the same γ subunit site as LKB1. Figure adapted from [179]: Hardie, D.G., Salt, I.P., Hawley, S.A. and Davies, S.P. (1999) AMPK-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. Biochem. J. 338, 717–722.

Regulation of AMPK

Regulation of AMPK activity is critical to the body’s capacity to accommodate variations in oxygen demand and supply during sleep and ascent to altitude is exemplified by the fact that adaptation of mammals to hypoxia at altitude is initially characterized by progressive increases in ventilatory drive, which partially restore arterial PO2 and protect against apnoea [30]. Ventilatory movements are delivered by motor neuronal pathways that are informed by respiratory central pattern generators (rCPGs), which are distributed bilaterally in the pons and ventral medulla of the brainstem (Figure 2) [31]. These semi-autonomous neural networks comprise core circuits of excitatory and inhibitory interneurons that deliver rhythmic patterns of activity [32], and confer a set-point about which respiratory rhythm is continuously modulated through the integration of inputs from those central [32,33] and peripheral chemosensors [34] which monitor oxygen, carbon dioxide and pH. It is generally accepted that the carotid bodies, which reside at the bifurcation of the common carotid artery, represent the primary peripheral chemoreceptors [34] and that the acute hypoxic ventilatory response is delivered by increased afferent discharge from the carotid bodies to the rCPGs via, in great part, catecholaminergic networks within the caudal brainstem (Figure 3) [35,36].

To assess the role of LKB1 and AMPK in this process, we used the tyrosine hydroxylase promoter to drive deletion of AMPK-α1 and -α2 genes in all catecholaminergic cells [4], including those of the hypoxic ventilatory response, and which ultimately led to hypoventilation rather than hyperventilation and frequent prolongation of apnoeas.

Upon hypoxia at altitude or during sleep, activation of LKB1/AMPK signalling pathways may therefore aid appropriate ventilatory adjustments and thus protect against acute ventilatory instability [30], although deficiency of either may confer greater susceptibility to disordered breathing. In this respect it is notable that, of the two available α subunits, selective loss of the AMPK-α1 catalytic subunit was the primary precipitant of ventilatory dysfunction during hypoxia [4,29] that was characterized by marked attenuation of the hypoxic ventilatory response, and which ultimately led to hyperventilation rather than hypoventilation and frequent prolongation of apnoeas.

Given that it is widely accepted that the carotid bodies drive the entire ventilatory response to a fall in arterial PO2, we had always presumed that this organ would be the primary site of AMPK action in this respect. Not for the first time, however, the
AMPK and oxygen supply

A PINCH OF PTOLEMY – AMPK AND THE CAROTID BODY

The carotid bodies were identified as sensory organs by De Castro in 1928 [39], after which Heymans and Bouckaert [40] established that they mediated hyperventilation in response to a fall in arterial $PO_2$ and thus defined these organs as the primary peripheral arterial chemoreceptors. The carotid body type I (glomus) cells underpin chemosensory activity [41], when upon exposure to hypoxia and/or hypercapnia they release a variety of neurotransmitters which elicit increases in afferent fibre discharge along the carotid sinus nerve and thereby govern cardiorespiratory reflexes that elicit corrective changes in ventilation [42–45]. Recent evidence now suggests that the aortic bodies, which are located at the aortic arch, are similarly activated during hypoxia and/or hypercapnia and may also contribute to the hypoxic ventilatory response [37]. Type I cells of the carotid and aortic bodies therefore define a class of oxygen-sensing cells, in which the $PO_2$ at which mitochondrial oxidative phosphorylation is inhibited during hypoxia ($\leq 60$ mmHg oxygen) is higher than in other cell types [46–48]. Once this threshold is breached hypoxia-induced changes in cell activity increase in a manner related to the degree of hypoxia [46,49], that is until these activities begin to fail under near anoxic conditions ($<2\%$ oxygen) [50]; at this point mitochondrial oxidative phosphorylation is inhibited in cells that do not function to monitor oxygen supply [47]. In short, all oxygen-sensing cells function to respond to deficits in oxygen supply over the physiological range of $PO_2$.

Mitochondria underpin hypoxia-response coupling in carotid body type I cells

A significant body of evidence now argues in favour of the view that type I cell activation during hypoxia is consequent to the inhibition of mitochondrial function. In retrospect the initial clue to this fact was provided by the seminal work of Heymans and Bouckaert [40], in that they demonstrated that cyanide mimicked and occluded the activation by hypoxia of the carotid body. However, the first direct evidence was obtained through the analysis of the respiratory chain redox status [51]. By relating outcomes to afferent sinus nerve discharge during hypoxia, it was shown that an increase in the NAD(P)H/NAD(P) + ratio correlated with changes in afferent nerve activity over the physiological range of oxygen levels. At the time it was proposed that mitochondria of most cells may utilize a high-affinity (i.e. normal) cytochrome $a_3$, whereas the cytochrome $a_3$ incorporated in mitochondria of oxygen-sensing cells may have a low affinity for oxygen. Consistent with this hypothesis, recent investigations have demonstrated that NDUFA4L2 [52] and COX4I2 [53,54], two nuclear-encoded atypical subunits of the mitochondrial electron transport chain, are constitutively expressed in carotid body type I cells under normoxia [55]. This contrasts with a number of other cell types where NDUFA4L2 and COX4I2 expression is ordinarily low, but is increased during prolonged
hypoxia [52–54]. Both NDUFA4L2 and COX4I2 reduce the capacity for mitochondrial oxygen consumption and act to limit mitochondrial ROS production during hypoxia, by reducing the activity of complex I and cytochrome c oxidase respectively. In this respect it is interesting to note that allosteric modulation of cytochrome c oxidase (COX) is delivered by COX4 in a subtype-specific manner, with COX4I1 but not COX4I2 conferring COX inhibition by ATP [54,56], i.e. in carotid body type I cells it seems unlikely that the rate of oxygen consumption and thus ATP supply via mitochondrial oxidative phosphorylation will increase during hypoxia as ATP levels fall [53,56–58]. It has been suggested, therefore, that constitutive expression of NDUFA4L2 and COX4I2 by carotid body type I cells might determine the affinity of their mitochondria for oxygen and thus confer, in part, the capacity of these cells to monitor changes in arterial oxygen supply. Intriguingly, COX4I2 is also constitutively expressed by pulmonary arterial myocytes [58,59] and neurons of the central nervous system [56], which may in some instances also function to monitor oxygen supply (see below).

That mitochondria may be the site of oxygen-sensing within type I cells of the carotid body is supported by the fact that, in addition to cyanide, all inhibitors and uncouplers of mitochondrial electron transport both mimic and occlude the effects of hypoxia [60]. Moreover, recent studies have shown that conditional deletion in type I cells of Ndufs2, a mitochondrial complex I gene that participates in ubiquinone binding, blocks carotid body activation during hypoxia [61].

ATP, LKB1, AMPK and hypoxia-response coupling in carotid body type I cells

What remains open to debate is the precise nature of the signalling pathway(s) which couples inhibition by hypoxia of mitochondrial oxidative phosphorylation to the activation of oxygen-sensing cells, such as type I cells, and whether or not all oxygen-sensing cells utilize a common signalling pathway. At the very least one would expect an initial fall in ATP supply and associated ADP accumulation that would be compensated for, in the immediate term, by the adenylate kinase reaction, leading to consequent increases in the AMP/ATP ratio [62,63]. When one considers this and the fact that AMPK is intimately coupled to mitochondrial metabolism via both increases in the AM(D)P/ATP ratio and LKB1, the possibility that AMPK may contribute to hypoxia-response coupling is immediately apparent. If this were the case, then one would naturally expect any contribution of AMPK to ventilatory control to be delivered at the level of the carotid body type I cell and through the consequent inhibition of those ‘oxygen-sensing’ potassium channels known to underpin their chemosensory response. Not least because thereafter the generally held viewpoint is that carotid body afferent inputs to the brainstem activate subordinate relays that modulate rCPG activities and thus increase ventilation.

Our preliminary investigations into the role of the LKB1/AMPK signalling pathway appeared entirely consistent with this view, in that conditional deletion of LKB1 virtually abolished the capacity for type I cell activation during hypoxia, increases in afferent discharge and, like AMPK deletion, attenuated the hypoxic ventilatory response [64,65]. Contrary to these findings and against our expectations, however, AMPK deletion failed to attenuate afferent discharge from the carotid body, yet caused even greater attenuation of the hypoxic ventilatory response [4] when compared with LKB1 deletion (unpublished work). This runs counter to our previous pharmacological studies, which suggested that 5-amino-4-imidazolecarboxamide riboside (AICAR), an AMPK agonist [66], activated carotid body type I cells and increased afferent discharge [67], and that this action was inhibited by the AMPK antagonist compound C. However, compound C is a very non-selective kinase inhibitor, which in a screen of 70 protein kinases was shown to inhibit at least ten other kinases more potently than AMPK [68]. Moreover, off-target effects of other pharmacological tools have also been identified, such as inhibition by AICAR of adenosine transporters [69] (adenosine receptors being key modulators of type I cell activity [70]) and/or AICAR-mediated reductions in the adenylate pool and ATP [71,72]. One must therefore conclude that AMPK is not necessary for type I cell activation by hypoxia. Consistent with this view, recent studies on the actions of two different AMPK activators, AICAR and A769662 [73], suggest that these agents neither precisely mimic the effects of hypoxia nor induce pronounced activation of carotid body type I cells [74,75], and our own most recent investigations now support this view (unpublished work).

Nevertheless it would appear that we have inadvertently uncovered a split in the dependency on LKB1 and AMPK respectively of carotid body activation during hypoxia on the one hand and the hypoxic ventilatory response on the other. The reasons for this remain to be resolved, but experimental outcomes perhaps point to hierarchical control of the respiratory network by LKB1, AMPK and one or more of the 12 AMPK-related kinases [76]. Given that afferent discharge is, in great part, triggered by exocytotic release of ATP from type I cells [77], it is quite plausible that LKB1 may maintain, in an AMPK-independent manner, the capacity for ATP synthesis and/or exocytosis within type I cells, and thus afferent discharge from the carotid body. This is entirely in keeping with the fact that LKB1 may govern glucose homeostasis [78,79] and mitochondrial function [80,81] independently of AMPK, perhaps via constitutive phosphorylation of an AMPK-related kinase [76,82,83], given that LKB1 deletion has been shown to decrease mitochondrial membrane potential and basal ATP levels in other cell types [80,81,84]. It is therefore possible that any cell lacking LKB1, such as carotid body type I cells, may be unable to sustain appropriate cellular energy charge and activity due to defective mitochondrial function, either at rest or during exposure to metabolic stresses such as hypoxia.

So where does this leave us? Well one backward look takes us to ATP, ADP and AMP levels and the inhibition during hypoxia of type I cell K+ channels, which ultimately triggers exocytosis [85–87]. The principal players in this respect are the large conductance voltage- and Ca2+-activated K+ current (BKCa) [88,89] and the voltage-independent TASK-like leak channel [88,89], which ultimately triggers exocytosis. Consistent with this view, recent studies on the actions of two different AMPK activators, AICAR and A769662 [73], suggest that these agents neither precisely mimic the effects of hypoxia nor induce pronounced activation of carotid body type I cells [74,75], and our own most recent investigations now support this view (unpublished work).
In short, type I cell activation during hypoxia is probably precipitated by changes in the adenylate pool and ATP [99], and membrane depolarization due to subsequent inhibition of K⁺ currents carried by TASK1/3 heterodimers [91,100]. However, the primacy of this view has recently been challenged by three alternative hypotheses:

(1) It has been suggested that type I cell activation may be triggered by increases in hydrogen sulfide production consequent to a fall in carbon monoxide synthesis during hypoxia [101], although the findings of others suggest that activation of carotid body type I cells by exogenous hydrogen sulfide results from direct inhibition of mitochondrial oxidative phosphorylation [102]. The effects on type I cells of hydrogen sulfide may not, therefore, be inconsistent with the conclusion drawn above. This perspective has recently received support from single-cell transcriptome analysis of mouse type I cells which identified few to no reads of the enzymes responsible for generating either carbon monoxide or hydrogen sulfide [55], respectively, haem oxygenase-2, or cystathionine-γ-lyase and cystathionine-β-synthase.

(2) As mentioned previously, conditional deletion in tyrosine hydroxylase-positive cells of Ndufs2, a mitochondrial complex I gene which encodes a protein that participates in ubiquinone binding, has also been shown to selectively block carotid body activation during hypoxia (but not hypercapnia or hypoglycaemia) and thus the hypoxic ventilatory response [61]. The authors concluded that this probably results from loss, during hypoxia, of the capacity for signalling via increased generation of mitochondrial ROS. However this study did not address the impact of Ndufs2 deletion on oxidative phosphorylation in type I cells, the capacity for inhibition of type I cell mitochondrial oxidative phosphorylation during hypoxia and consequent modulation of TASK-like potassium currents by alterations in the adenylate pool (see also [103]). Furthermore, and as discussed above, NDUFA4L2 and COX4I2 are constitutively expressed by type I cells and act to limit mitochondrial ROS production during hypoxia [53,54]. That aside, it is important to note that conditional deletion of Ndufs2 in catecholaminergic cells blocked the hypoxic ventilatory response even though the capacity for both basal and activated transmitter release was retained by type I cells (see below for further discussion).

(3) Most recently a novel chemosensory signalling pathway has been proposed to be a prerequisite for type I cell activation during hypoxia, namely lactate-dependent activation of olfactory receptor 78 (Olfr78) [104]. In this study global deletion of Olfr78 was found to block carotid body activation during hypoxia and thus the hypoxic ventilatory response of mice. By virtue of a requirement for lactate production and release consequent to induction of anaerobic glycolysis, the proposed model for lactate-dependent activation of Olfr78 during hypoxia is consistent with the mitochondrial hypothesis, but is inconsistent with a mechanism in which type I cell activation is determined by TASK K⁺ channel inhibition through alterations in the adenylate pool [74]. That is, unless, of course, these two pathways converge. Once again, however, it may be worthy of note that the hypoxic ventilatory response was blocked by global Olfr78 deletion despite the fact that basal afferent discharge from the carotid body was retained (see below for further discussion).

Putting due scrutiny of the aforementioned signalling pathways to one side, it is clear from our own findings that all pathways key to carotid body type I cell activation during hypoxia must be, in some way, dependent on the continued expression of LKB1, but not AMPK, and a sufficiency of mitochondrial function and/or ATP supply.

So how can it be that both LKB1 and AMPK deletion block the hypoxic ventilatory response, when deletion of the latter does not adversely affect carotid body activation during hypoxia [4,29,64]? For such a proposal runs contrary to the generally held view that increased afferent discharge from carotid body to brainstem determines the ventilatory response to a fall in arterial PO₂ [34]. Well there is substantial evidence to support an alternative yet inclusive perspective, namely that the hypoxic ventilatory response is determined by the co-ordinated action of the carotid body and a hypoxia-responsive circuit within the brainstem. We will see that this must now be borne in mind when drawing conclusions from all studies described above that employed either global knockout strategies or conditional gene deletion in catecholaminergic cells.

**A DASH OF COPERNICUS – AMPK AND THE BRAIN-CENTRED CHEMOSENSORY NETWORK**

From here on in our aim is to be a little more provocative if not heretical, at least in the eyes of some respiratory physiologists, by giving emphasis to a matter that has long been quietly considered by a minority of the field. In actual fact, our investigation is merely the latest in a long line to have described experimental observations that run counter to the standard model for the control of ventilation by peripheral chemosensors, and the pre-eminence of the carotid bodies in this respect.

Not surprisingly, in retrospect, the possibility that peripheral chemosensors may not be the sole arbiters of the hypoxic ventilatory response has been suggested by investigations on the evolution of ventilatory control systems, most notably with respect to the demonstration that oxygen-sensing occurs and a component of the hypoxic ventilatory response arises at the level of the caudal brainstem in amphibians, with both the location and influence of the primary peripheral chemosensors changing during the ascent from gill-breathing tadpole to lung-assisted air-breathing adult [105,106]. In fact one could quite reasonably argue that evolutionary pressures have periodically led to the reconfiguration of peripheral chemoreceptor inputs [106] about a common ancestral hypoxia-sensor within the caudal brainstem, that underpins signal integration and thus acts as the ‘gatekeeper’ of respiratory adjustments during hypoxia. That said, the possibility that neural networks within the brainstem of mammals might respond to central hypoxia was first raised over 35 years ago by the work of Dampney and Moon [107], during their investigations on the central ischaemic vasomotor response. Thereafter, during their investigations on Cushing’s reflex [108], Sun and Reiss demonstrated that both cyanide and hypoxia activated neurons within the rostral ventrolateral medulla [109,110], mirroring Heymans and Bouckaert’s earlier work on the carotid body. Moreover extensive evidence has been provided in support of the view that increases in ventilation are initiated by brainstem hypoxia in the presence of only basal normoxic afferent input from the carotid bodies [111,112], and it has been suggested that different aspects of the brainstem respiratory network may exhibit different sensitivities to hypoxia [113].

To date, however, little emphasis has been placed on the role of hypoxia-sensing at the brainstem, perhaps because the hypoxic ventilatory response is so effectively abolished by resection of the carotid sinus nerve in humans [114]. Yet extensive investigations have demonstrated that following carotid body resection, hypoxia-responsive catecholaminergic neurons of the caudal brainstem may underpin partial recovery of the hypoxic ventilatory response [115], at least in rodents, and it is recognized that loss of these neurons underpins ventilatory dysfunctions associated with...
Rett syndrome, including hypoventilation and apnoea, which are exacerbated during hypoxia [116].

Consistent with outcomes of the aforementioned studies, our findings strongly suggest that AMPK governs the activation of previously identified hypoxia-responsive nuclei within the caudal brainstem [110,117], and thus supports the delivery of increased respiratory drive during hypoxia that is required to protect against hypoventilation and apnoea. The most convincing evidence of this was provided by examination of brainstem function in AMPK knockout mice by functional magnetic resonance imaging (fMRI), which identified reduced activation during hypoxia of discrete dorsal and ventral nuclei of the caudal brainstem, despite the fact that carotid body afferent discharge was retained [4]. This was corroborated by analysis of immediate early gene (c-fos) expression.

The caudal location relative to Bregma of the dorsal active region is consistent with areas of the nucleus tractus solitarius (NTS) that are activated by hypoxia and which represent the primary site of receipt of carotid body afferent input [35,117,118]. Here AMPK deletion selectively attenuated c-fos expression during hypoxia by mixed subpopulations of C2 neurons and A2 neurons (SubP; SubM) within the medial subnucleus proximal to the midline and the area postrema (AP) [4], which have been previously shown to be activated during hypoxia [38]. A2 neurons of the AP/NTS provide afferent input to and determine, together with the carotid body, activation by hypoxia of A1/C1 neurons within the ventrolateral medulla [38,119], the position of which [119] aligns well with the ventral active region identified by fMRI analysis [4]; by contrast projections of the NTS mostly avoid key components of the rCPGS [119], namely the Bötzinger and pre-Bötzinger complexes [120]. Analysis of c-fos expression at the level of the ventrolateral medulla suggested that AMPK deletion selectively reduced the activation of A1 neurons during hypoxia, although it should be noted that there is significant overlap between the most caudal C1 and the most rostral A1 neurons [121]. Our findings therefore suggest that the hypoxic ventilatory response, including that provided by afferent inputs from peripheral chemosensors, is attenuated by loss of AMPK function at the level of the caudal brainstem, within a neuronal circuit spanning the C2/A2 neurons of the NTS and A1 neurons of the ventrolateral medulla. This is consistent with optogenetic and pharmacological interventions at the level of the NTS [117,122], and the proposal that NTS neurons lie on the sensory side of the central respiratory network [123,124]. We cannot rule out the possibility that suppression of the hypoxic ventilatory response in AMPK knockouts may be allied to exacerbation of the Cushing reflex [35,108]. However, this reflex is only elicited under anaesthesia and by ischaemic hypoxia (~1% O2), and is maintained or enhanced by hypercapnia [35,108,125]. By contrast, hypoxic ventilatory depression was evident in conscious AMPK knockouts during mild and severe hypoxia, as were deficits in brainstem activity, and was reversed rather than exacerbated by hypercapnia.

Surprisingly, we observed pronounced right–left asymmetry of brainstem activation during hypoxia, which may provide for specialization sufficient to prevent delays in respiratory responses to hypoxic stress by limiting conflicting outputs from each side of the brain [126], as has been proposed previously with respect to cognitive performance [127]. Further investigation will be required to determine how right–left asymmetry may be orchestrated by the complex interplay of neurotransmitters deployed during hypoxia and the role of AMPK in such processes of selection. In this respect it is notable that C2 and A2 neurons are both catecholaminergic and glutamatergic [123,128], and that 6–10% of tyrosine hydroxylase-positive C2, A2 and A1 neurons also express neuronal nitric oxide synthase, which supports the hypoxic ventilatory response by synthesizing NO [129] and/or S-nitrosothiols [130], and in a manner that may be facilitated by AMPK [131].

It could be argued that AMPK deletion in catecholaminergic cells simply leads to the failure of central integration and transduction of peripheral chemoeffector input and consequent failure of the hypoxic ventilatory response, due to the inability of affected neurons to maintain appropriate levels of activity when exposed to metabolic stress [132]. However, following AMPK deletion, carotid body afferent discharge remained exquisitely sensitive to a fall in Po2 and ventilatory responses to hypercapnia remained unaffected even during severe (8%) hypoxia, which clearly demonstrates that AMPK deletion does not compromise the capacity during hypoxia for activation of chemosensory catecholaminergic neurons, exocytosis nor effective delivery of increased respiratory drive. This is consistent with the observation that neuronal integrity during hypoxia may be preserved, in part, by AMPK-independent mechanisms [133] that maintain ATP supply by accelerating glycolysis and in a manner supported by mobilization of astrocyte glycogen stores [134]. If one accepts this position, then AMPK must aid the modulation by hypoxia of discrete nuclei within the caudal brainstem that deliver increased drive to breathe via neural networks that modulate the rCPGs [36], and which may also co-ordinate functional hyperaemia [135].

THE CASE FOR SIGNAL INTEGRATION AT AN OXYGEN-SENSING NUCLEUS WITHIN THE BRAINSTEM

The phrase ‘to say more would be pure speculation’ is often uttered and rightly so, at least when interpreting experimental outcomes. In the present context, however, we are happy to invite ridicule and scorn for the sake of greater debate and experimental inquisition, and to achieve this goal we bring to centre stage the possibility that a cluster of hypoxia-responsive neurons proximal to the NTS form a nucleus that acts as the ‘gatekeeper’ of the hypoxic ventilatory response.

If this nucleus does indeed exist, then why has it not been located by the extensive efforts of so many specialists in the field? Perhaps we are dealing with an interdependent circuit mechanism, with multiple points of signal integration? When it comes down to hand waving, either a single node or multi-nodal system of signal integration appears plausible, i.e. there may be no discrete nucleus to find. In this context and in light of all things above, we need now consider why:

(1) The degree of block by AMPK deletion of the hypoxic ventilatory response is increased in a manner directly related to the severity of hypoxia [4].
(2) The hypoxic ventilatory response can be triggered by central nervous system hypoxia alone, providing there is continued receipt of basal (normoxic) afferent input from the carotid bodies [136].
(3) The hypoxic ventilatory response may be blocked by interference at any point within this circuit, e.g. carotid body resection [114] or AMPK deletion [4].

We propose (Figure 4) that LKB1/AMPK signalling pathways support coincidence detection and thus signal integration at either a single node or multiple nodes within and thus activation of a hypoxia-responsive circuit that encompasses, at the very least, C2/A2 neurons within the NTS and ventrolateral A1 neurons, due to the capacity for AMPK activation by increases in the AM(D)/P/ATP ratio and LKB1 [3] that may be determined by ‘local hypoxic stress’ (decreased ATP supply) and in a manner...
that is coupled to ‘applied metabolic stress’ (increased ATP usage) delivered via afferent inputs from peripheral chemoreceptors to the NTS, and, in turn, to ventrolateral A1 neurons and perhaps also to downstream aspects of the cardiorespiratory network. Afferent input and brainstem hypoxia could thereby determine, each in part, the set-point about which AMPK and thus the brainstem respiratory network are activated during hypoxia. Therefore AMPK-dependent modulation of cellular metabolism [3], ion channels and thus neuronal firing frequency [21], and/or transmitter release [130,131] may facilitate efferent output and thereby deliver increased drive to breathe, in a manner that may be attenuated or augmented by appropriate regulation of AMPK expression.

In essence then, our proposal is that the LKB1/AMPK signalling pathway monitors changes in adenylate charge centrally as an index of local hypoxic stress and integrates with this applied metabolic stresses delivered by afferent chemosensory inputs, which are in turn providing an index of peripheral hypoxic (metabolic) status. If so, then perhaps we can garner more from our considerations on the regulation of afferent output from the peripheral chemoreceptors, namely the carotid and aortic bodies, in terms of their role in monitoring changes in adenylate charge and thus in the provision of an index of peripheral hypoxic stress.

As discussed in detail previously, hypoxia depolarizes type I cells through inhibition of TASK1/3 K⁺ channels, leading to voltage-gated Ca²⁺ entry, exocytosis and ultimately ATP release. Subsequently ATP stimulates postsynaptic P2X₂ receptors on afferent (petrosal) nerve terminals causing excitation, but at the same time activates P2Y₂ receptors on adjacent glial-like type II cells [77,137]. P2Y₂ receptor activation then triggers further ATP release from type II cells into the synaptic cleft, where ATP (from both type II and type I cells) is broken down by extracellular 5'-ectonucleotidase into adenosine, which primarily activates adenosine A₂A receptors on type I cells [77]. Activation of A₂A receptors leads to further inhibition of TASK1/3 channels and enhanced type I cell depolarization [138], and further augments ATP release during hypoxia [77]; a similar system probably operates at the aortic bodies (C. Nurse, personal communication). It seems quite possible, therefore, that LKB1 may govern a set-point for metabolic homeostasis about which both carotid and aortic bodies monitor adenylate charge as an index of hypoxic stress, by integrative inhibition of TASK1/3 channels consequent to deficits in mitochondrial ATP production that are allied to purinergic cross-talk between type I and type II cells. Via their tripartite synapse with afferent petrosal neurons [77], type I and type II cells may therefore act in concert to relay information on changes in the ‘peripheral adenylate pool’ (ATP, ADP, AMP and adenosine) to the brainstem. During the transit of re-oxygenated blood from the heart to the brainstem, the NTS may thereby coordinate the integration of information on adenylate charge, as an index of arterial oxygen saturation, via at least four separate and highly vascularized nodes, namely the aortic and carotid bodies, the AP/NTS and the ventrolateral medulla, in order to appropriately coordinate cardiorespiratory function (Figure 4).

IN ELLIPTICAL ORBIT – AMPK AND THE REGULATION OF BLOOD FLOW AND GASEOUS EXCHANGE

Hypoxia without hypercapnia induces pulmonary vasoconstriction, and thus assists ventilation-perfusion matching by diverting blood from oxygen-deprived to oxygen-rich areas of the lung [139,140]. By contrast, systemic arteries dilate in response to tissue hypoxemia, in order to match local perfusion to local metabolism [141]. Whichever we consider, it is now evident that AMPK may be key to the regulation of vascular reactivity during metabolic stress [23,142] and may thus facilitate gaseous exchange across the body.

AMPK and ventilation-perfusion matching at the lung

Quite unlike the hypoxic ventilatory response, hypoxic pulmonary vasoconstriction is governed locally and is mediated by mechanisms intrinsic to pulmonary arterial smooth muscles and endothelial cells. This is evident from the fact that neither central nor local regulation of the autonomic nervous system contributes to hypoxic pulmonary vasoconstriction [143–145], which remains unaffected following denervation in humans [146]. However, here...
too the nature of the principal signalling pathway(s) involved remains open to debate [103], although it is clear that this response relies on the modulation by hypoxia of mitochondrial metabolism [48]; pulmonary arterial smooth muscle cells depleted, by ethidium bromide, of mitochondrial DNA and thus of functional mitochondria do not respond to hypoxia [147], although inhibitors of mitochondrial oxidative phosphorylation either mimic or occlude hypoxic pulmonary vasoconstriction [148,149]. As mentioned previously and consistent with findings on carotid body type I cells [55], COX4I2 is constitutively expressed by pulmonary arterial myocytes [59] and may also act here to limit mitochondrial oxygen consumption and ROS production during hypoxia and confer, in part, the capacity of these cells to monitor oxygen supply [55].

In the light of the evidence in support of a role for mitochondria in hypoxia-response coupling, it was therefore proposed that the LKB1/AMPK signalling pathway might couple inhibition by hypoxia of mitochondrial metabolism to hypoxic pulmonary vasoconstriction [1,23,150]. Consistent with this view, AMPK-α1 activity was found to be greater in pulmonary than systemic (mesenteric) arterial smooth muscles [23] and this may in its own right afford a degree of pulmonary selectivity in terms of the capacity and nature of the response to physiological levels of hypoxia, over and above that which might be conferred by COX4I2 expression. Indeed exposure of pulmonary arterial smooth muscle to hypoxia (15–20 mmHg oxygen) precipitated increases in the AMP/ATP ratio, marked activation of AMPK and phosphorylation of acetyl-CoA carboxylase [23]; which may go some way to explain why cellular ATP levels remain remarkably stable in the presence of hypoxia [148]. Inhibition of mitochondrial oxidative phosphorylation by phenformin [151] and AICAR [66] precipitated AMPK activation and acetyl-CoA carboxylase phosphorylation within pulmonary arterial myocytes [23]. Regardless of their respective mechanism of action, hypoxia, phenformin and AICAR also induced an increase in the intracellular calcium concentration and contraction of acutely isolated pulmonary arterial myocytes, and did so by mobilizing sarcoplasmic reticulum stores via ryanodine receptors. Most significantly AICAR evoked a sustained and reversible constriction of pulmonary artery rings, which exhibited characteristics strikingly similar to hypoxic pulmonary vasoconstriction; not least clearly defined contributions from both smooth muscles and the endothelium. Furthermore, hypoxic pulmonary vasoconstriction was inhibited by compound C [152].

In this instance it would appear that the pharmacology held true, for our most recent studies on knockout mice suggest that LKB1 and AMPK, but not CaMKK-β, are indeed required for hypoxic pulmonary vasoconstriction [153] and that dysfunction within the AMPK signalling pathway may precipitate pulmonary hypertension. Further support for this view has recently been provided by our demonstration that upon inhibition of mitochondrial oxidative phosphorylation, AMPK directly phosphorylates K, 1.5 channels, and inhibits K+ currents carried by Kv1.5 in pulmonary arterial myocytes [24]. This is evident from the fact that down-regulation of K, 1.5 expression and activity is a hallmark not only of hypoxic pulmonary vasoconstriction but also of pulmonary hypertension [154–162], and may contribute to increased survival of smooth muscle cells due to attenuation of K+ channel-dependent apoptosis [163–165] and also facilitate the phenotypic switch from a contractile to a proliferative state [166,167].

Consistent with the above, Zhou and co-workers have suggested that AMPK activation promotes survival of pulmonary arterial myocytes during hypoxia and thus cell proliferation by a dual mechanism, incorporating activation of autophagy by AMPK-α1 and reductions in cell death conferred by AMPK-α2 acting to reduce apoptosis via different pathways [168]. Contrary to this latter proposal, however, up-regulation of mTORC2 signalling has been proposed to underpin smooth muscle proliferation and the progression of both idiopathic and hypoxic pulmonary arterial hypertension [169], by promoting smooth muscle cell survival in a manner, at least in part, dependent on down-regulation of AMPK and consequent activation of mammalian target of rapamycin complex 1 (mTORC1). One possible explanation for these contrary prepositions could be that AMPK action is context-dependent and/or that the progression of pulmonary hypertension at different stages is governed by temporal fluctuations in AMPK activity.

Regulation of utero-placental blood flow during hypoxia

AMPK has most recently been implicated in the regulation of uterine artery reactivity during hypoxia [170]. AMPK may, therefore, link maternal metabolic and cardiovascular responses during pregnancy and govern oxygen and nutrient supply to the foetus, thus determining foetal growth. Consistent with this view, PRKAA1 variants most common to Andeans are positively associated with birth weight, uterine artery diameter and to alterations in the expression of genes in the mammalian target of rapamycin (mTOR) pathway that have been previously implicated in altitude-associated foetal growth restriction [2].

CONCLUSION

In summary a growing body of evidence now supports the proposal that AMPK is key to oxygen and thus energy (ATP) supply to the body as a whole, through its contribution to the governance of the hypoxic ventilatory response, ventilation–perfusion matching at the lung and local regulation of blood and thus oxygen supply to the body systems. Aberrant AMPK expression or activity may therefore compromise system responses to hypoxia or other metabolic stressors and precipitate, for example, pulmonary hypertension [171], sleep-disordered breathing [172], hypertension [173] or foetal growth restriction [170], which are associated with either ascent to altitude [2,30] and/or metabolic syndrome-related disorders [172,174,175]. Therefore, further investigations on the role of AMPK in the regulation of ventilatory and vascular function in health and disease are warranted, in order that we may identify new therapeutic strategies allied to our growing understanding of the potential for development of subunit-selective small-molecule regulators of AMPK [73,176–178].

FUNDING

This work was supported by the Wellcome Trust [grant number WT081195MA], and the British Heart Foundation [grant number RG/12/14/29885].

REFERENCES

1 Evans, A.M. (2008) AMP-activated protein kinase and the regulation of Ca2+ signalling in O2-sensing cells. J. Physiol. 574, 113–123 CrossRef PubMed

2 Bigham, A.W., Julian, C.G., Wilson, M.J., Vargas, E., Brown, V.A., Shriver, M.D. and Moore, L.G. (2014) Maternal PRKAA1 and EDNRA genotypes are associated with birth weight, and PRKAA1 with uterine artery diameter and metabolic homeostasis at high altitude. Physiol. Genomics 46, 687–697 CrossRef PubMed
AMPK and oxygen supply

3 Hardie, D.G. (2014) AMPK-sensing energy while talking to other signaling pathways. Cell Metab. 20, 939–952 CrossRef PubMed

4 Mahmoud, A.D., Lewis, S., Juricic, L., Udoh, U.A., Hartmann, S., Jansen, M.A., Oguntayo, O.A., Puggioni, P., Holmes, A.P., Kumar, P. et al. (2016) AMP-activated protein kinase deficiency blocks the hypoxic ventilatory response and thus precipitates hypoventilation and apnea. Am. J. Respir. Crit. Care Med. 193, 1032–1043 CrossRef PubMed

5 Hardie, D.G. (2014) AMPK-sensing energy while talking to other signaling pathways. Cell Metab. 20, 939–952 CrossRef PubMed

6 Hardie, D.G. (2015) AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. Curr. Opin. Cell Biol. 33, 1–7 CrossRef PubMed

7 Reference deleted

8 Ross, F.A., MacKintosh, C. and Hardie, D.G. (2016) AMP-activated protein kinase: a cellular energy sensor that comes in twelve favours. FEBS J. doi: 10.1111/febs.13698

9 Gowans, G.J., Hawley, S.A., Ross, F.A., Hardie, D.G. (2013) AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. Cell Metab. 18, 556–566 CrossRef PubMed

10 Ross, F.A., Jensen, T.E. and Hardie, D.G. (2016) Differential regulation by AMP and ADP of AMPK complexes containing different gamma subunit isoforms. Biochem. J. 473, 189–199

11 Sakamoto, K., Gohansson, G., Hardie, D.G. and Alessi, D.R. (2004) Activity of LKB1 and AMPK-related kinases in skeletal muscle: effects of contraction, photophorin, and AICAR. Am. J. Physiol. Endocrinol. Metab. 287, E310–E317 CrossRef PubMed

12 Hardie, D.G. (2007) AMP-activatedSNF1 protein kinase: conserved guardians of cellular energy. Nat. Rev. Mol. Cell Biol. 8, 774–785 CrossRef PubMed

13 Emerling, B.M., Weinberg, F., Snyder, C., Burgess, Z., Mulli, G.M., Viollet, B., Budinger, G.R. and Chandel, N.S. (2009) Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radic. Biol. Med. 46, 1386–1391 CrossRef PubMed

14 AeCiello, F.R., Ross, F.A., Iwakuma, N. and Hardie, D.G. (2014) Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. FEBS Lett. 588, 3361–3366 CrossRef PubMed

15 Hawley, S.A., Ross, F.A., Chevrefoll, C., Green, K.A., Evans, A., Fogarty, S., Towler, M.C., Brown, L.J., Oguntayo, O.A., Evans, A.M. and Hardie, D.G. (2010) Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab. 11, 554–565 PubMed

16 Colenza, J.L. and Carlson, M. (1986) A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science 233, 1175–1180 CrossRef PubMed

17 Colenza, J.L., Eng, F.J. and Carlson, M. (1989) Molecular analysis of the SNF4 gene of Saccharomyces cerevisiae: evidence for physical association of the SNF4 protein with the SNF1 protein kinase. Mol. Cell. Biol. 9, 5045–5054 CrossRef PubMed

18 Woods, A., Munday, M.R., Scott, J., Yang, X., Carlson, M. and Carling, D. (1994) Yeast SNF1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase in vivo. J. Biol. Chem. 269, 19509–19515 PubMed

19 Mitchellhill, K.I., Stapleton, D., Gao, G., House, D., Michal, B., Katsis, F., Willers, L.A. and Kemp, B.E. (1994) Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. J. Biol. Chem. 269, 2361–2364 PubMed

20 Haurie, V., Boucherie, H. and Sagliocco, F. (2003) The Snf1 protein kinase controls the hypoxic ventilatory response in vivo. J. Physiol. 550, 3–11 CrossRef PubMed

21 Andersen, M.N., Skitbbye, L., Tang, C., Petersen, F., MacAulay, N., Rasmussen, H.B. and Jespersen, T. (2015) PKC and AMPK regulation of Kv1.5 potassium channels. Channels 9, 121–128 CrossRef PubMed

22 Mita, S., Munoz, C., Palakalak, T., Strakay, G., Voelkl, I., Alesatan, I. and Lang, F. (2012) Downregulation of Kv1.5 K channels by the AMP-activated protein kinase. Cell. Physiol. Biochem. 30, 1039–1050 CrossRef PubMed

23 Chang, T.J., Chen, W.P., Yang, C., Li, P.H., Liang, Y.C., Su, M.J., Lee, S.C. and Chuang, L.M. (2009) Sarine-385 phosphorylation of inwardly rectifying K+ channel subunit (Kir2.6) by AMP-dependent protein kinase plays a key role in rosiglitazone-induced closure of the KATP channel and insulin secretion in rats. Diabetologia 52, 1112–1121 CrossRef PubMed

24 Mahmoud, A.D. and Evans, A.M. (2012) LKB1 expression in carotid body type I cells is required for the ventilatory response to mice to hypoxia but not hypercapnia. Proc. Natl. Acad. Sci. U.S.A. 109, 15211–15216 CrossRef PubMed

25 Sper, K.M. (2009) To breathe or not to breathe? That is the question. Exp. Physiol. 94, 1–10 CrossRef PubMed

26 Smith, J.C., Abdala, A.P., Borgmann, A., Rybak, I.A. and Paton, J.F. (2013) Brainstem respiratory networks: building blocks and microcircuits. Trends Neurosci. 36, 152–162 CrossRef PubMed

27 Day, T.A. and Wilson, R.J. (2007) Brainstem PC02 modulates phrenic responses to specific carotid body hypoxia in an in situ dual perfused rat preparation. J. Physiology 578, 843–857 CrossRef PubMed

28 Nurse, C.A. (2014) Synchronous and paracrine mechanisms at carotid body arterial chemoreceptors. J. Physiology 592, 3419–3426 CrossRef PubMed

29 Guyenet, P.G. (2000) Neural structures that mediate sympathetic vasoconstriction during hypoxia. Respir. Physiol. 121, 147–162 CrossRef PubMed

30 Guyenet, P.G. (2014) Regulation of breathing and autonomic outflows by chemoreceptors. Compr. Physiol. 4, 1511–1562 CrossRef PubMed

31 Piskuric, N.A. and Nurse, C.A. (2012) Effects of chemostimuli on [Ca2+]i responses of rat aortic body type I cells and endogenous local neurons: comparison with carotid body cells. J. Physiology 590, 2121–2135 CrossRef PubMed

32 Hirooka, Y., Polson, J.W., Potts, P.D. and Dampney, R.A. (1997) Hypoxia-induced Fos expression in neurons projecting to the pressor region in the rostral ventrolateral medulla. Neuroscience 88, 1209–1224 CrossRef PubMed

33 De Castro, F. (1928) Sur la structure et l'innervation du sinus carotidien de l'homme et des mammiferes: nouveau faits sur l'innervation et la fonction du glomus caroticum. Trab. Lab. Invest. Biol. Univ. Madrid 24, 330–380

34 Heymans, C. and Bouckaert, J.J. (1930) Sinus caroticus and respiratory reflexes: I. Cerebral blood flow and respiration. Adrenaline aponoe. J. Physiology 69, 254–266 CrossRef PubMed

35 Verna, R., Roumy, M. and Leitner, L.M. (1975) Loss of chemoreceptive properties of the rabbit carotid body after destruction of the glomus cells. Brain Res. 100, 13–23 CrossRef PubMed

36 Gonzalez, C., Almaraz, L., Obeso, A. and Rigual, R. (1994) Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol. Rev. 74, 829–898 CrossRef PubMed

37 Nurse, C.A. (2010) Neurotransmitter and neuromodulatory mechanisms at peripheral arterial chemoreceptors. Exp. Physiol. 95, 557–667 CrossRef PubMed

38 Iturriaga, R. and Alcayaga, J. (2004) Neurotransmission in the carotid body: transmitters and modulators between glomus cells and petrosal ganglion nerve terminals. Brain Res. Brain Res. Rev. 47, 46–53 CrossRef PubMed

39 Zhang, M., Zhong, H., Vollmer, C. and Nurse, C.A. (2000) Co-release of ATP and ACh mediates hypoxic signaling at rat carotid body chemoreceptors. J. Physiology 525, 143–158 CrossRef PubMed

40 Millis, E. and Jobstis, F.F. (1970) Simultaneous measurement of cytochrome a3 reduction and chemoreceptor afferent activity in the carotid body. Nature 225, 1147–1149 CrossRef PubMed

41 Dipp, M., Thomas, J.M., Gallione, A. and Evans, A.M. (2003) A P02 window for smooth muscle cADPR accumulation and constriction by hypoxia in rabbit pulmonary artery smooth muscle. Proc. Physiol. Soc. 547P, C72

42 Mills, E. and Jobstis, F.F. (1972) Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension. J. Neurophysiol. 35, 405–428 PubMed
52 Tello, D., Balsa, E., Acosta-Iborra, B., Fuertes-Yebra, E., Elorza, A., Ordóñez, A., Corral-Escariz, M., Soro, I., Lopez-Bernardo, E., Perales-Clemente, E. et al. (2011) Induction of the mitochondrial NDUF4L2 protein by HIF-1α enhances oxygen consumption by inhibiting Complex I activity. Cell Metab. 14, 768–779

CrossRef PubMed

53 Fukuda, R., Zhang, H., Kim, J.W., Shimoda, L., Dang, C.V. and Semenza, G.L. (2007) HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 129, 111–122 CrossRef PubMed

54 Huttemann, M., Kadenbach, B. and Grossman, L.I. (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. Gene 267, 111–122 CrossRef PubMed

55 Zhou, T., Chien, M.S., Kaleem, S. and Matsuumi, H. (2016) Single cell transcriptome analysis of mouse carotid body glomus cells. J. Physiol. doi: 10.1113/JP217936

56 Horvat, S., Beyer, C. and Arnold, S. (2006) Effect of hypoxia on the transcription pattern of subunit isoforms and the kinetics of cytochrome c oxidase in cortical astrocytes and cerebellar neurons. J. Neurochem. 99, 937–951 CrossRef PubMed

57 Kocha, K.M., Reilly, K., Purpura, D.S., McDonald, J. and Morey, C.D. (2015) Evolution of the oxygen sensitivity of cytochrome c oxidase subunit 4. Am. J. Physiol. Regul. integrat. Comp. Physiol. 398, R305–R320 CrossRef PubMed

58 Aras, S., Pak, O., Sommer, N. Finley, Jr, R., Huttemann, M., Weismann, N. and Grossman, L.I. (2013) Oxygen-dependent expression of cytochrome c oxidase subunit 4–2 gene expression is mediated by transcription factors RBP4, CXXC5 and CHCHD2. Nucleic Acids Res. 41, 2255–2266 CrossRef PubMed

59 Huttemann, M., Lee, I., Gao, X., Pecina, P., Pecinova, A., Liu, J., Aras, S., Sommer, N., Sanderson, T.H., Tost, M. et al. (2012) Cytochrome c oxidase subunit 4 isomer 2-knockout mice show reduced enzyme activity, altered hypoxic hyporesponsiveness, and lung pathology. FASEB J. 26, 9196–9206 CrossRef PubMed

60 Wyatt, C.N. and Buckler, K.J. (2004) The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. J. Physiol. 556, 175–191 CrossRef PubMed

61 Fernandez-Aguera, M.C., Gao, L., Gonzalez-Rodriguez, P., Pintado, C.O., Arias-Mayenco, I., Garcia-Flores, P., Garcia-Perganeda, A., Pascual, A., Ortega-Saenz, P. and Lopez-Barneo, J. (2015) Oxygen sensing by arterial chemoreceptors depends on mitochondrial complex I signaling. Cell Metab. 22, 825–837 CrossRef PubMed

62 Deja, P.P. and Terzic, A. (2003) Phosphotransfer networks and cellular energetics. J. Exp. Biol. 206, 2039–2047 CrossRef PubMed

63 Panayotou, C., Solaroli, N. and Karlsson, A. (2014) The many isomers of human adenylate kinases. Int. J. Biochem. Cell Biol. 49, 75–83 CrossRef PubMed

64 Evans, A.M. (2012) The LKB1-AMPK signalling pathway is required for regulation of breathing by hypoxia and thereby energy supply to the whole body. Proc. Physiol. Soc. 27, S5A5

65 Mahmoud, A.D., Lewis, S., Junicić, L., Forêt, M., Viiolet, B., Marshall, I. and Evans, A.M. (2015) AMPK couples oxygen to energy supply at the whole-body level by delivering increased drive to breathe during hypoxia and thus protects against apnoea. Proc. Physiol. Sci. 34, PC041 CrossRef PubMed

66 Corton, J.M., Gillespie, J.G., Hawley, S.A. and Hardie, D.G. (2007) AMP-activated protein kinase mediates carotid body chemosensor responses to hypoxia. J. Physiol. 587, R305–R320 CrossRef PubMed

67 Wyatt, C.N., Mustard, K.J., Pearson, S.A., Dallas, M.L., Atkinson, L., Kumar, P., Peers, C., Hardie, D.G. and Evans, A.M. (2007) AMP-activated protein kinase mediates carotid body cell death by hypoxia. J. Biol. Chem. 282, 8092–8098 CrossRef PubMed

68 Bain, J., Plater, L., Elliott, M., Shpiro, N., Hastie, C.J., McLauchlan, H., Klevernic, I., Corton, J.M., Gillespie, J.G., Hawley, S.A. and Hardie, D.G. (2005) LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310, 1642–1646 CrossRef PubMed

69 Shaw, R.J., Lamia, K.A., Vaquero, D., Roo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M. and Cantley, L.C. (2005) The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310, 1642–1646 CrossRef PubMed

70 Galan, B., Hu, J., Jiang, S., Sahin, E., Zeng, L., Flier, J., Shpiro, N. and Bardeesy, N. (2010) LKB1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. Nature 468, 701–704 CrossRef PubMed

71 Gurumurthy, S., Xie, S.Z., Aijagesan, B., Kim, J., Yusuf, R.Z., Saez, B., Tzabos, A., Ozsolak, F., Milos, P., Ferrante, F. et al. (2010) The Lkb1 metabolic sensor maintains hematopoietic stem cell survival. Nature 468, 655–663 CrossRef PubMed

72 Patel, K., Forêt, M., Marion, A., Campbell, D.G., Gourley, R., Boudou, N., Tourier, M., Tilchenell, P., Pogge, M., Deak, M. et al. (2014) The LKB1-salt-inducible kinase pathway functions as a key gluconeogenic suppressor in the liver. Nat. Commun. 5, 4535 CrossRef PubMed

73 Choi, S., Lim, D.S. and Chung, J. (2015) Feeding and tasting signals converge on the LKB1-SIK pathway to regulate lipid metabolism in drosophila. PLoS Genet. 11, e1005263 CrossRef PubMed

74 Swisa, A., Granot, Z., Tamarina, N., Sayers, S., Bardeesy, N., Philipson, L., Hudson, D.J., Wikstrom, J.D., Rutter, G.A., Leibowitz, G. et al. (2015) Loss of liver kinase b1 (Lkb1) in beta cells enhances glucose-stimulated insulin secretion despite profound mitochondrial defects. J. Biol. Chem. 290, 20934–20946 CrossRef PubMed

75 Lopez-Barneo, J., Lopez-Lopez, J.R., Urena, J. and Gonzalez, C. (1993) Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. Science 241, 580–582 CrossRef PubMed

76 Stea, A. and Nurse, C.A. (1991) Whole-cell and perforated-patch recordings from O2-sensitive rat carotid body cells grown in short- and long-term culture. Pflugers Arch. 418, 93–101 CrossRef PubMed

77 Delpiano, M.A. and Hescheler, J. (1989) Evidence for a PO2-sensitive K+ channel in the type-I cell of the rabbit carotid body. FEBS Lett. 249, 195–198 CrossRef PubMed

78 Hescheler, J., Delpiano, M.A., Acker, H. and Pletschick, F. (1989) Ionic currents on type-I cells of the rabbit carotid body measured by voltage-clamp experiments and the effect of hypoxia. Brain Res. 486, 79–86 CrossRef PubMed

79 Peers, C. (1990) Hypoxic suppression of K+ currents in type I carotid body cells: selective effect on the Ca2+ dependent activated K+ current. Neurosci. Lett. 119, 253–256 CrossRef PubMed

80 Buckler, K.J. (1997) A novel oxygen-sensitive potassium current in rat carotid body type I cells. J. Physiol. 498, 649–662 CrossRef PubMed

81 Kim, D., Cavanaugh, E.J., Kim, I. and Carroll, J.L. (2009) Heteromic TASK-1/TASK-3 is the major oxygen-sensitive background K+ channel in rat carotid body glomus cells. J. Physiol. 587, 2963–2975 CrossRef PubMed

82 Ortega-Saenz, P., Levklyzky A.L., Marcos-Almara, M.T., Bonilla-Henao, V., Pascual, A. and Lopez-Barnes, J. (2010) Carotid body chemosensory responses in mice deficient of TASK1 and TASK3 channels. J. Gen. Physiol. 135, 379–392 CrossRef PubMed

83 Perez-Garcia, M.T., Collins, O., Miguel-Velado, E., Moreno-Dominguez, A. and Lopez-Lozano, J.R. (2004) Characterization of the Kv channels of mouse carotid body chemoreceptor cells and their role in oxygen sensing. J. Physiol. 557, 457–471 CrossRef PubMed

84 Lopez-Lopez, J.R., De Luis, D.A. and Gonzalez, C. (1993) Properties of a transient K+ current in chemoreceptor cells of rabbit carotid body. J. Physiol. 469, 15–32 CrossRef PubMed

85 Hatton, C.J., Carpenter, E., Pepper, D.R. Kumar, P. and Peers, C. (1997) Developmental changes in isolated rat type I carotid body cell K+ currents and their modulation by hypoxia. J. Physiol. 501, 49–58 CrossRef PubMed

© 2016 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution Licence 4.0 (CC BY).

A.M. Evans and others

2570
Wasicko, M.J., Bretwistle, G.E., Kim, I. and Carroll, J.L. (2006) Postnatal development of carotid body glomus cell response to hypoxia. Respir. Physiol. Neurobiol. 154, 356–371 CrossRef PubMed

Varas, R., Wyatt, C.N. and Buckler, K.J. (2007) Modulation of TASK-like background polassium channels in rat arterial chemoreceptor cells by intracellular ATP and other nucleotides. J. Physiol. 583, 521–536 CrossRef PubMed

Duncan, P.J., Sengü, S., Tabak, J., Ruth, P., Bertram, R. and Shipston, M.J. (2015) Large conductance Ca(2)(+)-activated (K +) (BK) channels promote secretagogue-induced transition from spiking to bursting in murine anterior pituitary corticotrophs. J. Physiol. 593, 1197–1211 CrossRef PubMed

Buckler, K.J. and Turner, P.J. (2013) Oxygen sensitivity of mitochondrial function in rat arterial chemoreceptor cells. J. Physiol. 591, 3549–3563 CrossRef PubMed

Turner, P.J. and Buckler, K.J. (2013) Oxygen and mitochondrial modulates both monomeric and heteromeric TASK-1 and TASK-3 channels in murine carotid body type-1 cells. J. Physiol. 591, 5977–5998 CrossRef PubMed

Yuan, G., Vasavada, C., Peng, Y.J., Makarenko, V.V., Raghuraman, G., Naduru, J., Gadalla, M.M., Semenza, G.L., Kumar, G.K., Snyder, S.H. and Prabhakar, N.R. (2015) Protein kinase G-regulated production of H2S governs oxygen sensing. Sci. Signal. 8, ra37 CrossRef PubMed

Alheid, G.F., Jiao, W. and McCrimmon, D.R. (2011) Caudal nuclei of the rat nucleus of the solitary tract (NTS) project to lateral hypothalamus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R266–R275 CrossRef PubMed

Vardhan, A., Kachroo, A. and Sapru, H.N. (1995) Excitatory amino acid receptors in commissural nucleus of the NTS mediate carotid chemoreceptor responses. Am. J. Physiol. 264, R41–R50 PubMed

Aicher, S.A., Saravay, R.H., Cravo, S., Jeske, I., Morrison, S.F., Reis, D.J. and Milner, T.A. (1996) Monosynaptic projections from the nucleus tractus solitarii to C1 adrenergic neurons in the rostral ventrolateral medulla: comparison with input from the caudal ventrolateral medulla. J. Comp. Neurol. 373, 62–75 CrossRef PubMed

Harris, A.P., Helou, S., Traysman, R.J., Jones, Jr, M.D. and Koehler, R.C. (1998) Efficacy of the coughing response in maintaining cerebral blood flow in premature and near-term fetal sheep. Pediatr. Res. 43, 50–56 CrossRef PubMed

Varlitoglia, G., Rogers, J.A. and Bisazza, A. (1999) Possible evolutional origins of cognitive brain lateralization. Brain Res. Brain Res. Rev. 30, 164–175 CrossRef PubMed

Dadda, M., Zandona, E., Agnoli, C. and Bisazza, A. (2009) The costs of hemispheric specialization in a fish. Proc. Biol. Sci. 276, 4399–4407 CrossRef PubMed

Stornetta, R.L., Sevigny, C.P. and Guyenet, P.G. (2002) Visceral gluatamater transporer DNP/VEGUF2 mRNA is present in C1 and several other groups of brainstem catecholaminergic neurons. J. Comp. Neurol. 444, 191–200 CrossRef PubMed

Gozal, D., Gozal, E., Torres, J.E., Gozal, Y.M., Nuckton, T.J. and Hornby, P.J. (1997) Nitric oxide modulates ventilatory responses to hypoxia in the developing rat. Am. J. Respir. Crit. Care Med. 155, 1755–1762 CrossRef PubMed

Lipton, A.J., Johnson, M.A., Macnald, T., Lieberman, M.G., Gozal, D. and Gaston, B. (2001) S-nitrosothiols signal the ventilatory response to hypoxia. Nature 413, 171–174 CrossRef PubMed

Murphy, B.A., Fakra, K.A., Song, Z., Beuve, A. and Routh, V.H. (2000) AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. Am. J. Physiol. Cell Physiol. 279, C750–C758 CrossRef PubMed

Culmsee, C., Moning, J., Kemp, B.E. and Mattson, M.P. (2001) AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. J. Mol. Neurosci. 17, 45–58 CrossRef PubMed

Cheng, F., Xie, S., Guo, M., Fang, H., Li, X., Xin, J., Lu, G., Li, Y., Ji, X. and Yu, S. (2011) Altered glucose metabolism and preserved energy charge and neuronal structures in the brain of mouse intemittently exposed to hypoxia. J. Chem. Neuroanat. 42, 65–71 CrossRef PubMed

Almeida, A., Moncada, S. and Bolanos, J.P. (2004) Nitric oxide switches on glycysis through the AMP protein kinase and 6-phosphohuctro-2-g-yse pathway. Nat. Cell. Biol. 6, 45–51 CrossRef PubMed

Buchet, E.S., Fox, M.E., Kim, L., Kirkpatrick, D.C., Rodeborg, N.T., Belle, A.M. and Wightman, R.M. (2014) Medullary noradrenergic neurons mediate local oxygen concentrations in the bed nucleus of the stria terminals. J. Cereb. Blood Flow Metab. 34, 1128–1137 CrossRef PubMed

Smith, C.A., Johnson, M.A., Macnald, T., Lieberman, M.G., Gozal, D. and Gaston, B. (2001) S-nitrosothiols signal the ventilatory response to hypoxia. Nature 413, 171–174 CrossRef PubMed

O'Rourke, A.M., McMonagle, S. and Bolanos, J.P. (2009) AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. Am. J. Physiol. Cell Physiol. 297, C750–C758 CrossRef PubMed

Bujo, D., Michel, J.C., Torre, J.E., Gozal, Y.M., Nuckton, T.J. and Hornby, P.J. (1997) Nitric oxide modulates ventilatory responses to hypoxia in the developing rat. Am. J. Respir. Crit. Care Med. 155, 1755–1762 CrossRef PubMed

Wightman, R.M. (2014) Medullary noradrenergic neurons mediate local oxygen concentrations in the bed nucleus of the stria terminals. J. Cereb. Blood Flow Metab. 34, 1128–1137 CrossRef PubMed

Conde, S.V., Monteiro, E.C., Riquel, R., Obseo, A. and Gonzalez, C. (2012) Hypoxic sensitivity: a determinant for the contribution of ATP and adenosine to the genesis of carotid body chemosensory activity. J. Appl. Physiol. 112, 2002–2010 CrossRef PubMed

Xu, F., Xu, J., Tse, F.W. and Tse, A. (2006) Adenosine stimulates depolarization and rise in intracellular [Ca(2)(+)] in type I cells of cat reta body cells. Am. J. Physiol. Cell Physiol. 290, C1592–C1598 CrossRef PubMed

von Euler, U.S. and Liljestrand, G. (1946) Observations on the pulmonary arterial blood pressure in the cat. Acta Physiol. Scand. 12, 301–320 CrossRef PubMed

Bradford, J.R. and Dean, H.P. (1984) The pulmonary circulation. J. Physiol. 348, 434–458 CrossRef PubMed

Roy, C.S. and Sherrington, C.S. (1890) On the regulation of the blood-supply of the brain. J. Physiol. 11, 117–158 CrossRef PubMed

Goirand, F., Solar, M., Atteia, Y., Voltz, B., Mateo, P., Forlin, D., Lecster, J., Hoeter, J., Ventura-Clapier, R. and Garnier, A. (2007) Activation of AMP kinase alpha1 subunit increases aortic vasorelaxation in mice. J. Physiol. 581, 1163–1171 CrossRef PubMed

Nicol, D. (1970) The influence of blood gases on the pulmonary vessels of the cat. Acta Physiol. Scand. 23, 85–90 CrossRef PubMed

Naeije, R., Lejeune, P., Leeman, M., Melot, C. and Closset, I. (1989) Pulmonary vascular responses to surgical chemodenervation and chemical sympathetomy in dogs. J. Appl. Physiol. 66, 42–50 PubMed
Lejeune, P., Vachery, J.L., Leeman, M., Brimouille, S., Hallemans, R., Melot, C. and Naeije, R. (1989) Absence of parasympathetic control of pulmonary vascular pressure-flow plots in hyperoxic and hypoxic dogs. Respir. Physiol. 78, 123–133 CrossRef PubMed

Robin, E.D., Theodore, J., Burke, C.M., Oesterle, S.N., Fowler, M.B., Jamieson, S.W., Baldwin, J.C., Morris, A.J., Hunt, S.A., Vankessel, A. et al. (1987) Hyperoxic pulmonary vasoconstriction persists in the human transplanted lung. Clin. Sci. (Lond.) 72, 283–287 CrossRef PubMed

Waya, G.B., Chandel, N.S. and Schumacker, P.T. (2001) Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ. Res. 88, 1259–1266 CrossRef PubMed

Leach, R.M., Hill, H.M., Snedket, V.A., Robertson, T.P. and Ward, J.P. (2001) Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction: role of identity of the hypoxic sensor. J. Physiol. 538, 211–224 CrossRef PubMed

Weissmann, N., Ebert, N., Ahrens, M., Gohfarini, H.A., Scherrmuth, R.T., Hanze, J., Fink, L., Rose, F., Conzen, J., Seeger, W. and Grimminger, F. (2003) Effects of mitochondrial inhibitors and uncouplers on hypoxic vasoconstriction in rabbit lungs. Am. J. Respir. Cell Mol. Biol. 29, 721–732 CrossRef PubMed

Evans, A.M., Hardie, D.G., Galicic, A., Peers, C., Kumar, P. and Wyatt, C.N. (2006) AMP-activated protein kinase couples mitochondrial inhibition by hypoxia to cell-specific Ca2+ signaling mechanisms in oxygen-sensing cells. Novartis Found. Symp. 272, 234–252 CrossRef PubMed

Owen, M.R., Doran, E. and Halestrap, A.P. (2000) Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem. J. 348, 607–614 CrossRef PubMed

Robertson, T.P., Mustard, K.J., Lewis, T.H., Clark, J.H., Wyatt, C.N., Blanco, E.A., Peers, C., Hardie, D.G. and Evans, A.M. (2008) AMP-activated protein kinase and hypoxic pulmonary vasoconstriction. Eur. J. Pharmacol. 595, 39–43 CrossRef PubMed

Moral-Sanz, J., Lewis, S., Thomson, A., Moran, C., Viollet, B., Forest, M. and Evans, A.M. (2015) AMP-activated protein kinase is necessary for hypoxic pulmonary vasoconstriction. Proc. Physiol. Soc. 34, PC265

Yuan, J.X., Aldinger, A.M., Juhaszova, M., Wang, J., Conte, J.V.J., Gare, S.P., Oren, J.B. and Rubin, L.J. (1998) Dysfunctional voltage-gated K+ channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. Circulation 98, 1400–1406 CrossRef PubMed

Morales-Cano, D., Menendez, C., Moreno, E., Moral-Sanz, J., Lewis, S., Sahasrabudhe, P., Magyar, R., Rajamohan, F., Reyes, A., Frisbie, R.K. and Hoth, L.R. (2005) Sleep apnea is a manifestation of the metabolic syndrome. Sleep Med. Rev. 9, 1323–1330 CrossRef PubMed

Vgontzas, A.N., Bixler, E.O. and Chrousos, G.P. (2005) Adenosine monophosphate-activated protein kinase is required for pulmonary artery smooth muscle cell survival and the development of hypoxic pulmonary hypertension. Am. J. Respir. Cell Mol. Biol. 40, 615–632 CrossRef PubMed

Bremova, E.E., Platoshyn, O., Zhang, S. and Yuan, J.X. (2004) Overexpression of human KCNAs increases IKV and enhances apoptosis. Am. J. Physiol. Cell Physiol. 287, C715–C722 CrossRef PubMed

Knick, S., Platoshyn, O., Sweeney, M., Kim, H. and Yuan, J.X. (2001) Activation of K+ channels induces apoptosis in vascular smooth muscle cells. Am. J. Physiol. Cell Physiol. 280, C970–C979 PubMed

Moudgil, R., Michelakis, E.D. and Archer, S.L. (2006) The role of K+ channels in determining pulmonary vascular tone, oxygen sensing, cell proliferation, and apoptosis: implications in hypoxic pulmonary vasoconstriction and pulmonary arterial hypertension. Microcirculation 13, 615–632 CrossRef PubMed

Cidat, P., Jimenez-Perez, I., Garcia-Arribas, D., Miguel-Velado, E., Tajada, S., Ruiz-McCavitt, C., Lopez-Lopez, J.R. and Perez-Garcia, M.T. (2012) Kv1.3 channels can modulate cell proliferation during phenotypic switch by an ion-flux independent mechanism. Arterioscler. Thromb. Vasc. Biol. 32, 1299–1307 CrossRef PubMed

Cidat, P., Miguel-Velado, E., Ruiz-McCavitt, C., Alonso, E., Jimenez-Perez, I., Asaja, A., Carmona, Y., Garcia-Arribas, D., Lopez, J., Marroquin, Y. et al. (2014) Kv1.3 channels modulate human vascular smooth muscle cells proliferation independently of mTOR signaling pathway. Pflugers Arch. 467, 1711–1722 Pubmed

Ibe, J.C., Zhou, Q., Chen, T., Tang, H., Yuan, J.X., Rui, J.U. and Zhou, G. (2013) Adenosine monophosphate-activated protein kinase is required for pulmonary artery smooth muscle cell survival and the development of hypoxic pulmonary hypertension. Am. J. Respir. Cell Mol. Biol. 49, 609–618 CrossRef PubMed

Goncharov, D.A., Kudyashova, T.V., Zai, H., Ihida-Stansbury, K., DeLisser, H., Krymskaya, V.P., Tuder, R.M., Kawat, S.M. and Goncharova, E.A. (2014) Mammalian target of rapamycin complex 2 (mTORC2) coordinates pulmonary artery smooth muscle cell metabolism, proliferation, and survival in pulmonary arterial hypertension. Circulation 129, 864–874 CrossRef PubMed

Sheffington, K.L., Higgins, J.S., Mahmood, A.D., Evans, A.M., Sterzuzi-Perti, A.N., Fowder, A., Yung, H.W., Burton, G.J., Giussani, D.A. and Moore, L.G. (2016) Hypoxia, AMPK activation and uterine artery vasoactivity. J. Physiol. 594, 1357–1369 CrossRef PubMed

Latham, T., Tudor, R.M. and Petracek, I. (2014) Progress in solving the sex hormone paradox in pulmonary hypertension. Am. J. Physiol. Lung Cell Mol. Physiol. 307, L7–L26 CrossRef PubMed

Nahar, E.H., Lam, D., Wong, M., Mokhlesi, B. and Chung, F. (2012) Obesity hypoventilation syndrome: a review of epidemiology, pathophysiology, and perioperative considerations. Anesthiology 117, 188–205 CrossRef PubMed

Schneider, H., Schubert, K.M., Blodow, S., Kreutz, C.P., Erdogmus, S., Wiedenmann, M., Qiu, J., Fey, T., Ruth, P., Lubomirov, L.T., Pitzer, G., Mederos, Y.S.M., Hardie, D.G., Gudermuth, T. and Pohl, U. (2015) AMPK dilates resistance arteries via activation of SERCA and BKCa channels in smooth muscle. Hypertension 66, 108–116 CrossRef PubMed

Ruderman, N.B., Carling, D., Pretinki, M. and Cacciado, J. (2013) AMPK, insulin resistance, and the metabolic syndrome. J. Clin. Invest. 123, 2764–2772 CrossRef PubMed

Vignitzas, A.N., Bioler, E.O. and Chrousos, G.P. (2005) Sleep apnea is a manifestation of the metabolic syndrome. Sleep Med. Rev. 9, 211–224 CrossRef PubMed

Rajamohan, F., Reyes, A.R., Frisbie, R.K., Hoth, L.R., Safarabdaband, P., Magyar, R., Landro, J.A., Wilhka, J.M., Caspers, N.L., Calabrese, M.F. et al. (2016) Probing the enzyme kinetics, allosteric modulation and activation of alpha-1 and alpha-2 subunit containing AMP-activated protein kinase (AMPK) heterotrimers by pharmacological and physiological activators. Biochem. J. 473, 581–592 CrossRef PubMed

Gomez-Galeno, J.E., Dang, Q., Nguyen, T.H., Boyer, S.H., Grote, M.P., Sun, Z., Chen, M., Craigio, W.A., van Poelje, P.D., MacKenna, D.A. et al. (2010) A potent and selective AMPK activator that inhibits de novo lipogenesis... ACS Med. Chem. Lett. 1, 478–482 CrossRef PubMed

Scott, J.W., Ling, N., Issa, S.M., Dite, T.A., O’Brien, M.T., Chen, Z.P., Galic, S., Langendorf, C.C., Steinberg, G.R., Kemp, B.E. and Oaktill, J.S. (2014) Small molecule drug A-769662 and AMP synergistically activate naive AMPK independent of upstream kinase signaling. Chem. Biol. 21, 619–627 CrossRef PubMed

Hardie, D.G., Salt, I.P., Hawley, S.A. and Davies, S.P. (1999) AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. Biochem. J. 347, 581–592 CrossRef PubMed

Rekling, J.C. and Feldman, J.L. (1998) PreBotzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. Annu. Rev. Physiol. 60, 385–405 CrossRef PubMed