AMPK and Pulmonary Hypertension: Crossroads Between Vasoconstriction and Vascular Remodeling

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Pulmonary hypertension (PH) is a debilitating and life-threatening disease characterized by increased blood pressure within the pulmonary arteries. Adenosine monophosphate-activated protein kinase (AMPK) is a heterotrimeric serine-threonine kinase that contributes to the regulation of metabolic and redox signaling pathways. It has key roles in the regulation of cell survival and proliferation. The role of AMPK in PH is controversial because both inhibition and activation of AMPK are preventive against PH development. Some clinical studies found that metformin, the first-line antidiabetic drug and the canonical AMPK activator, has therapeutic efficacy during treatment of early-stage PH. Other study findings suggest the use of metformin is preferentially beneficial for treatment of PH associated with heart failure with preserved ejection fraction (PH-HFpEF). In this review, we discuss the "AMPK paradox" and highlight the differential effects of AMPK on pulmonary vasoconstriction and pulmonary vascular remodeling. We also review the effects of AMPK activators and inhibitors on rescue of preexisting PH in animals and include a discussion of gender differences in the response to metformin in PH.

Keywords: AMPK, pulmonary hypertension, pulmonary vascular remodeling, hypoxic pulmonary vasoconstriction, metformin

INTRODUCTION

Pulmonary hypertension (PH) is a disease characterized by high blood pressure that affects the vessels in lungs. These changes result in right ventricular failure and ultimately, premature death (Maron and Leopold, 2015). Adenosine monophosphate-activated protein kinase (AMPK) is a central regulator of energy homeostasis. It is activated under a variety of conditions, including hypoxia, nutrient starvation, and toxin exposure (Towler and Hardie, 2007; Kim et al., 2016; Herzig and Shaw, 2018). AMPK exerts most of its biological effects *via* catalytic α-subunits (α1 and α2) that are ubiquitously expressed in pulmonary vessels (Mihaylova and Shaw, 2011; Hardie, 2013; Kim et al., 2016). AMPK α1 is the predominant subunit in small pulmonary artery-derived pulmonary microvascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). AMPK α2 is the predominant subunit in conduit pulmonary artery-derived ECs and VSMCs (Evans et al., 2005; Creighton et al., 2011). The AMPK α1 and AMPK α2 subunits have different effects on survival of pulmonary VSMCs and hypoxic pulmonary vasoconstriction. For example, activation of AMPK α1 stimulates autophagy in pulmonary artery VSMCs, but AMPK α2 activation prevents apoptosis

(Ibe et al., 2013). Under conditions of mild hypoxia, AMPK α1 is activated by liver kinase B1 (LKB1) and is required for hypoxic pulmonary vasoconstriction; the AMPK α2 subunit is required under conditions of severe hypoxia (Moral-Sanz et al., 2018). Because of these characteristics, studies found that use of AMPK-targeting agonists and antagonists results in contradictory effects on PH development. Some studies found that AMPK activators [i.e., metformin (Agard et al., 2009; Dean et al., 2016; Lai et al., 2016; Omura et al., 2016; Zhai et al., 2018; Zhang et al., 2018; Wang et al., 2020), 5-aminoimidazole-4-carboxamide (AICAR) (Huang et al., 2014; Chen et al., 2016; Dean et al., 2016), rosiglitazone/pioglitazone (Hansmann et al., 2007; Satoh et al., 2009; Kim et al., 2010; Legchenko et al., 2018), and apelin (Chandra et al., 2011; Kim, 2014)] are protective against experimental PH. Other studies found that AMPK activation induces hypoxic pulmonary vasoconstriction (Evans, 2006; Robertson et al., 2008; Evans et al., 2009; Moral-Sanz et al., 2018) and that inhibition of AMPK by compound C prevents PH (Ibe et al., 2013). Results from human clinical studies are not currently conclusive on the precise role of AMPK in PH because studies on PH treatment using metformin are currently phase two clinical trials (NCT01884051 and NCT03629340). The AMPK paradox remains relevant.

AMPK: STRUCTURE AND REGULATION

AMPK Structure

Adenosine monophosphate-activated protein kinase is a highly conserved serine/threonine protein kinase complex consisting of a catalytic α-subunit, a scaffolding β-subunit, and a regulatory γ-subunit (**Figure 1**). In eukaryotes, each subunit has multiple distinct isoforms encoded by different genes. The α-subunit has two isoforms, α1 and α2, encoded by genes *Prkaa1* and *Prkaa2*, respectively (Stapleton et al., 1996). It contains a canonical N-terminal Ser/Thr kinase domain (KD), an auto-inhibitory domain (AID), and an adenine nucleotide sensor segment termed an α-linker (Herzig and Shaw, 2018; Yan et al., 2018). AMPK activation requires phosphorylation of critical residues (Thr174 in the AMPK α1 subunit and Thr172 in the AMPK α2 subunit) within the activation loop of the KD in the AMPKα catalytic subunit that is phosphorylated by upstream kinases LKB1 (Hudson et al., 2003), Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ) (Woods et al., 2005), or TGF-beta-activated kinase-1 (TAK-1) (Momcilovic et al., 2006). AMPK auto-inhibition requires an AID, which interacts with the KD and causes AMPK to be maintained as an inactive conformation (Chen et al., 2013; Kim et al., 2016). The β-subunit also has two isoforms, β1 and β2, encoded by *Prkab1* and *Prkab2*, respectively (Hudson et al., 2003). The γ-subunit has three isoforms, γ1, γ2, and γ3, encoded by *Prkag1*, *Prkag2*, and *Prkag3*, respectively (Cheung et al., 2000). The γ-subunits contain four tandem cystathionine-β-synthase domains, which enable AMP, ADP, or ATP binding (Xiao et al., 2007). Binding of AMP, and to a lesser extent ADP, to the γ-subunit is an important regulatory feature of the conformational switch that activates the AMPK complex (Hardie et al., 2011; Gowans et al., 2013; Ross et al., 2016a). Each
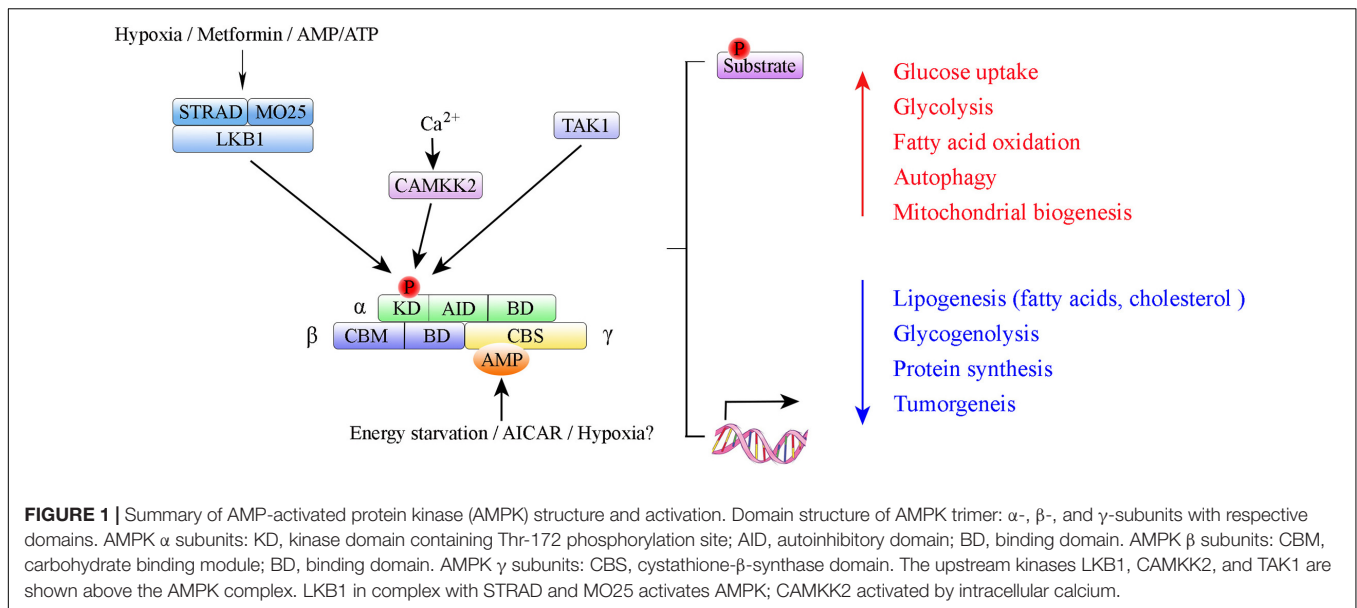
AMPK complex consists of one α-subunit, one β-subunit, and one γ-subunit, and all 12 heterotrimeric combinations are possible (Ross et al., 2016b). Different subunits have distinct organ preferences and expression patterns. For example, the AMPK α1 subunit is mainly expressed in adipose tissue (Ruderman et al., 2003; Rutter et al., 2003; Kelly et al., 2004). The AMPK α2 subunit is predominantly expressed in skeletal muscle and cardiac myocytes (Sakamoto et al., 2005, 2006; Thomson et al., 2007). Isoform-specific roles of AMPK α1/AMPK α2 contribute to the pathogenesis of different diseases (e.g., cardiovascular disease (Ahmad et al., 2005; Sakamoto et al., 2006; Zarrinpashneh et al., 2006; Arad et al., 2007), osteoclastogenesis (Wang et al., 2016), and Alzheimer's disease (Zhao et al., 2020)).

AMPK Signaling Transduction

Adenosine monophosphate-activated protein kinase can be phosphorylated directly by small molecules that mimic cellular decreased ATP-to-ADP or ATP-to-AMP ratios or three upstream AMPK kinases (i.e., LKB1, CaMKKβ and TAK1) (**Figure 1**). Upon changes in ATP/ADP and ATP/AMP ratios that occur during nutrient starvation, AMP binds to the AMPK γ subunit to cause allosteric activation *via* modulation of the phosphorylation state of Thr172 (Xiao et al., 2011; Oakhill et al., 2012; Gowans et al., 2013). LKB1, in a complex with the pseudokinase STRAD and the scaffolding protein MO25, directly phosphorylates AMPK at Thr172 (Lizcano et al., 2004). Study results indicate that LKB1 is the principal route *via* which AMPK is activated in many organs (e.g., skeletal muscle, adipose tissue, and liver) (Shackelford and Shaw, 2009). Whereas, CaMKKβ activates AMPK in response to Ca^{2+} signaling pathways (Hawley et al., 2005; Hong et al., 2005; Hurley et al., 2005). In 2006, TAK1 (i.e., MAPKK kinase-7, MAP3K7) was identified as the third kinase capable of direct AMPK activation (Momcilovic et al., 2006).

Adenosine monophosphate-activated protein kinase can be activated by hypoxia in various tissue and cell types (Mungai et al., 2011; Sallé-Lefort et al., 2016), but long-term hypoxia exposure inhibits AMPK activation (de Theije et al., 2018). Many studies found that activation of AMPK under hypoxia primarily implies LKB1 activity, because AMPK activation is abrogated in LKB1-deleted cells and knockout of CaMKK2, or another upstream kinase, has no effect on AMPK activation in VSMCs under hypoxic conditions (Moral-Sanz et al., 2018). Some studies found that LKB1 seems to only activate the AMPK α2 subunit, because the AMPK α1 subunit remains phosphorylated in LKB1-deficient heart muscle cells (Sakamoto et al., 2006). This result might be explained by differences in abundances and preferences of the AMPK upstream kinase in different cells and organs. Increased production of reactive oxygen species (ROS) in hypoxic conditions contributes to activation of AMPK (Choi et al., 2001; Emerling et al., 2009; Zmijewski et al., 2010; Hinchy et al., 2018). Hypoxia-inducible factor-prolyl-4-hydroxylases (HIF-P4Hs) have a role in the activation of AMPK (Yan et al., 2012; Dengler and Gäbel, 2019).

Once activated, AMPK phosphorylates key proteins in multiple pathways (Marsin et al., 2000; Inoki et al., 2003;

FIGURE 1 | Summary of AMP-activated protein kinase (AMPK) structure and activation. Domain structure of AMPK trimer: α-, β-, and γ-subunits with respective domains. AMPK α subunits: KD, kinase domain containing Thr-172 phosphorylation site; AID, autoinhibitory domain; BD, binding domain. AMPK β subunits: CBM, carbohydrate binding module; BD, binding domain. AMPK γ subunits: CBS, cystathione-β-synthase domain. The upstream kinases LKB1, CAMKK2, and TAK1 are shown above the AMPK complex. LKB1 in complex with STRAD and MO25 activates AMPK; CAMKK2 activated by intracellular calcium.

Gwinn et al., 2008) or directly regulates key enzymes involved in these pathways. These processes occur over time *via* targeting of transcriptional regulators (Koo et al., 2005; Lamia et al., 2009; Bungard et al., 2010; **Figure 1**). The most important aspect of AMPK biology is its role in maintaining the balance between catabolism and anabolism in response to metabolic stress (Towler and Hardie, 2007; Hardie, 2008; Fogarty and Hardie, 2010). Studies have revealed the roles of AMPK in lipid homeostasis [e.g., acetyl-CoA carboxylase (Munday et al., 1988) and HMG-CoA reductase (Carling et al., 1987)], glucose metabolism [e.g., thioredoxin-interacting protein (TXNIP) (Wu et al., 2013) and 6-phosphofructo-2-kinase (Bando et al., 2005)], insulin signaling (Galic et al., 2011; Li Y. et al., 2011; Fullerton et al., 2013; Wu et al., 2015; Emilio et al., 2016; Myers et al., 2017), and food intake and body weight (Kahn et al., 2005; Kola et al., 2005; Kola, 2008). Given those functional attributes in metabolism, AMPK is a major therapeutic target for treatment of metabolic diseases (e.g., type 2 diabetes) and obesity (Viollet et al., 2009; Rojas et al., 2011; Hardie, 2013; Day et al., 2017). A growing body of evidence also points to specific regulation of AMPK and mitochondrial homeostasis, including *via* stimulation of mitochondrial biogenesis (Bergeron et al., 2001; Zong et al., 2002; Garcia-Roves et al., 2008), regulation of mitochondrial dynamics (Ducommun et al., 2015; Toyama et al., 2016), and mitophagy (Wang et al., 2001; Egan et al., 2011).

AMPK and Cardiovascular Disease

Adenosine monophosphate-activated protein kinase has pivotal roles in cardiovascular physiology and in cardiovascular disease states. AMPK α1 is the predominant subunit in VSMCs, ECs, monocytes/macrophages, and adipocytes. AMPK α2 is the predominant subunit in cardiomyocytes (Shirwany and Zou, 2010; Wu and Zou, 2020). The functions of AMPK in cardiovascular disease include contributions to atherosclerosis and to heart failure and hypertension, which have been extensively reviewed elsewhere (Shirwany and Zou, 2010; Wu and Zou, 2020).

PULMONARY HYPERTENSION

Categories

Pulmonary hypertension is a general term used to describe increased blood pressure (mean pulmonary arterial pressure, mPAP, exceeds 25 mmHg at rest) in the lungs (Galiè et al., 2009). At the 5th and 6th World Symposium on PH, it was classified into five groups: pulmonary artery hypertension (PAH, Group 1), PH associated with left heart disease (Group 2), PH associated with lung disease and/or hypoxia (Group 3), PH associated with chronic thromboembolic disease (Group 4), and PH with unclear or multifactorial mechanisms, or both (Group 5) (Galiè and Simonneau, 2013; Simonneau et al., 2019). Each group represents a very broad spectrum of disease etiology, pathobiology, hemodynamic characteristics, and therapeutic approaches (**Table 1**). The detailed features and treatments of pulmonary hypertensive vascular disease in humans have been reviewed elsewhere (Maron and Galiè, 2016; Thenappan et al., 2018). AMPK deficiency has been identified in metabolic syndrome-associated PH (PH-HFpE) (Lai et al., 2016). However, in PAH, AMPK activity and expression can be either inhibited or promoted depending on cell type and branch pulmonary artery diameter (Ibe et al., 2013; Omura et al., 2016; Zhang et al., 2018), which is discussed in section "Clinical Trials of Pulmonary Hypertension Treatment Using Metformin."

Pathology of PH

Although the exact causes of PH remain to be determined, study findings indicate that it results from a combination of sustained pulmonary vasoconstriction and pulmonary vascular remodeling (Stenmark and McMurtry, 2005; Stenmark et al., 2006).

TABLE 1 | Animal models of pulmonary hypertension.

Cause	Histological features	Animal models
Group 1: Pulmonary arterial hypertension (PAH)		
Idiopathic PAH	Pulmonary artery intimal proliferation	Su-Hx rat/mouse
Heritable PAH	Pulmonary artery medical hypertrophy	MCT rat
Drugs/Toxin/Others	Plexiform lesions	Su-Hx-Normoxia
Group 2: Pulmonary hypertension with left heart disease		
Left-sided heart disease	Pulmonary medical hypertrophy	SU5416/Obese ZSF1 rat
	Pulmonary vein arterialization	
	Pulmonary interstitial edema	
Group 3: Pulmonary hypertension associated with lung disease and/or hypoxemia		
High altitudes	Hypoxic pulmonary vasoconstriction	Su-Hx rat/mouse
COPD/Pulmonary fibrosis Obstructive sleep apnea	Muscularization of arterioles	Hypoxia rat/mouse
Group 4: Pulmonary hypertension due to chronic thrombotic and/or embolic disease		
Pulmonary emboli	Thrombi or embolism	Vena cava ligation
Other clotting disorders	Recanalized organized thrombi	
Group 5: Pulmonary hypertension triggered by other health conditions		
Heterogeneous	Heterogeneous	

Pulmonary vasoconstriction is the major contributor to the early phase of the disease; pulmonary vascular structural remodeling becomes progressively more dominant and important over time (Shimoda and Laurie, 2013). Hypoxic pulmonary vasoconstriction is a reflex contraction of vascular smooth muscle in the pulmonary circulation to optimize lung blood flow from low ventilated areas to well-oxygenated areas, and thereby optimize gas exchange and oxygen delivery (Moudgil et al., 2005; Dunham-Snary et al., 2017; Tarry and Powell, 2017). Unlike the systemic circulation, which dilates in the presence of hypoxia, pulmonary arteries constrict in response to alveolar hypoxia (Detar, 1980; Waypa and Schumacker, 2010). Hypoxic pulmonary vasoconstriction is an important homeostatic mechanism used to match regional perfusion and ventilation in the lung (Dunham-Snary et al., 2017; Tarry and Powell, 2017).

Sustained pulmonary vasoconstriction initiates pulmonary vascular structural changes. These changes are characterized by thickening of the intimal and/or medial layers of muscular vessels, which results in concentric pulmonary vascular remodeling (Heath and Edwards, 1958; Tuder, 2017). In human beings, pulmonary vascular remodeling is attributed to lesions that mainly occur in distal pre-capillary arteries, ranging in diameter from 500 to 700 μm. Remodeling involves a change in the maximal lumen diameter (interior and exterior) and accumulation of different vascular cell types in the pulmonary arterial wall (pulmonary artery ECs, VSMCs, and fibroblasts). Pulmonary endothelial dysfunction is the key trigger that drives

PH development. It is characterized by either impairment of endothelial-dependent vasodilatation, reduced anticoagulant properties, ROS production, or active EC metabolic changes (Budhiraja et al., 2004; Attinà et al., 2005; Klinger et al., 2013; Ranchoux et al., 2018). Various stimuli (e.g., hypoxia, smoking, disturbed blood flow, and oxidative stress) can lead to endothelial dysfunction (Dummer et al., 2018; Ranchoux et al., 2018). In PH, progressive accumulation of resident VSMCs in pulmonary arteries contributes to expansion of the tunica media. Accumulating evidence also supports involvement of increased VSMC proliferation and inhibition of apoptosis in pulmonary vascular medial layer thickening (Tuder et al., 2007; Lyle et al., 2017; Humbert et al., 2019). Better understanding of the molecular mechanisms underlying pulmonary endothelial dysfunction and VSMC adaptation will greatly enhance our understanding of the pathogenesis of PH, which may help identify new therapeutic strategies. Other promising targets (e.g., fibroblast cell activation and immune system dysregulation) have also been identified as contributing to the pathogenesis of PH (Li M. et al., 2011; Rabinovitch et al., 2014; Plecitá-Hlavatá et al., 2016; Nicolls and Voelkel, 2017).

Animal Models of PH

A variety of pre-clinical PH animal models are available to study this complex disease of diverse etiologies and histopathological features. Each model has its own hemodynamic and microanatomic histological characteristics (**Table 1**). The chronic hypoxia rat/mouse model is the one most widely used to study PH. Exposure of rats/mice to hypoxia causes increased mPAP, pulmonary vasoconstriction, and vascular medial hypertrophy that mimic the pathological features of human PH. However, right ventricular failure is absent (Zhao, 2010; Ryan et al., 2013). Monocrotaline (MCT) is a toxic alkaloid that causes a widespread pneumotoxicity and endothelial injury (Kay et al., 1967; Wilson et al., 1989). A single dose of MCT (60 mg/Kg) is sufficient to induce PH in rats by modulating two key pathological features of human PH, pulmonary vascular remodeling and right ventricular failure (Schoental and Head, 1955; Jasmin et al., 2001; Dumitrascu et al., 2008; Gomez-Arroyo et al., 2012). Sugen 5416 is a vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor. Sugen 5416/hypoxia (Su/Hx) induces severe PH in both rats and mice that is characterized by pulmonary angioobliteration and right ventricular failure (Taraseviciene-Stewart et al., 2001; Sakao and Tatsumi, 2011). These three PH animal models are well-recognized models of Group 1 PH and Group 3 PH (Ryan et al., 2013; Colvin and Yeager, 2014; Sztuka and Jasińska-Stroschein, 2017). Lai et al. (2016) developed a two-hit model of PH associated with heart failure with preserved ejection fraction (PH-HFpEF). It includes giving a single injection of SU5416 to obese ZSF1 rats. The SU5416/obese ZSF1 rats develop PH that includes a preserved ejection fraction and right and left ventricular hypertrophy. PH-HFpEF develops as a more advanced corollary of PH and diastolic HF, leading to more severe symptoms than those with HFpEF and suffers significant exercise intolerance, frequent hospitalization, and reduced survival (Thenappan et al., 2011; Hoeper et al., 2016).

AMPK AND PULMONARY HYPERTENSION

Role of AMPK in the Predisposition and Development of PH

Researches have revealed the role of AMPK in hypoxic pulmonary vasoconstriction and pulmonary vascular remodeling. Two clinical trials (NCT01884051 and NCT03629340) focusing on PAH treatment with metformin are in progress. However, in various animal models, AMPK has contradictory effects on PH, as both inhibition and activation of AMPK are protective for the development of PH. These seemingly opposing results can be partly explained by the different effects of AMPK signaling in pulmonary vasoconstriction and pulmonary vascular remodeling.

Role of AMPK in Hypoxic Pulmonary Vasoconstriction

Until 1871, it was universally believed that the pulmonary vessels did not respond to a vasomotor system. However, Brown-Séquard (1871) published some results indicating that such a system exists. Subsequently, Bradford and Dean (1894) reported that asphyxia causes PH. von Euler and von Liljestrand (1946) reported that acute hypoxia promotes pulmonary vasoconstriction to increase pulmonary arterial pressure. This study (von Euler and von Liljestrand, 1946) launched the current era of study of hypoxia and pulmonary vasoconstriction.

Hypoxic pulmonary vasoconstriction is an important homeostatic physiological mechanism that optimizes ventilation/perfusion matching, gas exchange, and systemic oxygen delivery. In response to alveolar hypoxia, intrapulmonary arteries constrict to divert blood to better-oxygenated lung segments (Bradford and Dean, 1894; von Euler and von Liljestrand, 1946; McMurtry et al., 1976; Madden et al., 1985; Sylvester et al., 2012; Dunham-Snary et al., 2017). Hypoxic pulmonary vasoconstriction relies on a group of specialized pulmonary VSMCs, which are located in pulmonary arterial segments stripped of the tunica intima and tunica media, but not in similar segments of pulmonary veins or systemic arteries (Bergofsky et al., 1967; Murray et al., 1990a,b; Madden et al., 1992; Weir and Archer, 1995). Hypoxic pulmonary vasoconstriction is triggered by mitochondrial redox signaling that involves voltage-gated potassium channels (Kv) and calcium channels (Weir and Archer, 1995). Hypoxia inhibits Kv channels in pulmonary VSMCs, causing membrane depolarization and opening of voltage-gated calcium channels to initiate Ca^{2+}-mediated pulmonary vasoconstriction (Weir and Archer, 1995; Archer and Michelakis, 2002; Sommer et al., 2008; Dunham-Snary et al., 2017).

Adenosine monophosphate-activated protein kinase has a critical role in hypoxic pulmonary vasoconstriction by linking the oxygen sensor to its effectors (**Figure 2**). Evans et al. (2005, 2006) and Evans (2006) found that physiological hypoxia increases the AMP/ATP ratio in pulmonary VSMCs, followed by increased AMPK activity and phosphorylation of a classical AMPK substrate, acetyl CoA carboxylase (a well-validated marker for AMPK activation). This process is likely to be
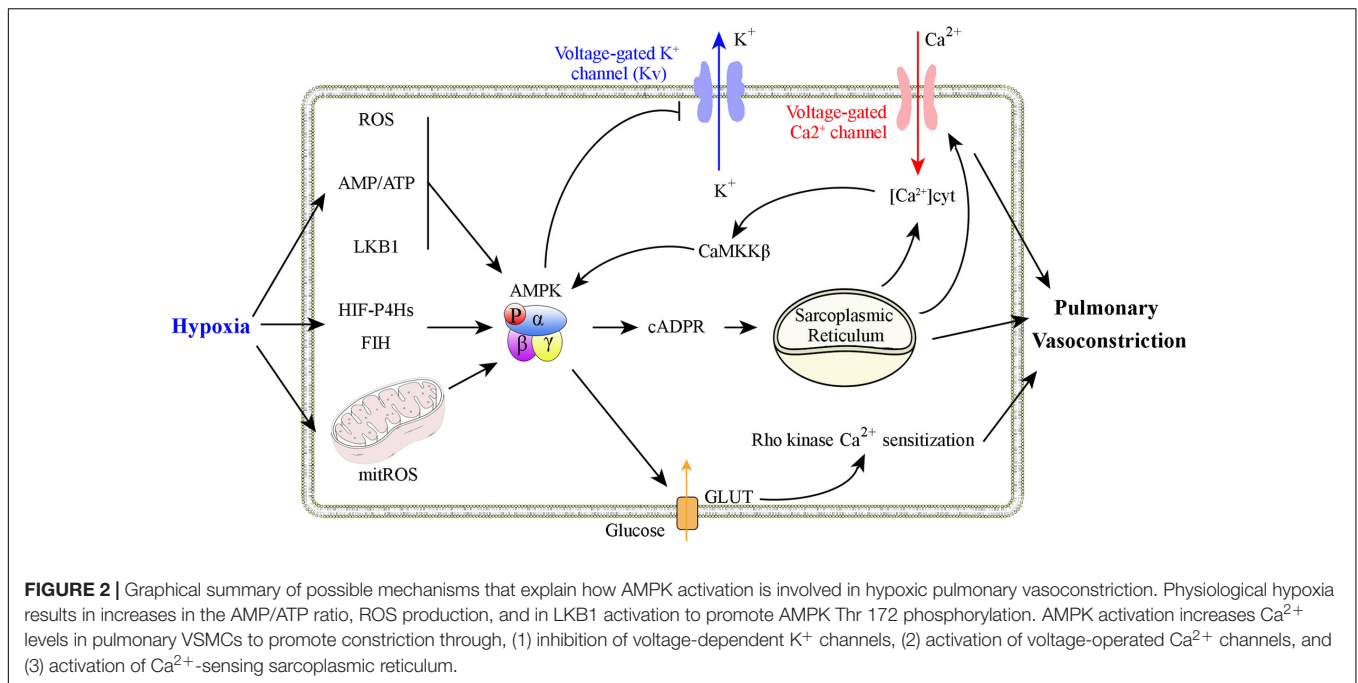
mediated by binding of AMP to the AMPK γ subunit, which triggers activation of the kinase by, (1) promoting AMPK Thr 172 phosphorylation *via* allosteric regulation (Scott et al., 2002; Kemp, 2004; Oakhill et al., 2010), (2) inhibiting AMPK Thr 172 dephosphorylating (Davies et al., 1995), and (3) facilitating phosphorylation of Thr 172 by the upstream kinase LKB1 (Hawley et al., 2003; Woods et al., 2003; Shaw et al., 2004, 2005). Additional studies found that AMPK activation evokes a slow, sustained, and reversible increase in Ca^{2+} influx *via* cyclic adenosine diphosphate-ribose (cADPR)-dependent mobilization of sarcoplasmic reticulum stores in pulmonary VSMCs and the consequent induction of constriction of pulmonary artery rings (Evans et al., 2005). Consistent with these findings, two different AMPK activators, AICAR and phenformin, evoke intracellular Ca^{2+} influx and reversible constriction of the pulmonary artery rings. The characteristics of this process are strikingly similar to those of hypoxic pulmonary vasoconstriction (Evans et al., 2005). The hypoxia-associated pulmonary vasoconstriction and Ca^{2+} influx is inhibited by the non-selective AMPK antagonist, compound C, upon inhibition of the sarcoplasmic reticulum store-refilling current (Robertson et al., 2008). When hypoxia occurs, AMPK can directly phosphorylate voltage-gated potassium channels (Kv1.5 channels), followed by inhibition of K^+ currents in pulmonary VSMCs. The entry of voltage-dependent Ca^{2+} to initiate the hypoxia-related pulmonary vasoconstriction is thus activated (Moral-Sanz et al., 2016). Downregulation of Kv1.5 expression and activity is also a hallmark of PH (Yuan et al., 1998; Lv et al., 2013). Strong support for this mechanism results from *in vivo* studies performed by Moral-Sanz et al. (2018), who found a key *in vivo* role of AMPK in hypoxic pulmonary vasoconstriction using a combination of AMPK isoform deletion strategies and spectral Doppler ultrasound. Under conditions of mild hypoxia (8% O_2), deletion of AMPK α1, but not AMPK α2, in smooth muscle cells block induction of hypoxia-related pulmonary vasoconstriction. When conditions of severe hypoxia (5% O_2) are present, either AMPK α1 or AMPK α2 deletion attenuates hypoxia-related pulmonary vasoconstriction (Moral-Sanz et al., 2018). The findings that SNPs in the *Prkaa1* gene have been identified in populations that live at high altitudes and who have attenuated hypoxic pulmonary vasoconstriction are consistent with these results (Penaloza and Arias-Stella, 2007; Bigham et al., 2014). In summary, a growing body of evidence supports the hypothesis that AMPK activation is a primary mediator of hypoxic pulmonary vasoconstriction.

Role of AMPK in Pulmonary Vasculature Remodeling

In PH, pulmonary arteries and veins undergo structure changes. This pulmonary vascular remodeling is characterized by proliferation of pulmonary ECs and VSMCs. AMPK has a key role in the pathogenesis of pulmonary vasculature remodeling (**Figure 3**).

AMPK in Endothelial Cells and PH

Both AMPK subunits (AMPK α1 and AMPK α2) are expressed in pulmonary ECs. However, AMPK α1 is mainly expressed in capillary-derived pulmonary microvascular ECs and AMPK α2 is mainly expressed in conduit-derived pulmonary artery ECs

FIGURE 2 | Graphical summary of possible mechanisms that explain how AMPK activation is involved in hypoxic pulmonary vasoconstriction. Physiological hypoxia results in increases in the AMP/ATP ratio, ROS production, and in LKB1 activation to promote AMPK Thr 172 phosphorylation. AMPK activation increases Ca^{2+} levels in pulmonary VSMCs to promote constriction through, (1) inhibition of voltage-dependent K^+ channels, (2) activation of voltage-operated Ca^{2+} channels, and (3) activation of Ca^{2+}-sensing sarcoplasmic reticulum.
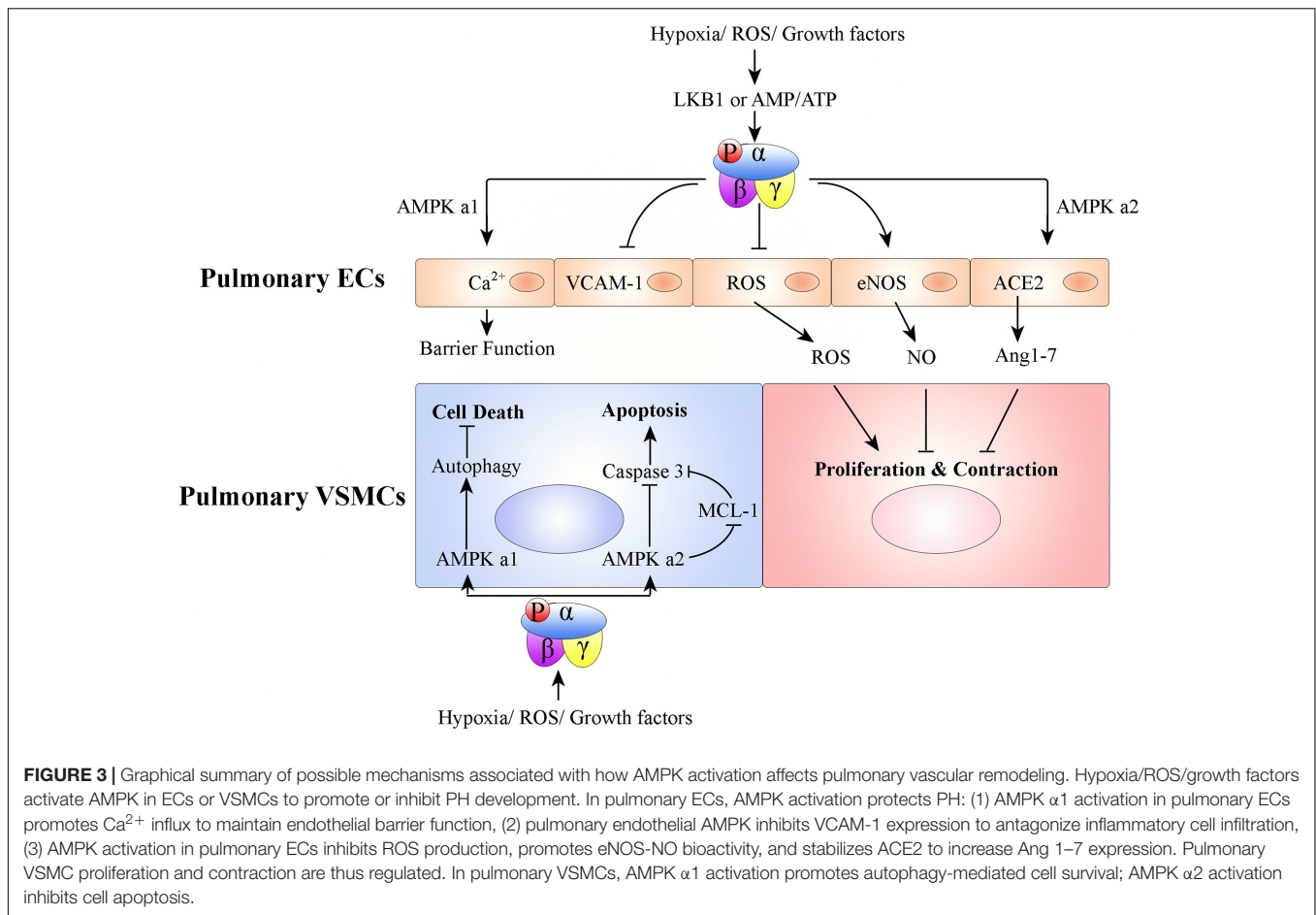
(Creighton et al., 2011). Pulmonary endothelial AMPK is down-regulated in pulmonary hypertensive patients and hypoxia-induced PH animals, compared with that from healthy donors or non-PH animals, respectively (Teng et al., 2013; Afolayan et al., 2016; Omura et al., 2016; Rana et al., 2020). EC-specific AMPK knockout mice (EC-AMPK$^{-/-}$) (Omura et al., 2016) and EC-specific AMPK α2 knockout mice (EC-AMPK α2$^{-/-}$) (Zhang et al., 2018) have accelerated development of PH under conditions of hypoxia. Endothelial AMPK exerts protective effects against PH via, (1) paracrine growth mechanism (e.g., PDGF-BB and FGF-2) mediation of the interaction between pulmonary artery ECs and pulmonary VSMCs (Omura et al., 2016), (2) positive regulation of nitric oxide (NO) production *via* endothelial NO synthase (eNOS) phosphorylation (Zhang et al., 2006; Chen et al., 2009; Afolayan et al., 2016), (3) stabilization of angiotensin-converting enzyme 2 (ACE2) to increase angiotensin 1–7 (Ang 1–7) expression and eNOS-derived NO bioavailability (Zhang et al., 2006), (4) Ca^{2+} influx in pulmonary microvascular ECs, which promotes endothelial barrier function (Creighton et al., 2011), (5) notch ligand promotion of angiogenesis (200), and (6) alleviation of EC apoptosis (Ido et al., 2002; Nagata et al., 2009; Enkhjargal et al., 2014).

AMPK in Vascular Smooth Muscle Cells and PH
During PH development, the remodeling process universally involves medial thickening driven by VSMC proliferation/hypertrophy and deposition of extracellular matrix within the tunica media of pulmonary arteries (Lyle et al., 2017). Pulmonary VSMCs express both the AMPK α1 and AMPK α2 subunits of AMPK (Creighton et al., 2011; Ibe et al., 2013). However, AMPK α1 is the predominant subunit in pulmonary VSMCs and contributes up to 80% of total AMPK activity (Evans et al., 2005; Xue et al., 2017). AMPK α1 catalytic

activity is much higher in VSMCs from small pulmonary arteries than in those from the main pulmonary arteries (Evans et al., 2005). Unlike AMPK in pulmonary ECs, studies of AMPK in pulmonary VSMCs have found contradictory results. Some studies found that phosphorylated AMPK is increased, while total AMPK levels remain the same, in pulmonary VSMCs from pulmonary hypertensive patients and hypoxia-induced PH mice, compared with those from healthy donors or non-PH mice, respectively (Krymskaya et al., 2011; Ibe et al., 2013). Mechanistically, hypoxia-activated AMPK promotes pulmonary VSMC survival, but AMPK activity pharmacologically inhibited by either compound C or 9-β-d-arabinofuranosyl adenine (Ara-a) abrogates hypoxia-induced pulmonary VSMC proliferation and PH (Ibe et al., 2013; Xue et al., 2017). Ibe et al. (2013) found that although suppression of either AMPK α1 or α2 in pulmonary VSMCs leads to increased cell death, AMPK α1 and AMPK α2 have differential roles. Activation of AMPK α1 stimulates autophagy and promotes pulmonary VSMC survival; activation of AMPK α2 regulates myeloid cell leukemia sequence 1 (MCL-1) to prevent apoptosis (Ibe et al., 2013). In contrast to these results, another series of studies found that phosphorylated AMPK is decreased in pulmonary VSMCs from patients with PH and from mice with hypoxia-induced PH, compared with those from healthy donors or non-PH mice, respectively (Goncharov et al., 2014). AMPK inhibition promotes pulmonary VSMC proliferation and survival, but AMPK pharmacologically activated by metformin or AICAR inhibits hypoxia-induced pulmonary VSMC proliferation and chronic PH (Agard et al., 2009; Goncharov et al., 2014; Wu et al., 2014; Ke et al., 2016; Song et al., 2016; Gui et al., 2017; Liu et al., 2019).

Seemingly contradictory results should be interpreted with caution. In the study that found elevated AMPK phosphorylation in PH and AMPK inhibition-attenuated

FIGURE 3 | Graphical summary of possible mechanisms associated with how AMPK activation affects pulmonary vascular remodeling. Hypoxia/ROS/growth factors activate AMPK in ECs or VSMCs to promote or inhibit PH development. In pulmonary ECs, AMPK activation protects PH: (1) AMPK α1 activation in pulmonary ECs promotes Ca^{2+} influx to maintain endothelial barrier function, (2) pulmonary endothelial AMPK inhibits VCAM-1 expression to antagonize inflammatory cell infiltration, (3) AMPK activation in pulmonary ECs inhibits ROS production, promotes eNOS-NO bioactivity, and stabilizes ACE2 to increase Ang 1–7 expression. Pulmonary VSMC proliferation and contraction are thus regulated. In pulmonary VSMCs, AMPK α1 activation promotes autophagy-mediated cell survival; AMPK α2 activation inhibits cell apoptosis.

PH (Krymskaya et al., 2011; Ibe et al., 2013), the researchers used pulmonary VSMCs isolated from large-diameter arteries located in a segment of pulmonary arteries just proximal to where lung entry occurs (diameter ≥ 0.8 mm). In the study that found AMPK reduction in PH and PH mitigation by AMPK activation (Goncharov et al., 2014), pulmonary VSMCs isolated from small-diameter arteries located in distal pulmonary artery segments (type III, diameter ≤ 0.1 mm) were used. Therefore, these discrepancies may be due to different functions of AMPK or different AMPK isoforms, or both, in pulmonary arteries with different diameters. Hypoxic pulmonary vasoconstriction is more vigorous in small pulmonary arteries (Dawson et al., 1977; Grimm et al., 1978; Sylvester et al., 2012), where the AMPK α1 catalytic subunit is predominantly expressed (Evans et al., 2005). The non-selective nature of AMPK activators or inhibitors may be another factor that contributes to these apparent inconsistencies.

AMPK and Pulmonary Hypertension Treatment

AMPK Inhibition Is Preventive for Development of Pulmonary Hypertension

Ibe et al. (2013) found that inhibition of AMPK by compound C prevents development of hypoxia-induced PH. When mice treated with compound C one day before hypoxia exposure (10% oxygen for 3 weeks), compound C prevents hypoxia-induced PH, pulmonary arterial wall thickening, and right ventricular hypertrophy. The activation of AMPK α1 stimulates autophagy, promoting pulmonary VSMCs survival, whereas the activation of AMPK α2 increases the expression of myeloid cell leukemia sequence 1 (MCL-1), inhibiting pulmonary VSMCs apoptosis (Ibe et al., 2013). Consistent with these results, Robertson et al. (2008) and Evans et al. (2009) pre-incubated intrapulmonary arteries (3rd and 4th order branches of the pulmonary arterial tree, 0.2–0.5 mm internal diameter) with compound C (40 mM). Compound C reversed/inhibited hypoxic pulmonary vasoconstriction in a concentration-dependent manner. Functionally, AMPK phosphorylates voltage-gated K^+ channel (Kv2.1) and thereby confers a leftward shift in both the activation and inactivation curves of Kv2.1, which precipitates an increase in the intracellular Ca^{2+} concentration (Evans et al., 2009).

AMPK Activation Is Preventive for Development of Pulmonary Hypertension

A significant body of evidence suggests that AMPK activation is preventive for development of PH. Metformin, the first-line medication for treatment of type 2 diabetes and the canonical

TABLE 2 | Pulmonary hypertension (PH) animal experiments of AMPK.

Therapy	Dose (mg/kg/day)	Route	Time (day)	PH models	Sex	References
AMPK activator with beneficial effects on PH						
Metformin	100–150	p.o.	21–28	MCT rat	M	Zhai et al., 2018; Yoshida et al., 2020
Metformin	100–150	i.p.	21–28	MCT rat	M	Agard et al., 2009; Li et al., 2016
Metformin	100	i.p.	30	MCT rat		Sun et al., 2019
Metformin	100	i.p.	21	Hypoxia rat		Liu et al., 2019
Metformin	100	p.o.	21	Su/Hx rat	F	Dean et al., 2016
Metformin	300	p.o.	31–28	Obese ZSF1 rat	M	Lai et al., 2016
Metformin	300	p.o.	98	SU5416/Obese ZSF1 rat	F	Wang et al., 2020
Metformin	150	i.p.	14	Su/Hx mouse	M	Zhang et al., 2018
Metformin	100	p.o.	21	Hypoxia mouse	M	Omura et al., 2016
AICAR	1?	i.p.	28	Hypoxia rat	M	Huang et al., 2014; Chen et al., 2016
AMPK antagonist with beneficial effects on PH						
Compound C	20	i.p.	21	Hypoxia mouse	M	Ibe et al., 2013
AMPK activators without effects on PH						
Metformin	100	p.o.	14	SuHx mouse	M	Goncharov et al., 2018
Metformin	300	p.o.	42	SuHx rat	M	Goncharov et al., 2018
AICAR	500	p.o.	42	SuHx rat	M	Goncharov et al., 2018

p.o., oral administration (per os); i.p., Intraperitoneal injection.

AMPK activator, demonstrates therapeutic efficacy on PH in animal models. AMPK activation by metformin prevents MCT-induced PH in rats (Agard et al., 2009; Li et al., 2016; Zhai et al., 2018; Sun et al., 2019; Yoshida et al., 2020). In these experimental models, rats were injected with one dose of MCT (60 mg/kg) to induce PH. Metformin (100–150 mg/kg/day, drinking water or intraperitoneal injection, 21–30 days) treatment significantly reduced right ventricular systolic pressure and pulmonary vascular remodeling in rats with MCT-induced PH (Agard et al., 2009; Li et al., 2016; Zhai et al., 2018; Sun et al., 2019; Yoshida et al., 2020). Consistent with these results, metformin has protective effects on hypoxia-induced PH in mice and rats (Huang et al., 2014; Omura et al., 2016; Liu et al., 2019), and a more pronounced PH with angioobliterative lesions in Sugen 5416/hypoxia (SuHx) mice/rats (Dean et al., 2016; Zhang et al., 2018) and SU5416/Obese ZSF1 rats (Lai et al., 2016; Wang et al., 2020). The AMPK activator, AICAR, also prevents PH development in rats with hypoxia-induced PH (Huang et al., 2014; Chen et al., 2016).

In contrast to the results that metformin has protective effects, other researchers reported that their findings did not support the efficacy of metformin in PAH therapy (Goncharov et al., 2018). Goncharov et al. (2018) findings suggested that metformin treatment may be preferentially beneficial for PH with heart failure with preserved ejection fraction (PH-HFpEF, group 2 PH), but that it has limited efficacy for PAH. They induced PAH in a male C57BL/6J mouse model using a 3-week exposure to SuHx. They then gave metformin (100 mg/kg/day, 14 days) in drinking water for 2-weeks post hypoxia exposure. Goncharov et al. found no changes in right ventricular systolic pressure, right ventricular hypertrophy, or pulmonary vascular remodeling in the metformin-treated SuHx mice. They also evaluated the preventive effects of metformin and AICAR in PAH. In these animal models, metformin (300 mg/kg/day, drinking water,

42 days) or AICAR (500 mg/kg/day, intraperitoneal, 42 days) were administrated 1 day before SuHx exposure. They found no changes in right ventricular systolic pressure, right ventricular hypertrophy, or pulmonary vascular remodeling in either the metformin- or AICAR-treated SuHx rats. However, they did not measure phosphorylation levels of AMPK or downstream AMPK pathways. Metformin-induced AMPK activation requires full activation of an upstream kinase (e.g., LKB1), especially at low doses (Choi et al., 2001; Emerling et al., 2009; Zmijewski et al., 2010; Hinchy et al., 2018). Therefore, it is unknown whether the lack of metformin efficacy for PH treatment was associated with AMPK activation.

Different characteristics that likely contribute to apparently contradictory results are presented in **Table 2**. In Dean et al. (2016), AMPK activation by metformin (100 mg/kg/day, oral gavage, 21 days) seems to reverse the PH phenotype induced by SuHx in female rats, in contrast to the findings of Goncharov et al. (2018) that was performed using male rats. Thus, a sex difference might affect the response to metformin treatment of PH. Effects of this difference have been described for other diseases (e.g., obesity, aging, and spontaneous tumorigenesis) (Anisimov et al., 2010; Quan et al., 2016; Park et al., 2017; Bramante et al., 2021).

Clinical Trials of Pulmonary Hypertension Treatment Using Metformin

There has been significant interest in the use of metformin for PH treatment. Liao et al. (2018, 2019) found that a combination therapy using metformin (500 mg, twice daily, 3 months) and bosentan (endothelin receptor antagonist) improves 6-min walk distance and right heart hemodynamics, decreases serum pro-brain natriuretic peptide (pro-BNP) levels, and ameliorates pulmonary vasoconstriction in patients with PH associated with congenital heart defects. Two phase I/II clinical trials of

metformin for PH treatment are in progress (clinicaltrials.gov, NCT01884051 and NCT03629340). Results to date indicate good tolerability and potential clinical efficacy for improvement in right ventricular function in patients with PH who receive metformin therapy (2 g/day, 8 weeks) (Brittain et al., 2020). However, metformin use did not change the 6-min walk distance in those patients (Brittain et al., 2020). Although not yet complete, this clinical study provides new insights into the potential benefits of metformin use on right ventricular failure in patients with PH and indicates the need for more studies of the use of metformin therapeutic intervention in patients with PH and PH-HFpEF.

CONCLUSION AND PERSPECTIVES

In this review, we discussed some seemingly contradictory study results for AMPK and PH development. AMPK has a key role in PH, either during the early process of hypoxic pulmonary vasoconstriction or later during pulmonary vasculature remodeling, or both. However, whether AMPK activation or inhibition is protective against PH remains unclear: (1) AMPK activation triggers hypoxia-induced pulmonary artery constriction. AMPK activator use (e.g., AICAR and Ara-a) prevents hypoxia-induced pulmonary artery constriction and PH. (2) EC-specific deletion of AMPK exaggerates hypoxia-induced PH *in vivo*. This result indicates endothelial AMPK has a protective role during PH development. (3) VSMCs from large pulmonary arteries with AMPK activation have accelerated proliferation and inhibited apoptosis. VSMCs from distal small pulmonary arteries with AMPK inhibition have similar potential. (4) Some animal studies found that the AMPK activators, AICAR and metformin, have beneficial effects on PH treatment. Other study findings suggest that metformin therapy for PH may be limited to use for PH-HFpEF. AMPK activation might have less pronounced pulmonary vascular effects than right ventricular effects, as much evidence has been published suggesting that

AMPK activation exerts a protective effect in cardiac dysfunction, ischemic heart, heat failure, and cardiac hypertrophy (Russell et al., 2004; Miller et al., 2008; Ma et al., 2010; Kim et al., 2011; Morrison et al., 2011). (5) Sex differences in the response to metformin used for PH treatment may affect outcomes.

In conclusion, studies found seemingly contradictory results for the relationship between AMPK and PH. In one series of studies, inhibition of AMPK resulted in attenuated hypoxic pulmonary vasoconstriction and pulmonary VSMC proliferation. In another series of studies, activation of AMPK resulted in improved EC function, VSMC apoptosis, and decreased pulmonary vasculature tone. Given that AMPK a1 and AMPK a2 have different expression patterns and different functions in pulmonary arteries of different sizes, the role of AMPK in PH should be studied using a cell-specific and pathological process-specific approach. Studies involving genetically- and specifically-modified AMPK α1 and α2 subunits are needed to clarify their specific roles in PH pathogenesis and treatment.

AUTHOR CONTRIBUTIONS

QZ drafted the manuscript and figures. PS and M-HZ revised the manuscript. All authors contributed to the article and approved the submitted version.

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