11 Modulation of the LKB1-AMPK Signalling Pathway Underpins Hypoxic Pulmonary Vasoconstriction and Pulmonary Hypertension

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Abstract

Perhaps the defining characteristic of pulmonary arteries is the process of hypoxic pulmonary vasoconstriction (HPV) which, under physiological conditions, supports ventilation-perfusion matching in the lung by diverting blood flow away from oxygen deprived areas of the lung to oxygen rich regions. However, when alveolar hypoxia is more widespread, either at altitude or with disease (e.g., cystic fibrosis), HPV may lead to hypoxic pulmonary hypertension. HPV is driven by the intrinsic response to hypoxia of pulmonary arterial smooth muscle and endothelial cells, which are acutely sensitive to relatively small changes in pO_2 and have evolved to monitor oxygen supply and thus address ventilation-perfusion mismatch. There is now a consensus that the inhibition by hypoxia of mitochondrial oxidative phosphorylation represents a key step towards the induction of HPV, but the precise nature of the signalling pathway(s) engaged thereafter remains open to debate. We will consider the role of the AMP-activated protein kinase (AMPK) and liver kinase B1 (LKB1), an upstream kinase through which AMPK is intimately coupled to changes in oxygen supply via mitochondrial metabolism. A growing body of evidence, from our laboratory and others, suggests that modulation of the LKB1-AMPK signalling pathway underpins both hypoxic pulmonary vasoconstriction and the development of pulmonary hypertension.

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Keywords

LKB1 • AMPK • Hypoxia • Pulmonary artery • Vasoconstriction • Kv1.5

11.1 Introduction

Although there had been some prior comment, the first definitive description of hypoxic pulmonary vasoconstriction (HPV) was provided by Bradford and Dean in 1894 (Bradford and Dean 1894). They described not only a rise in pulmonary vascular pressure upon asphyxia, but concluded that this was driven by the reaction to asphyxia of the blood vessels themselves, because the measured increase in pressure upon asphyxia persisted after transection of the spinal cord. Fifty years later, von Euler and Liljestrand showed that hypoxia without hypercapnia induced pulmonary vasoconstriction, and hypothesised that HPV may assist ventilation-perfusion matching in the lung (von Euler and Liljestrand 1946); by contrast, systemic arteries dilate in response to tissue hypoxemia, in order to match local perfusion to local metabolism (Roy and Sherrington 1890).

That HPV was largely, or entirely independent of the autonomic nervous system (Nisell 1951) was evident after chemical sympathectomy (using 6-hydroxy-dopamine), surgical denervation of the carotid and aortic chemoreceptors or after bilateral cervical vagotomy (Lejeune et al. 1989; Naeije et al. 1989). Most significantly, bilateral lung transplants established that HPV remains unaffected following denervation in man (Robin et al. 1987). Therefore, neither central nor local regulation of the autonomic nervous system plays a role in mediating HPV.

In 1951 it was demonstrated that HPV was not induced when the lung was perfused with hypoxic blood at a constant, normoxic alveolar oxygen tension (Duke and Killick 1952), and later work confirmed that a fall in airway/alveolar pO_2 triggered a pronounced increase in pulmonary vascular perfusion pressure (Bergofsky et al. 1968). Consistent with this, Kato and Staub demonstrated, using unilobar hypoxia, that the small precapillary resistance arteries contributed most to the increase in pulmonary vascular perfusion pressure during alveolar hypoxia, and that the magnitude of HPV was inversely related to pulmonary artery diameter (Kato and Staub 1966). In isolated arteries HPV is biphasic (see Fig. 11.2a) with a threshold for initiation of ~60 mmHg (Dipp and Evans 2001). Thereafter the magnitude of constriction increases in a manner proportional to the degree of hypoxia, until it fails under near anoxic conditions (Dipp et al. 2003). The two phases of HPV observed in isolated arteries are discrete, comprising a transient constriction (Phase 1; 5–10 min) followed by a slow tonic constriction (Phase 2; peak after 30–40 min), both of which are initiated immediately upon exposure to hypoxia (Dipp et al. 2001; Leach et al. 1994).

It is generally accepted that hypoxia triggers pulmonary artery constriction via signalling pathways intrinsic to the smooth muscle and endothelial cells. Initially, HPV is driven by calcium release from the smooth muscle sarcoplasmic reticulum via ryanodine receptors and in a manner that does not require calcium influx (Dipp et al. 2001), although it is clear that subsequent activation of the store-refilling current aids the maintenance of constriction (Evans et al. 2005; Lu et al. 2008; Wang et al. 2004; Wilson et al. 2002). Thereafter, constriction is augmented via myofilament calcium sensitisation, which is initiated in response to the release of a vasoconstrictor from the endothelium (Evans and Dipp 2002; Robertson et al. 2000, 2001). At the molecular level, there is also clear evidence that hypoxia modulates the activity of voltage-gated potassium channels (K_V) in the plasma membrane of the smooth muscle cells, although the functional consequence of potassium channel regulation remains unclear (Archer et al. 1993; Dipp et al. 2001; Post et al. 1992; Remillard et al. 2007). That aside, the nature of the principle signalling

pathway(s) involved remains open to debate (Evans et al. 2011), but clearly relies on the modulation by hypoxia of mitochondrial metabolism (see for example Sommer et al. 2010).

11.2 Mitochondria and Oxygen Sensing

A requirement for functional mitochondria in the process of oxygen-sensing was first identified by investigations into the function of the carotid bodies, which noted that cyanide (Cooper and Brown 2008) mimicked and occluded activation by hypoxia of the carotid body (Heymans et al. 1930). The first direct evidence of this fact was provided by spectrophotometric analysis of the respiratory chain redox status and fluorometric measurement of the NAD(P)H/NAD(P)$^+$ ratio (Mills and Jobsis 1972). 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 afferent fibre discharge frequency over what is considered to be the physiological range of arterial pO_2. It was therefore proposed that mitochondria of most cells may utilise a high affinity (i.e. normal) cytochrome a_3, while the cytochrome a_3 incorporated in mitochondria of oxygen-sensing cells may have a low affinity for oxygen. This proposal gained support from the work of Duchen and Biscoe (1992a, b) and has gathered further momentum of late, not least due to the detailed investigations of Buckler and co-workers (Turner and Buckler 2013; Wyatt and Buckler 2004). However, the strongest evidence in favour of an absolute requirement for functional mitochondria in oxygen-sensing comes from studies on immortalised neonatal adrenomedullary chromaffin cells that incorporate or lack functional mitochondria (Buttigieg et al. 2006; Thompson et al. 1997, 2007). Those with functional mitochondria were found to respond to hypoxia and to inhibitors of mitochondrial oxidative phosphorylation. By contrast, those cells lacking functional mitochondria failed to respond to either stimulus. Allied to these findings, it has been shown that pulmonary arterial smooth muscle cells depleted, by ethidium bromide, of mitochondrial DNA and thus a functional mitochondrial electron transport chain, do not respond to hypoxia (Waypa et al. 2001). Moreover, comprehensive data indicate that inhibitors of mitochondria (either uncouplers or blockers of specific respiratory chain complexes) mimic hypoxia in their ability to regulate ion channel function in a variety of oxygen-sensing cells, including pulmonary arterial smooth muscle cells (Leach et al. 2001; Weissmann et al. 2003). It should be noted, however, that we have yet to determine effectively the extent to which mitochondria of oxygen-sensing cells are uniquely sensitive to a fall in oxygen supply, or subject to the modulation of their activities, either directly or indirectly, by "local" metabolic intermediates (e.g. O_2 gradients, ATP, ADP; Brown 1992; Gnaiger et al. 1998; Jones 1986) and/or the signalling systems that they may modulate mitochondrial function. Nevertheless the weight of evidence suggests that the inhibition of mitochondrial oxidative phosphorylation is a key step towards the initiation of HPV.

11.3 The AMP-Activated Protein Kinase Mediates Hypoxic Pulmonary Vasoconstriction

Ten years ago the LKB1-AMPK signalling pathway was proposed to couple inhibition by hypoxia of mitochondrial metabolism to HPV (Evans 2006; Evans et al. 2005, 2006). This seemed logical not least because AMPK activity is intimately coupled to mitochondrial oxidative phosphorylation through the action of LKB1, the principle upstream kinase contributing to the activation of AMPK in response to metabolic stress (Hardie 2007; Oakhill et al. 2011). Moreover, AMPK is ubiquitously expressed and comprises catalytic α and regulatory β and γ subunits, of which there are multiple isoforms (Hardie 2007) (Fig. 11.1), that may confer at least 12 different subunit combinations and thus the capacity for the modulation of both substrate- and cell-specific functions (Steinberg and Kemp 2009). In response to metabolic stresses, such as the inhibition of mitochondrial oxidative

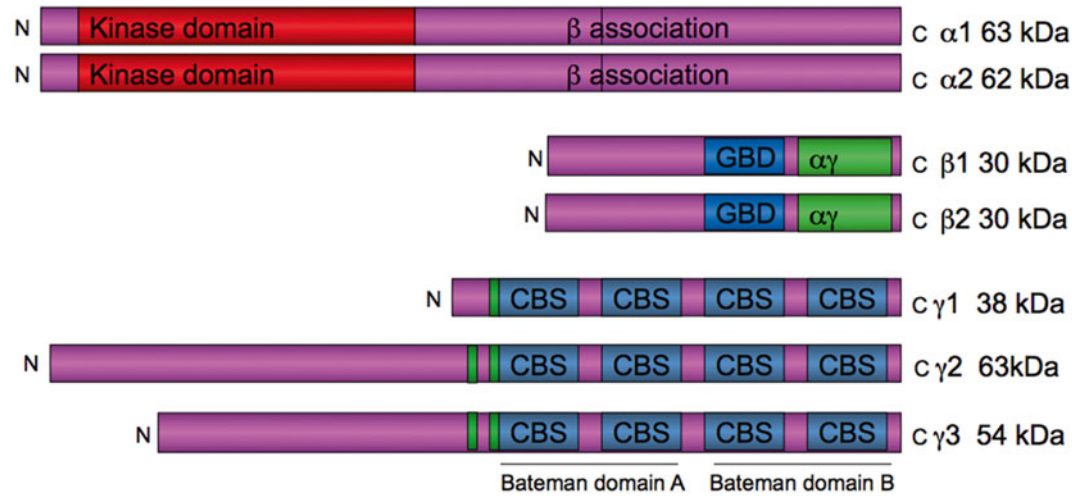

Fig. 11.1 The AMPK alpha, beta and gamma subunit isoforms GBD, Glycogen binding domain

phosphorylation, AMPK is activated by an increase in the ADP/ATP ratio, which is amplified by adenylate kinase into a much larger increase in the AMP/ATP ratio (Gowans et al. 2013; Hawley et al. 1995). Allosteric activation of AMPK by AMP binding to the γ subunit may confer a 10-fold increase in activity (Gowans et al. 2013). That aside, activation of AMPK by more than 100-fold is conferred by phosphorylation at Thr-172 within the α subunit by upstream kinases, of which the most important is the tumor suppressor, LKB1 (Hawley et al. 2003). LKB1 appears to phosphorylate Thr-172 constitutively, but in manner facilitated by binding of AMP to the γ subunit (Gowans et al. 2013). Moreover, binding of AMP or ADP to exchangeable sites (Oakhill et al. 2011; Xiao et al. 2011) on the γ subunit of AMPK inhibits dephosphorylation of Thr-172, thus augmenting the switch to the active, phosphorylated form. This multiplexing mechanism of AMPK activation ensures great sensitivity, with the combinatorial effects delivering graded activation up to 1,000-fold from very low activities observed in unstressed cells when ATP is bound to the γ subunit sites. Alternatively, AMPK may be activated by increases in intracellular calcium via CaMKK-β, which also phosphorylates Thr-172 and likely acts to increase energy supply, for example, during periods of high cellular activity (Woods et al. 2005). By these mechanisms AMPK can be activated within seconds (Tamas et al. 2006) in order to up-regulate catabolic processes and suppress non-essential ATP-consuming reactions in order to maintain ATP supply; even without any measurable fall in cellular ATP. AMPK is therefore rightly considered to act as a 'guardian' or 'fuel gauge' of cellular metabolism (Hardie 2007). Pertinent to this Chapter, however, is the concept that AMPK may contribute to the regulation of oxygen and thereby energy (ATP) supply at the whole-body level and in doing so may contribute to hypoxia-response coupling by regulating cell and system physiology (Evans 2006). That AMPK may regulate aspects of cell function other than metabolism in all cell types brings us back to the mitochondrial/metabolic hypothesis for oxygen sensing.

As mentioned above, all mitochondrial inhibitors tested thus far mimic the effects of hypoxia on pulmonary arterial smooth muscle cells at the level of the oxygen-sensitive delayed rectifier potassium (K_V) current (Firth et al. 2008). However only some mitochondrial inhibitors have been shown to mimic and occlude HPV in the perfused lung, while others have been shown to block but not mimic HPV in the perfused lung and isolated pulmonary arteries

(Leach et al. 2001; Weissmann et al. 2003). This has been a bone of contention in the field and has been cast as being inconsistent with the view that HPV may be triggered by inhibition of mitochondrial oxidative phosphorylation. In this respect it is important to note that HPV fails under near anoxic conditions (<1 % oxygen), i.e., there is a pO_2 window within which pulmonary artery constriction may be initiated by hypoxia. It is therefore notable, for example, that the NAD(P)H/NAD(P)$^+$ ratio in dorsal root ganglion neurones, which do not serve to monitor oxygen supply, exhibits no shift until the pO_2 falls to near anoxic levels (~5 mm Hg); i.e., the pO_2 at which HPV begins to fail (Dipp et al. 2003). Why might this be significant? Strictly speaking, it is the "anoxic" and not the "hypoxic" condition that mitochondrial inhibitors would mimic at concentrations that ablate oxidative phosphorylation. Therefore, an explanation for the inconsistency of outcome with respect to the effects of mitochondrial inhibitors on HPV, and the pO_2 window within which HPV is triggered, may ultimately be provided by a greater understanding of the impact on pulmonary vascular function of degrees of metabolic stress. After all, dilating pulmonary arteries in response to anoxia might be the "last gasp" for optimal gaseous exchange within the lungs and thereby oxygen supply to the body. These considerations bring us back nicely to AMPK, which is activated by all mitochondrial inhibitors in a manner dependent of the degree of inhibition of mitochondrial oxidative phosphorylation (Hawley et al. 2010).

Consider the possibility that physiological levels of hypoxia may activate AMPK and thereby precipitate, for example, HPV (Evans et al. 2005). It is quite possible that during more extreme metabolic stress, such as anoxia, AMPK may play its now classical role and "switch off" non-essential ATP-consuming processes in order to ensure cell survival, and may not under these conditions function itself to drive constriction. A case in point with respect to mitochondrial inhibitors may be that one such agent, metformin, provides for effective therapy of type II diabetes via AMPK activation, whereas a closely related and more potent inhibitor of mitochondrial respiration, phenformin, is no longer prescribed because of related contra-indications.

What of the evidence supporting a role for AMPK in HPV? Our initial studies (Evans et al. 2005) showed that exposure of pulmonary arterial smooth muscle to hypoxia (15–20 mm Hg) precipitates an increase in the AMP/ATP ratio (Fig. 11.2), concomitant activation of AMPK and phosphorylation of acetyl-CoA carboxylase (ACC; an established marker for AMPK action), despite the fact that cellular ATP levels remain remarkably stable in the presence of hypoxia (Fig. 11.3a). Moreover, inhibition of mitochondrial oxidative phosphorylation by phenformin (Owen et al. 2000), evoked increases in NAD(P)H autofluorescence (Fig. 11.3b), AMPK activation and ACC phosphorylation (Fig. 11.3a) in pulmonary arterial smooth muscle cells. AMPK activation and ACC phosphorylation were also induced by AICAR (Fig. 11.3c), which activates AMPK not by inhibiting the mitochondrial electron transport chain (Fig. 11.3b) but by uptake into cells and subsequent metabolism to the AMP mimetic, ZMP (AICAR monophosphate; Corton et al. 1995). Regardless of their respective mechanism of action, each agent induced an increase in the intracellular calcium concentration in acutely isolated pulmonary arterial smooth muscle cells and did so by mobilising sarcoplasmic reticulum stores via ryanodine receptors as does hypoxia (Fig. 11.4). Most significantly, AMPK activation by AICAR evoked a slow, sustained and reversible constriction of pulmonary artery rings (Fig. 11.5a, b); an action not mimicked by phenformin due to confounding effects on smooth muscle function (Evans, unpublished observation). Moreover the sustained phase of HPV and pulmonary artery constriction in response to AICAR exhibited strikingly similar characteristics, namely a requirement for smooth muscle SR calcium release via ryanodine receptors, and calcium influx into and vasoconstrictor release from the endothelium (Fig. 11.5c). Consistent with these findings HPV was blocked by the non-selective AMPK antagonist, compound C (Robertson et al. 2008).

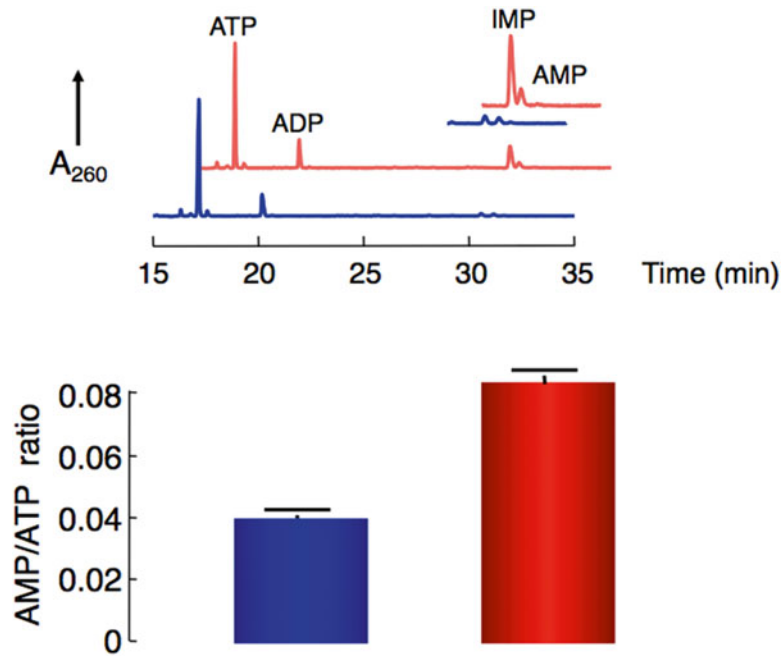

Fig. 11.2 Hypoxia increases the AMP/ATP ratio in pulmonary arterial smooth muscle. *Upper panel* shows an idealised representation of the assessment of relative nucleotide levels by capillary electrophoresis. *Lower panel* show the AMP/ATP ratio of pulmonary arterial smooth muscle during normoxia (*blue*) and hypoxia (*red*)

Most recently we have gathered further significant support for our original proposals by use of pharmacological activators of AMPK (e.g., A769662) and recombinant, thiophosphorylated and thus active, human α2β2γ1 heterotrimers. Intracellular dialysis of the activated human AMPK or extracellular application of A769662 resulted in the inhibition of recombinant currents carried by $K_V1.5$ channels (not shown) and, like hypoxia, inhibited K_V currents in acutely isolated pulmonary arterial smooth muscle cells (Fig. 11.6). Therefore, AMPK activation mimics the effects of hypoxia on pulmonary arterial smooth muscle cells, at the molecular, cellular and system level. This leaves us with perhaps the most important question – is the LKB1-AMPK signalling cascade necessary for HPV?

11.4 The Lkb1-AMPK Signalling Cascade and HPV

We have recently studied mice in which the genes for either LKB1, CaMKK-β or AMPK have been deleted in smooth muscles. Experimental outcomes from a range of studies on these mice are entirely consistent with the view that the LKB1-AMPK signalling pathway is required for HPV, including the response to hypoxia of the pulmonary vasculature and acutely isolated smooth muscle cells. By contrast, we find no evidence to suggest that CaMKK-β contributes to HPV. Nor have we found any evidence for the direct regulation of AMPK by hydrogen peroxide (Emerling et al. 2009), which appears to activate AMPK through inhibition of mitochondrial oxidative phosphorylation (Hawley et al. 2010).

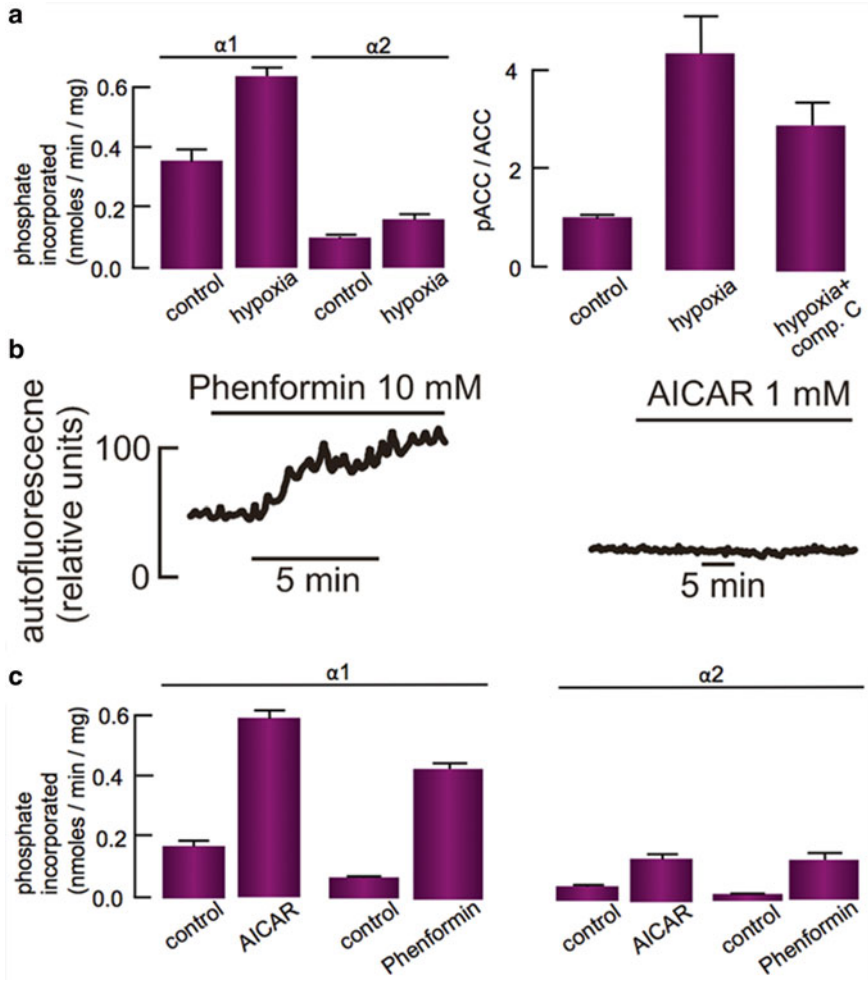

Fig. 11.3 AMPK activation and acetyl CoA carboxylase phosphorylation in response to hypoxia, mitochondrial inhibition and direct pharmacological activation. (**a**) *Left panel* shows the increases in activity of AMPK-α1 and AMPK-α2 containing heterotrimers during hypoxia, as determined by immunoprecipitate kinase assay. *Right panel* shows the increased phosphorylation of acetyl CoA carboxylase during hypoxia in the presence and absence of compound C, the non-selective AMPK antagonist. (**b**) NAD(P)H autofluorescence of pulmonary arterial smooth muscle cells is increased by phenformin but not AICAR. (**c**) Effect of AICAR and phenformin on the activity of AMPK-α1 (*left panel*) and AMPK-α2 (*right panel*) containing heterotrimers, as determined by immunoprecipitate kinase assay

11.5 The Lkb1-AMPK Signalling Cascade and the Development of Hypoxic Pulmonary Hypertension

Consistent with our findings, Zhou and co-workers have demonstrated that acute hypoxia-induced pulmonary hypertension may be prevented and partially reversed by the non-selective AMPK antagonist compound C (Ibe et al. 2013). Moreover they suggest that AMPK activation promotes pulmonary arterial smooth muscle survival and thus proliferation during hypoxia by a dual mechanism. Briefly, it was suggested that activation of autophagy by AMPK-α1 reduced cell death and that reduced apoptosis resulted from AMPK-α2 activation. Contrary to this latter proposal, however, upregulation of mTORC2 signalling has been proposed to underpin smooth muscle proliferation and the

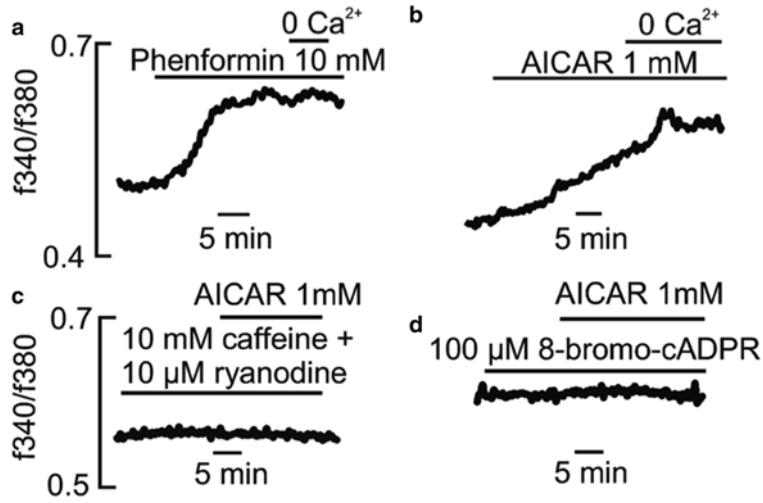

Fig. 11.4 AMPK activation initiates cADPR-dependent calcium release from the sarcoplasmic reticulum via ryanodine receptors. Activation of AMPK by either phenformin (**a**) or AICAR (**b**) increases the FURA-2 fluorescence ratio in acutely isolated pulmonary arterial smooth muscle cells, in a manner that is abolished by prior block of ryanodine receptors (**c**) or cADPR (**d**)

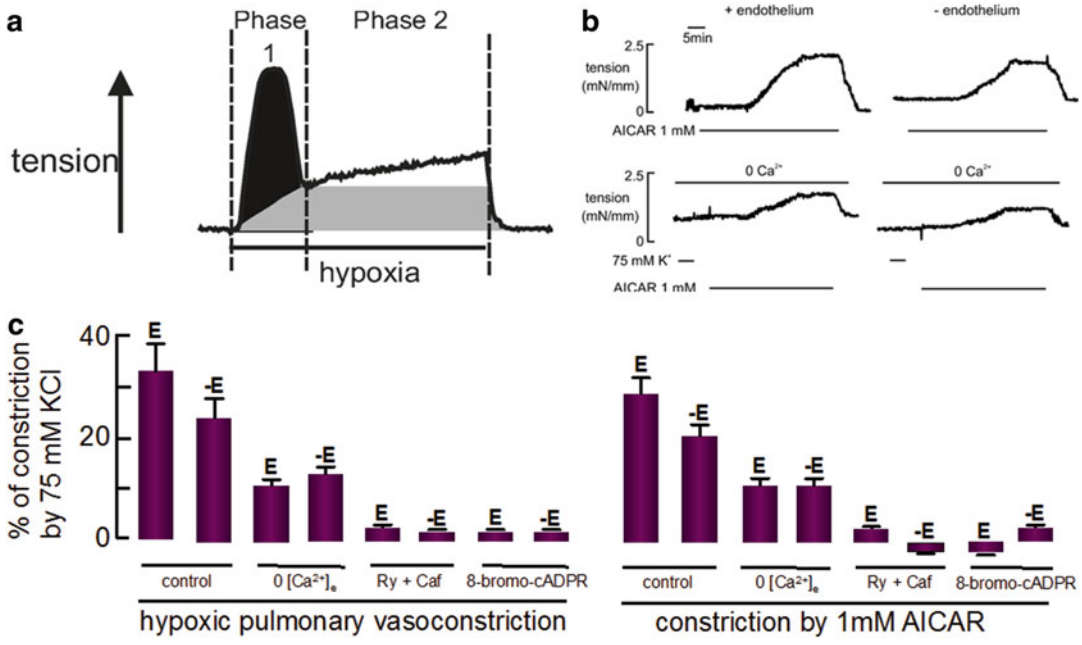

Fig. 11.5 Hypoxia and AMPK activation induce pulmonary artery constriction by similar mechanisms. (**a**) Record of Phase 1 and Phase 2 of hypoxia-induced constriction of a rat pulmonary artery ring, indicating the contribution to constriction of two components of calcium release from the smooth muscle sarcoplasmic reticulum (*black and grey*) and 3rd component driven by the release of a vasoconstrictor from the pulmonary artery endothelium (*white*). (**b**) Constriction of a pulmonary artery ring by AMPK activation with AICAR, in the presence and absence of the endothelium, with and without extracellular calcium. (**c**) Comparison of hypoxic pulmonary vasoconstriction and constriction by AICAR following the indicated experimental interventions: removal of the endothelium (-E); removal of extracellular calcium (0[Ca^{2+}]$_e$); block of ryanodine receptors with caffeine and ryanodine; block of cADPR with 8-bromo-cADPR

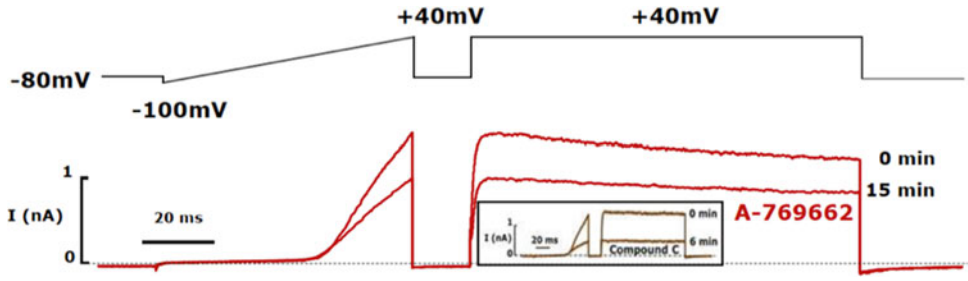

Fig. 11.6 AMPK activation by A769662 inhibits Kv current in pulmonary arterial smooth muscle cells. The effect of AMPK activation by A769662 on K_V currents recorded in acutely isolated pulmonary arterial smooth muscle cells. *Left*, current voltage relationship obtained by use of a ramp protocol between −100 mV and +40 mV, from a holding potential of −80 mV. *Right*, current activated by a voltage step to +40 mV from −80 mV. Insert shows confounding inhibition of Kv current by the AMPK antagonist compound C

progression of both idiopathic and hypoxic pulmonary arterial hypertension (Goncharov et al. 2014), by promoting smooth muscle cell survival in a manner, at least in part, dependent on down-regulation of AMPK and consequent activation of mTORC1. One possible explanation for these contrary findings could be that AMPK activation may be context-dependent and/or that the progression of pulmonary hypertension at different stages is governed by temporal fluctuations in AMPK activity.

11.6 Summary

We conclude that the inhibition of mitochondrial oxidative phosphorylation by acute hypoxia is coupled to pulmonary vasoconstriction through the LKB1-AMPK signalling pathway, which drives HPV by initiating and maintaining intracellular calcium release from the smooth muscle sarcoplasmic reticulum, promoting concomitant inhibition of the smooth muscle K_V current and by initiating vasoconstrictor release from the pulmonary artery endothelium. Thereafter modulation of the LKB1-AMPK signalling pathway may determine the progression of pulmonary hypertension. Further detailed characterisation of the regulation and role of AMPK signalling in the pulmonary vasculature is therefore vital to our understanding of the progression HPV and pulmonary hypertension, and may provide greater insight into those tissue-specific responses that are key to oxygen homeostasis.

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References

Archer SL, Huang J, Henry T, Peterson D, Weir EK (1993) A redox-based O_2 sensor in rat pulmonary vasculature. Circ Res 73:1100–1112

Bergofsky EH, Haas F, Porcelli R (1968) Determination of the sensitive vascular sites from which hypoxia and hypercapnia elicit rises in pulmonary arterial pressure. Fed Proc 27:1420–1425

Bradford JR, Dean HP (1894) The pulmonary circulation. J Physiol 16:34–158 25

Brown GC (1992) Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J 284:1–13

Buttigieg J, Zhang M, Thompson R, Nurse C (2006) Potential role of mitochondria in hypoxia sensing by adrenomedullary chromaffin cells. Adv Exp Med Biol 580:79–85, discussion 351–9

Cooper CE, Brown GC (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. J Bioenerg Biomembr 40:533–539

Corton JM, Gillespie JG, Hawley SA, Hardie DG (1995) 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? Eur J Biochem/FEBS 229:558–565

Dipp M, Evans AM (2001) Cyclic ADP-ribose is the primary trigger for hypoxic pulmonary vasoconstriction in the rat lung in situ. Circ Res 89:77–83

Dipp M, Nye PC, Evans AM (2001) Hypoxic release of calcium from the sarcoplasmic reticulum of pulmonary artery smooth muscle. Am J Physiol Lung Cell Mol Physiol 281:L318–L325

Dipp M, Thomas JM, Galione A, Evans AM (2003) A PO2 window for smooth muscle cADPR accumulation and constriction by hypoxia in rabbit pulmonary artery smooth muscle. Proc Physiol Soc 547P:C72

Duchen MR, Biscoe TJ (1992a) Mitochondrial function in type I cells isolated from rabbit arterial chemoreceptors. J Physiol 450:13–31

Duchen MR, Biscoe TJ (1992b) Relative mitochondrial membrane potential and $[Ca^{2+}]i$ in type I cells isolated from the rabbit carotid body. J Physiol 450:33–61

Duke HN, Killick EM (1952) Pulmonary vasomotor responses of isolated perfused cat lungs to anoxia. J Physiol 117:303–316

Emerling BM, Weinberg F, Snyder C, Burgess Z, Mutlu GM, Viollet B et al (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

Evans AM (2006) AMP-activated protein kinase and the regulation of Ca^{2+} signalling in O_2-sensing cells. J Physiol 574:113–123

Evans AM, Dipp M (2002) Hypoxic pulmonary vasoconstriction: cyclic adenosine diphosphate-ribose, smooth muscle Ca(2+) stores and the endothelium. Respir Physiol Neurobiol 132:3–15

Evans AM, Mustard KJ, Wyatt CN, Peers C, Dipp M, Kumar P et al (2005) Does AMP-activated protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O_2-sensing cells? J Biol Chem 280:41504–41511

Evans AM, Hardie DG, Galione A, Peers C, Kumar P, Wyatt CN (2006) AMP-activated protein kinase couples mitochondrial inhibition by hypoxia to cell-specific Ca^{2+} signalling mechanisms in oxygen-sensing cells. Novartis Found Symp 272:234–252, discussion 52–8, 74–9

Evans AM, Hardie DG, Peers C, Mahmoud A (2011) Hypoxic pulmonary vasoconstriction: mechanisms of oxygen-sensing. Curr Opin Anaesthesiol 24:13–20

Firth AL, Yuill KH, Smirnov SV (2008) Mitochondria-dependent regulation of Kv currents in rat pulmonary artery smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 295:L61–L70

Gnaiger E, Lassnig B, Kuznetsov A, Rieger G, Margreiter R (1998) Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome c oxidase. J Exp Biol 201:1129–1139

Goncharov DA, Kudryashova TV, Ziai H, Ihida-Stansbury K, DeLisser H, Krymskaya VP et al (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

Gowans GJ, Hawley SA, Ross FA, Hardie DG (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

Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8:774–785

Hawley SA, Selbert MA, Goldstein EG, Edelman AM, Carling D, Hardie DG (1995) 5′-AMP activates the AMP-activated protein kinase cascade, and Ca^{2+}/calmodulin activates the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. J Biol Chem 270:27186–27191

Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, Makela TP et al (2003) Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. J Biol 2:28

Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S et al (2010) Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab 11:554–565

Heymans C, Bouckaert JJ, Dautrebande L (1930) Sinus carotidien et refléxes respiratoires. II. Influences respiratoires réflexes de l'acidose, de l'alcalose, de l'anhydride carbonique, de l'ion hydrogéne et de l'anoxémie: sinus carotidiens et échanges respiratoires dans les poumons et au dela poumons. Arch Int Pharmacodyn Ther 39:400–408

Ibe JC, Zhou Q, Chen T, Tang H, Yuan JX, Raj JU et al (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

Jones DP (1986) Intracellular diffusion gradients of O_2 and ATP. Am J Physiol 250:C663–C675

Kato M, Staub NC (1966) Response of small pulmonary arteries to unilobar hypoxia and hypercapnia. Circ Res 19:426–440

Leach RM, Robertson TP, Twort CH, Ward JP (1994) Hypoxic vasoconstriction in rat pulmonary and mesenteric arteries. Am J Physiol 266:L223–L231

Leach RM, Hill HM, Snetkov VA, Robertson TP, Ward JP (2001) Divergent roles of glycolysis and the mitochondrial electron transport chain in hypoxic pulmonary vasoconstriction of the rat: identity of the hypoxic sensor. J Physiol 536:211–224

Lejeune P, Vachiery JL, Leeman M, Brimioulle S, Hallemans R, Melot C et al (1989) Absence of parasympathetic control of pulmonary vascular pressure-flow plots in hyperoxic and hypoxic dogs. Respir Physiol 78:123–133

Lu W, Wang J, Shimoda LA, Sylvester JT (2008) Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca^{2+} responses to

hypoxia. Am J Physiol Lung Cell Mol Physiol 295:L104–L113
Mills E, Jobsis FF (1972) Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension. J Neurophysiol 35:405–428
Naeije R, Lejeune P, Leeman M, Melot C, Closset J (1989) Pulmonary vascular responses to surgical chemodenervation and chemical sympathectomy in dogs. J Appl Physiol 66:42–50
Nisell O (1951) The influence of blood gases on the pulmonary vessels of the cat. Acta Physiol Scand 23:85–90
Oakhill JS, Steel R, Chen ZP, Scott JW, Ling N, Tam S et al (2011) AMPK is a direct adenylate charge-regulated protein kinase. Science 332:1433–1435
Owen MR, Doran E, Halestrap AP (2000) Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 348:607–614
Post JM, Hume JR, Archer SL, Weir EK (1992) Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. Am J Physiol 262:C882–C890
Remillard CV, Tigno DD, Platoshyn O, Burg ED, Brevnova EE, Conger D et al (2007) Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. Am J Physiol Cell Physiol 292:C1837–C1853
Robertson TP, Dipp M, Ward JP, Aaronson PI, Evans AM (2000) Inhibition of sustained hypoxic vasoconstriction by Y-27632 in isolated intrapulmonary arteries and perfused lung of the rat. Br J Pharmacol 131:5–9
Robertson TP, Ward JP, Aaronson PI (2001) Hypoxia induces the release of a pulmonary-selective, Ca(2+)-sensitising, vasoconstrictor from the perfused rat lung. Cardiovasc Res 50:145–150
Robertson TP, Mustard KJ, Lewis TH, Clark JH, Wyatt CN, Blanco EA et al (2008) AMP-activated protein kinase and hypoxic pulmonary vasoconstriction. Eur J Pharmacol 595:39–43
Robin ED, Theodore J, Burke CM, Oesterle SN, Fowler MB, Jamieson SW et al (1987) Hypoxic pulmonary vasoconstriction persists in the human transplanted lung. Clin Sci 72:283–287
Roy CS, Sherrington CS (1890) On the regulation of the blood-supply of the brain. J Physiol 11(85–158):17
Sommer N, Pak O, Schorner S, Derfuss T, Krug A, Gnaiger E et al (2010) Mitochondrial cytochrome redox states and respiration in acute pulmonary oxygen sensing. Eur Respir J 36:1056–1066
Steinberg GR, Kemp BE (2009) AMPK in health and disease. Physiol Rev 89:1025–1078
Tamas P, Hawley SA, Clarke RG, Mustard KJ, Green K, Hardie DG et al (2006) Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca^{2+} in T lymphocytes. J Exp Med 203:1665–1670
Thompson RJ, Jackson A, Nurse CA (1997) Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells. J Physiol 498:503–510
Thompson RJ, Buttigieg J, Zhang M, Nurse CA (2007) A rotenone-sensitive site and H_2O_2 are key components of hypoxia-sensing in neonatal rat adrenomedullary chromaffin cells. Neuroscience 145:130–141
Turner PJ, Buckler KJ (2013) Oxygen and mitochondrial inhibitors modulate both monomeric and heteromeric TASK-1 and TASK-3 channels in mouse carotid body type-1 cells. J Physiol 591:5977–5998
von Euler US, Liljestrand G (1946) Observations on the pulmonary arterial blood pressure in the cat. Acta Physiol Scand 12:301–320
Wang J, Shimoda LA, Sylvester JT (2004) Capacitative calcium entry and TRPC channel proteins are expressed in rat distal pulmonary arterial smooth muscle. Am J Physiol Lung Cell Mol Physiol 286:L848–L858
Waypa GB, Chandel NS, Schumacker PT (2001) Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ Res 88:1259–1266
Weissmann N, Ebert N, Ahrens M, Ghofrani HA, Schermuly RT, Hanze J et al (2003) Effects of mitochondrial inhibitors and uncouplers on hypoxic vasoconstriction in rabbit lungs. Am J Respir Cell Mol Biol 29:721–732
Wilson SM, Mason HS, Smith GD, Nicholson N, Johnston L, Janiak R et al (2002) Comparative capacitative calcium entry mechanisms in canine pulmonary and renal arterial smooth muscle cells. J Physiol 543:917–931
Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR et al (2005) Ca^{2+}/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab 2:21–33
Wyatt CN, Buckler KJ (2004) The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. J Physiol 556:175–191
Xiao B, Sanders MJ, Underwood E, Heath R, Mayer FV, Carmena D et al (2011) Structure of mammalian AMPK and its regulation by ADP. Nature 472:230–233