

Review Article

Ion channels as convergence points in the pathology of pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a fatal disease of the cardiopulmonary system that lacks curative treatments. The main pathological event in PAH is elevated vascular resistance in the pulmonary circulation, caused by abnormal vasoconstriction and vascular remodelling. Ion channels are key determinants of vascular smooth muscle tone and homeostasis, and four PAH channelopathies (*KCNK3*, *ABCC8*, *KCNA5*, *TRPC6*) have been identified so far. However, the contribution of ion channels in other forms of PAH, which account for the majority of PAH patients, has been less well characterised. Here we reason that a variety of triggers of PAH (e.g. *BMPR2* mutations, hypoxia, anorectic drugs) that impact channel function may contribute to the onset of the disease. We review the molecular mechanisms by which these ‘extrinsic’ factors converge on ion channels and provoke their dysregulation to promote the development of PAH. Ion channels of the pulmonary vasculature are therefore promising therapeutic targets because of the modulation they provide to both vasomotor tone and proliferation of arterial smooth muscle cells.

Introduction

Pulmonary arterial hypertension (PAH) is a progressive disease of the cardiopulmonary system with life-limiting consequences. PAH is defined by a mean pulmonary artery pressure above 25 mmHg at rest, and end-expiratory pulmonary artery wedge pressure of less than 15 mmHg. Disease factors include: (i) genetic origins (i.e. mutations in genes crucial for pulmonary vascular function); (ii) other pathologies including congenital heart disease, portal hypertension, HIV or COPD; (iii) drugs or toxins, or (iv) unknown factors (i.e. PAH of idiopathic origins) [1]. In all cases, a complex pathological cascade leads to narrowing and remodelling of arteries and/or increased vascular reactivity ultimately leading to increased vascular resistance and elevated pulmonary blood pressure which may precipitate in right heart failure and death [1]. The development of PAH-specific therapies significantly improved patients’ outcome and quality of life [2]. However, poor prognosis (57% five-year survival rate), resistance to current drugs, and lack of curative treatment means there is an unmet need for new and innovative targets in PAH pharmacotherapy [2].

Ion channels have an important homeostatic function whereby they regulate the resting membrane potential and cytoplasmic $[Ca^{2+}]$ of pulmonary arterial smooth muscle cells (PASMC), which is a key determinant of vasoconstriction and vascular cell proliferation [3,4]. Therefore, the dual involvement of ion channels in the control of arterial tone and remodelling makes them potential key mediators in the pathogenic cascade of PAH, which can be therapeutically targeted. Supporting this view is the notion that mutations in ion channel genes may lead to hereditary forms of PAH [4,5]. Ion channel dysregulation may also be secondary to alterations in the cellular environment that take place during PAH, and thus contribute to worsening of the condition [6,7]. We therefore suggest that dysregulation of ion channels could be a convergence point in PAH pathogenesis, independent of the pathological trigger.

Received: 23 June 2021
 Revised: 15 July 2021
 Accepted: 16 July 2021

Version of Record published:
 4 August 2021

Here we examine the pathophysiological roles of ion channels in PAH, and how their dysfunction, either directly caused by genetic alterations in ion channel genes ('intrinsic' dysregulation) or secondary to altered cellular microenvironment ('extrinsic' factors), can lead to PAH. Since a range of recent reviews has covered the pathophysiology of PAH channelopathies [4,5], we primarily focus this review on the extrinsic factors (e.g. hypoxia, endothelial dysfunction, drugs, HIV) that contribute to dysfunction of plasmalemmal channels in PASMCs during PAH.

Overview of channelopathies in PAH

A range of *de novo* and heritable mutations in PASMC ion channels have been implicated in familiar forms of PAH, these include mutations in the *KCNK3*, *ABCC8*, *KCNA5* and *TRPC6* genes. [4]

The TWIK-related acid-sensitive K^+ (TASK) channels belong to the superfamily of Two-Pore Domain K^+ (K2P) channels [8]. The TASK1 (*KCNK3*) channel is responsible for the background I_{KN} leak current that contributes to the resting membrane potential, vasomotor tone as well as proliferation of PASMCs [7,9]. So far, 12 loss-of-function missense mutations were identified in *KCNK3*, which was the first channelopathy discovered in PAH [5]. The lack of 'protective' heterodimerisation with TASK3 appears to underlie the lung-specific phenotype of *KCNK3* mutations [10]. Several *in vivo* studies confirmed that TASK1 loss-of-function precedes haemodynamic changes and is sufficient to cause PAH [7,9].

ATP-sensitive K^+ (K_{ATP}) channels promote hyperpolarisation and vasodilation in vascular smooth muscle cells [11]. Recently, 23 loss-of-function mutations in *ABCC8* (encoding the regulatory SUR1 subunit) were found in PAH patients [5,12]. These heterozygous mutations appear to impair channel trafficking and/or affect ATP sensitivity [13]. Several paradoxes surround this second PAH channelopathy, such as the lack of congenital hyperinsulinism, or the observation that patients with Cantú syndrome, with gain-of-function mutations in Kir6.1 and SUR2 subunits, also exhibit pulmonary hypertension [14]. The role of K_{ATP} in the pulmonary circulation therefore remains to be clarified.

In the pulmonary vasculature voltage-gated K^+ (K_v) channels also regulate the resting membrane potential, Ca^{2+} influx and vasoconstriction as well as cellular apoptosis, migration and proliferation [3,15]. Strong lines of evidence support a causal role for Kv1.5 dysfunction in PAH [16]. Sequencing of idiopathic PAH (IPAH) patients identified a number of mutations in the promoter and coding regions of *KCNA5* believed to affect its transcription, trafficking, and interaction with its β subunits [17,18]. A new variant has also been found in Pulmonary Hypertension of the Newborn (PPHN) [19]

Transient Receptor Potential Canonical (TRPC) channels are a subfamily of non-selective cation channels, which represent a major alternative route of Ca^{2+} entry outside voltage-gated Ca^{2+} channels, by functioning as both store-operated (SOC) and receptor-operated (ROC) channels [20]. Pharmacological blockade and knock-out experiments found a strong correlation between TRPC6 expression, capacitative Ca^{2+} entry, resting cytoplasmic $[Ca^{2+}]$, vascular tone and proliferation of rat PASMCs [21]. In PAH, increased TRPC6 expression promotes vasoconstriction and neomuscularisation of pulmonary arteries [22]. A genotypic analysis found that the 254(C \rightarrow G) single nucleotide polymorphism (SNP) was 2.85 times more common in IPAH patients [23]. It is of interest that another mutation in *TRPC6* (F443I) was recently found to associate with PPHN [19].

Extrinsic dysregulation of ion channels in PAH

Individuals with mutations in ion channels represent a minority of PAH patients, whereas dysregulation of ion channels is also observed in patients with other forms of PAH [6,7,24]. PAH can be induced by a wide variety of triggers, giving rise to different classifications (Table 1) [1]. In this section, the involvement of ion channels in the various forms of PAH is examined (Figure 1).

Germline mutations

BMPR2

Signalling events involving the transforming growth factor- β (TGF β) family, in particular bone morphogenetic proteins (BMP), are an important regulator of PASMC homeostasis. More than 806 loss-of-function variants in bone morphogenetic protein receptor type II (BMPR2) have been identified, which account for 75% and 25% of heritable PAH (HPAH) and IPAH cases, respectively [24,25].

Several lines of evidence link BMP signalling to regulation of K^+ channels in PASMC. Indeed, BMP2 up-regulates the expression of α subunits through Mothers Against Decapentaplegic Homologues (SMADs),

Table 1 Clinical classification of PAH

1. Pulmonary arterial hypertension (PAH)
1.1 Idiopathic PAH (IPAH)
1.2 Heritable/familial PAH (HFAH)
1.2.1 <i>BMPT2</i> mutations
1.2.2 Other mutations (e.g. <i>KCNK3</i> , <i>CAV1</i>)
1.3 Drug-induced PAH (DPAH) (e.g. fenfluramine)
1.4 PAH Associated with comorbidities (APAH)
1.4.1 Connective tissue disease (e.g. systemic sclerosis)
1.4.2 Human immunodeficiency virus (HIV) infection
1.4.3 Portal hypertension
1.4.4 Congenital heart disease
1.4.5 Schistosomiasis
1'. Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
1''. Persistent pulmonary hypertension of the newborn (PPHN)

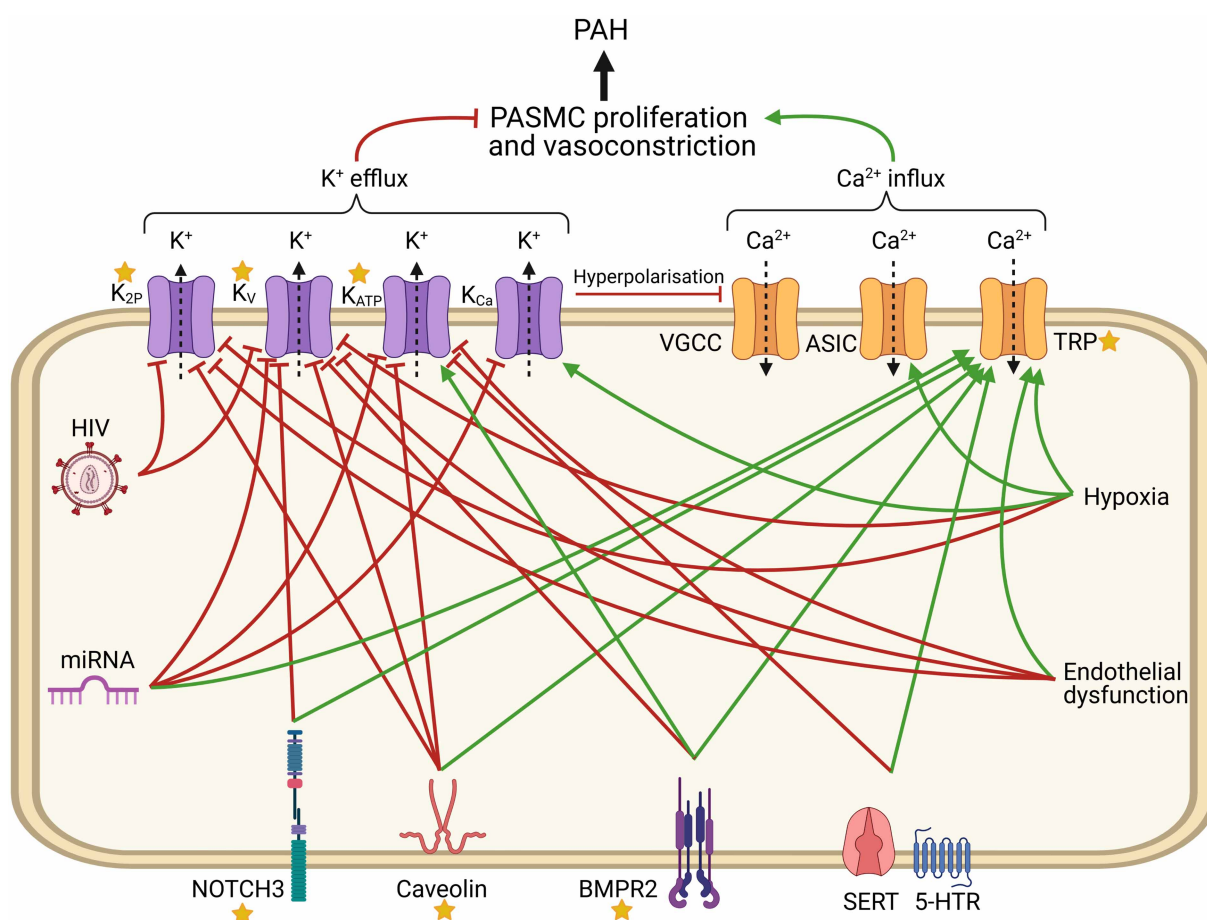


Figure 1. Dysregulation of PASM ion channels during PAH.

Red lines represent inhibitory influences vs activation for green arrows. Stars represent proteins with mutations associated with PAH.

while decreasing the expression of inhibitory β and γ subunits in human PASMC [26]. In a murine model of PAH, BMPR2 loss-of-function reduced Kv1.5 expression, and resulted in increased voltage-gated Ca^{2+} entry and vasoconstriction [27]. Additive effects between *BMPR2* and *KCNA5* mutations may underlie the earlier age of onset and higher disease severity in digenic patients, suggesting that mutational load and the presence of ‘second hits’ is important in PAH progression [28]. *BMPR2* deletion was also shown to down-regulate TASK1 and promote vasoconstriction in rat PASMC, although in this case modulation appears to be transcription-independent, but instead due to altered channel trafficking [25]. *ABCC8* mRNA expression was also up-regulated in lung biopsies obtained from *BMPR2* patients [29].

BMPR2 mutations also promote the formation of atypical heteromeric BMP receptors, shifting BMP signalling towards non-canonical MAPK-dependent pathways in murine PASMCs [30,31]. This shift is important, as BMP2 was shown to inhibit the expression of TRPC1/4/6 channels and proliferation of proximal rat PASMCs, whereas BMP4 up-regulated TRPC1/4/6 expression and distal PASMCs proliferation via non-canonical cascade PASMCs [32,33]. Overall, BMPR2 haploinsufficiency favours the formation of different BMP receptor complexes, suppressing the canonical, pro-apoptotic BMP2/SMAD signalling in proximal PASMCs, and potentiating the non-canonical, pro-proliferative BMP4/p38 axis in distal human PASMCs (Figure 2) [34,35].

Caveolin

The scaffolding protein caveolin is a structural component of caveolae, which are specialised dynamic lipid rafts important for microdomain functional coupling [36]. For instance, in the pulmonary vasculature, the caveolin ‘signalplex’ regulates arterial vasoconstriction by interacting with Ca^{2+} regulatory molecules [36]. Several mutations that decrease caveolin-1 (*CAV1*) expression were found in PAH, and *CAV1* knock-out in animal models leads to pulmonary hypertension [37,38]. Contrary to the loss of expression in pulmonary endothelium, *CAV1* expression is up-regulated in rat PASMCs and promotes proliferation [39]. Caveolin-1 directly influences the kinetics, assembly and regulation of several vascular channels via its scaffolding domain. For instance, binding of caveolins to Kir6.1, Kv1.5 and TASK1 reduced their functional activity in heterologous expression systems [40–42]. Caveolin-1 is also a positive regulator of TRPC assembly and SOCE in mice pulmonary vascular cells [43]. Increased caveolin expression in PASMCs of two rat models of PAH (chronic hypoxia and monocrotaline) was found to enhance agonist-induced SOCE/ROCE and vasoconstriction in pulmonary arteries [44].

NOTCH3

The transmembrane NOTCH3 receptor controls the differentiation of PASMCs from proliferative to contractile phenotype [45]. Three genetic variants in *NOTCH3* were linked to PAH [19,46]. Clinical studies found a causative role for the overexpression of NOTCH3 in the abnormal proliferation of PASMCs in PAH [47,48]. NOTCH3 enhanced Ca^{2+} entry in human PASMCs by (i) up-regulating TRPC6-dependent SOCE and (ii) inhibiting Kv1.2/Kv1.5 activity, which in turn promotes voltage-gated Ca^{2+} entry [45,49]. The resulting increased Ca^{2+} mobilisation leads to PAH by preferentially promoting vascular remodelling [47]. The fact that some of these effects were transcription-independent hints that non-canonical signalling of NOTCH3 may be involved in this pathological process [45,49]. Indeed, the two NOTCH3 missense mutations were shown to boost proliferative signalling of NOTCH3, despite actually decreasing its downstream transcriptional activity [46]. The NOTCH intracellular domain (NICD) has also been proposed to directly influence ion channel activity [45,49].

Epigenetics

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding and silencing the translation of target mRNAs. Several studies have shown a correlation between dysregulation of miRNAs, altered ionic currents and dysfunction of rat and human pulmonary artery *in vivo*. For example, the Kv1.2, Kv1.5, Kv7.5, BKCa β 1 and TASK1 channels are under direct negative control of miR-1, miR-23b-3p, miR-29b, miR-138, miR-190, miR-206 and miR-222, whose up-regulation in PAH (e.g. 4-fold for miR-1) decreased channel expression in human and rodent PASMCs, and promoted membrane depolarisation and arterial wall hypertrophy [50–52]. In addition, down-regulation of miR-135a-5p increased TRPC1 expression and promoted PASMC proliferation [53]. miRNAs are promising diagnostic markers of PAH, as well as potential therapeutic targets to correct ion channel dysfunction in PAH.

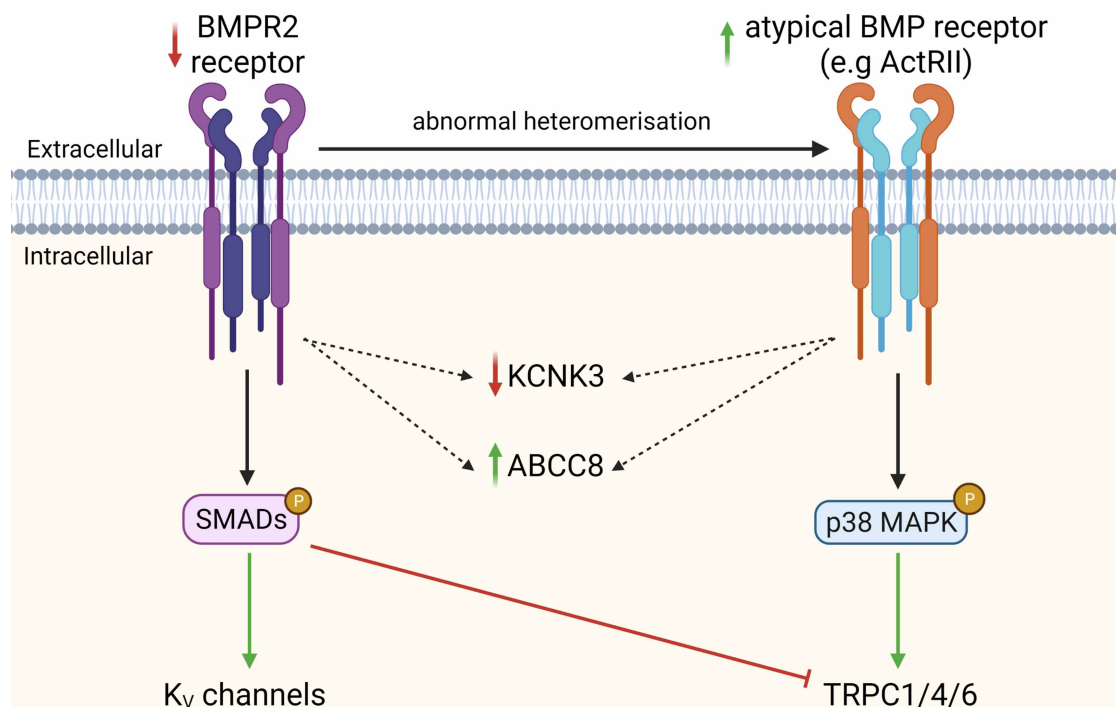


Figure 2. Consequences of shift from canonical BMPR2 to non-canonical BMP signalling on ion channel function in PSMCs.

Continuous arrows denote inhibition/down-regulation (red) or activation/up-regulation (green). Dotted arrows represent undefined links. 'P' denotes phosphorylated, activated mediator. MAPK, mitogen-activated protein kinase; ActRII, activin type 2 receptor.

Comorbidities and PAH

HIV

PAH is one of the most severe complications of HIV infection and a major cause of mortality in HIV-PAH patients, leading to a lower survival rate compared with other PAH patients [54]. HIV infection increases the incidence of PAH by 2500-fold in human subjects [54], and can trigger PAH in some laboratory animals (rats and macaques, but not mice) [55–57]. Two studies showed that HIV transgenes down-regulated the I_{KN} and I_{KV} currents (decreased Kv1.5, Kv7.1 and Kv7.4 expression) in rodent pulmonary arteries, possibly contributing to membrane depolarisation [55,57]. The Vpu protein of HIV-1 shares a high homology with the N-terminal of TASK1 channel, and it promotes abnormal oligomerisation of Vpu and TASK1 subunits, which leads to their degradation in heterologous expression systems [58]. The connection between HIV and PAH remains to be determined, one question being why only a small proportion (8.3%) of HIV patients have PAH.

Hypoxia

Hypoxia is a known trigger of pulmonary hypertension and ion channels are essential mediators of the hypoxic pulmonary vasoconstriction (HPV) [59]. The inhibition of Kv1.5 and Kv2.1 channels in the small resistance pulmonary arteries is a major contributor of HPV [15,60], but the underlying regulatory mechanisms remain controversial [61,62]. Although redox signalling is a key component of HPV, the extent of changes in the level of reactive oxygen species (ROS) during hypoxia are not fully defined, and both a decrease and an increase in ROS were shown to inhibit Kv channel opening [61]. The molecular mechanisms of oxygen sensing in PSMCs are also not fully defined. Mitochondria, which produce ROS in proportion to PO_2 levels, are likely involved in the PSMC response to hypoxia [62]. Cytoplasmic NADPH oxidases, which also produce ROS, reportedly lead to inhibition of Kv1.5 channel activity and modulation of channel trafficking via oxidation of C-terminal cysteine residues [63]. Also the TASK1 channel mediates an O_2 -sensitive current in carotid body

cells [64]. Conversely, hypoxia down-regulated both TASK1 expression (via the RELM β -STAT3-NFAT pathway) and activity (via the kinase Src), which promote human PASMCM proliferation and vasoconstriction [65,66].

Hypoxia led to activation of store-operated Ca²⁺ entry (SOCE) and elevation of cytoplasmic [Ca²⁺] in rodent PASMCMs [67]. Mice in which the *Trpc1* and *Trpc6* genes were deleted (knockouts) had impaired hypoxic response and loss of hypoxic vasoconstriction [22]. The rise in ROS up-regulates both the expression and assembly of SOCE channels to promote Ca²⁺ mobilisation in hypoxic PASMCMs [68,69]. There is evidence indicating that hypoxia directly modulates TRPC6 via the second messenger diacylglycerol and the enzyme AMP-activated protein kinase. Calcium Release-Activated Calcium (ORAI) channels are involved in the control of proliferation of rat PASMCMs, but their role in PAH requires further investigation [21].

The mechanisms of chronic hypoxia, more relevant to the context of PAH, may differ from those of HPV. Hypoxia-inducible factors (HIFs) are key transcription factors that contribute to remodelling of pulmonary artery during chronic hypoxia [70]. Selective down-regulation of the expression of Kv α subunits (Kv1.1, Kv1.5, Kv2.1, Kv4.3, Kv9.3) in PASMCM via HIF1 α occurs during chronic hypoxia; this signalling pathway is abnormally potentiated by ROS during PAH [71,72]. In contrast, hypoxia increased the expression of Ca²⁺-activated K⁺ (BKCa) channels in rat PASMCMs, which may explain the BKCa up-regulation seen in pulmonary arteries of PAH patients [73]. The underlying mechanism involves altered expression of the auxiliary β 1 subunit via HIF1 α , which enhances BKCa channel sensitivity to voltage and Ca²⁺, hence promoting PASMCM relaxation and thus constituting an endogenous protective mechanism [74].

Chronic hypoxia also selectively up-regulates a range of non-selective cation channels in PASMCMs including TRPC1 and TRPC6, via the HIF1 α and Notch signalling pathways, and the osmo-mechanosensitive cation channels, TRPV1/4 [75–77]. The family of acid-sensitive ion channels (ASIC) can also participate in SOCE in pulmonary vascular cells [67]. ASIC1-dependent Ca²⁺ entry during hypoxia is independent of changes in gene expression [67], but it depends on inactivation of the inhibitory effect of H₂O₂ on ASIC1 channel, as well as stimulation of forward trafficking via the protein RhoA [67,78]. The increase in cytoplasmic [Ca²⁺] in PASMCMs observed in hypoxia led to activation of transcription factor NFAT which down-regulates Kv1.5 expression and mitochondrial homeostasis, potentially hardwiring ionic dysfunction in a pathological feedback loop [79].

5-HT Signalling and anorectic drugs

Appetite-suppressants that potentiate serotonin signalling (e.g. fenfluramine, benfluorex) were taken off market after causing a drug-induced PAH epidemic in the 1960–70s [80]. The causal role of up-regulated 5-HT signalling in PASMCMs of PAH patients and animal models further reinforced this ‘serotonin hypothesis’ of PAH [81]. The serotonin transporter (SERT) is strongly implicated in the pathogenesis of PAH [82], and some studies have linked a gain-of-function polymorphism in SERT to an increased risk of PAH [83].

5-HT is both a potent vasoconstrictor and PASMCM mitogen, known to up-regulate TRPC1, TRPC6, TRPV4 currents in rat PASMCMs [84,85]. Anorectic drugs constrict pulmonary arteries by inhibiting Kv1.5 currents, although the underlying molecular mechanisms are not fully understood and may include direct K_V channel inhibition, activation of 5-HT₂ receptors or internalisation by SERT [86]. The pathways downstream of the 5-HT receptor and transporter seem to converge on second messengers like ROS [87] and kinases [86], which are known modulators of ion channel activity. For instance, 5-HT triggers the endocytosis of Kv1.5 channels in PASMCM through a 5-HT_{2A}R/tyrosine kinase/caveolin pathway [88].

Endothelial dysfunction

The endothelium produces several vasoactive mediators that play an important role in PASMCM homeostasis. A shift towards vasoconstrictive and mitogenic endothelial signals is one of the first events in PAH. It is still unclear whether endothelial dysfunction is a cause or consequence of PAH; however a monocrotaline-induced animal model of PAH presents pulmonary endothelium dysfunction, which is targeted by current PAH therapies (e.g. endothelin receptor antagonists, prostacyclin analogues) [89]. Endothelial dysfunction is accompanied by up-regulation of endothelin-1 and down-regulation of NO and PGI₂ signalling [89]. These factors modulate vascular ion channels through cyclic nucleotides and protein kinases [90]. Endothelin-1 promotes depolarisation in rodent and human PASMCMs and vasoconstriction by inhibiting I_{KV}, I_{KATP} and up-regulating I_{CaCl}, I_{BKCa} and TRPC currents through the PLC/PKC pathway [90]. Endothelin-1 also inhibits TASK1 in PASMCM, which could involve both gating inhibition in TASK1 as well as its phosphorylation-dependent internalisation [91,92]. The NO/cGMP/PKG pathway promotes vasorelaxation by activating I_{KV}, I_{KCa}, I_{KN}, I_{KATP} and

inhibiting I_{SOCE} [90,93]. Likewise, the PGI_2 /cAMP/PKA axis activates I_{Kv} , I_{KCa} , I_{KN} , I_{KATP} and inhibits TRPC channels [90,94].

Summary and implications for therapy

Ion channels are key determinants of the tone and the remodelling of the pulmonary artery. Over 50 genetic variants in four ion channels (*KCNK3*, *ABCC8*, *KCNA5* and *TRPC6*) have been found in PAH patients [4]. In this review, we show that ion channel dysfunction occurs in virtually in all forms of PAH (Figure 1).

A better characterisation of the regulation of ion channels in PAMSCs could provide new information about why so many different triggers (e.g. anorectic drugs, HIV, hypoxia) can all lead to PAH. Integrative ‘omic’ approaches hold hope for establishing a deep genotype-phenotype correlation of the disease. Identifying new pathways that regulate PAMSCs ion channels will be important to draw an accurate picture of their pathological relevance in the various forms of PAH (e.g. the recently proposed link between TASK1 and vitamin D deficiency) [95]. Also, the molecular mechanisms that dysregulate the Kv7 channel [96], the mechanosensitive Piezo1 channel [97] and the Ca^{2+} -activated Cl^- channel TMEM16A [98] in PAH remain poorly understood.

Multiple studies showed that activators of K^+ channels [7,99] and inhibitors of both Cl^- channels [98] and TRPC channels [100] can prevent and/or reverse PAH in animal models. Optimising the selectivity, potency and lung-specific delivery of these compounds will therefore be paramount to the development of new PAH therapies. Recent advances in the determination of structures for these channel types may also expedite drug discovery efforts towards this goal.

Perspectives

- **Highlight the importance of the field:** Pulmonary arterial hypertension is a multifactorial and severe disease with no curative treatments. Vasoconstriction and arterial remodelling both contribute to elevated resistance to blood flow and increased blood pressure.
- **A summary of the current thinking:** Ion channels are involved in the control of both arterial tone and proliferation of pulmonary artery smooth muscle cells. Dysfunction of ion channels plays a causative role in PAH, and may link the different pathological triggers of PAH to the onset of the disease.
- **A comment on future directions:** Ion channels are currently under-explored targets for PAH. Understanding the regulation and pathophysiological role of ion channels in PAH will provide new avenues for therapeutic intervention.

Acknowledgements

This work was supported by funding from the British Heart Foundation (PG/19/8/34168), the Biotechnology and Biological Sciences Research Council (BB/T007664/1 and BB/T002018/1) and the Wellcome Trust. T.J.-T. is a student on the OXION PhD Programme in Ion Channels and Disease. Figures were created with Biorender.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Open Access Statement

Open access for this article was enabled by the participation of University of Oxford in an all-inclusive Read & Publish pilot with Portland Press and the Biochemical Society under a transformative agreement with JISC.

Author contributions

All authors were involved in writing and editing the manuscript. T.J.-T. produced the figures.

Abbreviations

BMP, bone morphogenetic proteins; BMPR2, bone morphogenetic protein receptor type II; CAV1, caveolin-1; HIF, hypoxia-inducible factor; HPAH, heritable pulmonary arterial hypertension; HPV, hypoxic pulmonary vasoconstriction; IPAH, idiopathic pulmonary arterial hypertension; K_{ATP} , ATP-sensitive K^+ ; PAH, Pulmonary arterial hypertension; PASMC, pulmonary arterial smooth muscle cells; PPHN, Pulmonary Hypertension of the Newborn; ROS, reactive oxygen species; SERT, serotonin transporter; SOCE, store-operated Ca^{2+} entry; TASK1, TWIK-Related Acid Sensitive K^+ Channel 1; TRPC, Transient Receptor Potential Canonical.

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