Hypoxic pulmonary hypertension in chronic lung diseases: novel vasoconstrictor pathways

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Summary

Pulmonary hypertension is a well-recognised complication of chronic hypoxic lung diseases, amongst the commonest causes of death and disability worldwide; its development independently predicts reduced life expectancy. In COPD long-term oxygen therapy ameliorates pulmonary hypertension and significantly improves survival, although the correction of alveolar hypoxia and pulmonary hypertension is only partial. Recent advances in understanding of the regulation of vascular smooth muscle tone reveal that chronic vasoconstriction plays a more important role in the pathogenesis of hypoxic pulmonary hypertension than previously thought and that structural vascular changes contribute less. Trials of existing vasodilator agents show that pulmonary hypertension can be ameliorated and systemic oxygen delivery improved in carefully selected patients, although systemic hypotensive effects limit the doses used. We review recently discovered vasoconstrictor pathways that are selective for the pulmonary circulation. These pathways can be blocked to reduce hypoxic pulmonary hypertension, without causing systemic hypotension, and thus provide potential targets for novel therapeutic strategies.

Introduction

Chronic hypoxic lung diseases are among the commonest causes of death and disability worldwide. These diseases, which include chronic obstructive pulmonary disease (COPD), bronchiectasis, idiopathic pulmonary fibrosis, and restrictive chest wall abnormalities, are frequently complicated by the development of pulmonary hypertension i.e. World Health Organisation (WHO) Group 3 of the standard classification of pulmonary hypertension[1]. In COPD elevated pulmonary arterial pressure is an independent predictor of an increased risk of future exacerbations and reduced life expectancy[1,2]. In some COPD patients severe pulmonary hypertension occurs, which is out of proportion to the severity of the underlying lung disease, and is associated with a particularly poor prognosis[3]. Pulmonary hypertension is also associated with greater morbidity and mortality in cystic fibrosis and idiopathic pulmonary fibrosis[1,4].

The increased pulmonary vascular resistance and the resultant pulmonary hypertension in chronic hypoxic lung diseases arise as a result of two major pathogenic mechanisms. The first is reduction in the pulmonary vascular bed due to inflammation, destruction of the pulmonary parenchyma and obliteration of the vessels caused by the underlying disease process[3,5,6]. The second major mechanism causing increased pulmonary vascular resistance in these conditions is hypoxic pulmonary hypertension[3,6].

This review focuses on the latter mechanism i.e. hypoxic pulmonary hypertension in chronic lung diseases, and on the advances in our understanding of the underlying mechanisms made since the beginning of this millennium. These include recently discovered pathways controlling vascular smooth muscle contraction, cytoskeletal function and vascular compliance, which are major contributory mechanisms causing increased pulmonary vascular resistance and right ventricular overload. These mechanisms are regulated by signalling pathways that are selective for the pulmonary circulation and can be antagonised without causing systemic vasodilatation. We review the results of recently concluded clinical studies that examined the effects of vasodilators in pulmonary hypertension caused by chronic hypoxic lung diseases and highlight more recently discovered therapeutic targets.

Hypoxic pulmonary hypertension in chronic lung diseases

The importance of chronic alveolar hypoxia as a stimulus to the development of pulmonary hypertension is clearly demonstrated by the observation that healthy, native sea-level dwellers who move to high altitude develop increased pulmonary vascular resistance and elevated pulmonary arterial pressure in the absence of any pre-existing lung disease[7]. In most normal individuals, this elevation of pulmonary arterial pressure stabilises and is well tolerated over extended periods[7]. However, there is wide variability in the pulmonary response to hypoxia and in a minority of previously healthy people it causes the development of sub-acute mountain sickness, an inexorably progressive increase in pulmonary vascular resistance leading to right heart failure and ultimately death if the hypoxia is not alleviated[7]. Furthermore, in some high altitude adapted dwellers, severe, progressive pulmonary hypertension develops later in life and causes right ventricular failure, a condition known as chronic mountain sickness, which can be relieved by moving to low altitude[7]. Thus, in some individuals alveolar hypoxia in an otherwise healthy lung causes life threatening pulmonary hypertension.

The role of hypoxia in the pathogenesis of pulmonary hypertension associated with intrinsic lung disease has been definitively demonstrated in COPD, in which long term oxygen therapy reduces pulmonary hypertension and increases survival[1,3]. Furthermore, the potential for pulmonary hypertension *per se* to reduce life expectancy is well illustrated in patients with idiopathic pulmonary arterial hypertension. In this condition severe pulmonary hypertension develops in the absence of alveolar hypoxia or other known causes and, if untreated, typically leads to death in less than five years. In severely affected patients, atrial septostomy, which, without changing the underlying pulmonary vascular disease, leads to rapid relief of symptoms, improves right ventricular function and cardiac output, and prolongs survival, despite causing a significant reduction of arterial oxygen saturation[8]. These findings show that pulmonary hypertension reduces survival independently of any accompanying hypoxaemia.

It has been previously thought that the increased pulmonary vascular resistance of hypoxic pulmonary hypertension is predominantly caused by structural changes in

the pulmonary circulation. A key finding supporting this view is that, when native sea level dwellers moved to high altitude and developed stable chronic hypoxic pulmonary hypertension, acute normalization of alveolar O_2 did not produce an immediate, substantial reduction in resistance. Small changes in pulmonary arterial pressure were sometimes observed in response to relief of hypoxia, but these resulted from reductions in cardiac output; pulmonary vascular resistance remained unchanged[9,10]. Furthermore, conventional vasodilators such as calcium channel blockers or prostacyclin, did not substantially reduce pulmonary vascular resistance in chronically hypoxic lungs[11,12].

The development of hypoxic pulmonary hypertension is accompanied by thickening of all the layers of the walls of the pulmonary vessels, which can increase pulmonary vascular resistance by encroachment into, and narrowing of, the lumen[11]. A second major structural change previously thought to contribute to the elevated pulmonary vascular resistance was “rarefaction” i.e. a loss of pulmonary vessels[11]. Taken together with the absence of a significant acute vasodilator effect of supplemental oxygen, these findings supported the view that structural changes in the vasculature were the predominant underlying cause of hypoxic pulmonary hypertension.

Novel mechanisms in hypoxic pulmonary hypertension.

Although, long-term oxygen therapy in COPD, is effective in reducing pulmonary hypertension and prolonging survival, at best it produces only partial correction of alveolar hypoxia with small reductions in pulmonary arterial pressure[1,3]. Moreover, many patients consider oxygen therapy burdensome and compliance is frequently poor. Thus improved therapeutic approaches are needed and this has driven continued research into improving our understanding of the underlying mechanisms of hypoxic pulmonary hypertension. Our previously held views of these mechanisms must now be revised given more recent discoveries about the control of pulmonary vascular smooth muscle contraction and cytoskeletal regulation, in particular the key role of the small g-protein RhoA and its downstream effectors Rho-associated, coiled-coil containing protein kinases 1 and 2 (ROCK1 and ROCK2)[13].

Role of Rho kinase in vascular smooth muscle contraction

Contraction of vascular smooth muscle cells narrows the vessel lumen and increases resistance to blood flow. Two processes act in concert to bring about this contraction (i) the interaction of actin and myosin filaments in the classic "sliding filament" mechanism and (ii) the transmission of the tension developed by this actin-myosin interaction through the cytoskeleton and cellular adhesion complexes to adjacent cells and structural elements in the extracellular matrix[14].

Vascular smooth muscle cells connect to the extracellular matrix or to one another at sites of focal adhesions, so called dense plaques; there are also dense plaques throughout the cytoplasm[14]. The dense plaques are the sites of attachment of contractile actin at each end of an actin-myosin unit (Figure 1). Talins provide the important mechanical linkage between ligand-bound integrins in the cell membrane, dense plaques and the actin cytoskeleton and are required for the transmission of force from the cytoskeleton, through focal adhesion complexes, to adjacent cells and the extra-cellular matrix. Non-contractile actin forms the sub-sarcolemmal cytoskeletal network, which has an important structural role[14].

In smooth muscle cells actin-myosin interaction leading to contraction is activated by phosphorylation of the light chain of myosin (MLC) by MLC kinase (MLCK), which is activated by increased cytosolic Ca^{++} (Figure 2). Thus, one important method of regulating contraction is by regulation of cytosolic Ca^{++}. Relaxation of smooth muscle is produced by de-phosphorylation of MLCK though the action of myosin light chain phosphatase (MLCP). Regulation of MLCP activity is the second important mechanism contributing to the control of smooth muscle contraction. Vascular smooth muscle tension is determined at any time by the balance of MLCK and MLCP activities (Figure 2).

Activated ROCK1 and ROCK2 phosphorylate CPI-17, a smooth muscle phosphoprotein, and the MYPT1 subunit of MLCP, both of which actions inhibit phosphatase activity[13,15]. The result of this inhibition is enhanced MLC phosphorylation and activation at any given level of cytosolic Ca^{++}, so called "calcium sensitization" (Figure 2)[13]. ROCK1 and ROCK2 can also directly phosphorylate MLC and activate actin-myosin cross bridge formation[16].

An important mechanism of interaction between the RhoA ROCK signalling cascade and Ca^{++} induced contraction has more recently been identified by the demonstration that the sustained contraction of vascular smooth muscle produced by membrane depolarisation requires ROCK activation. This ROCK activation depends on inositol-3-phosphate induced calcium release from the sarcoplasmic reticulum, although the detailed steps in this pathway remain to be elucidated (Figure 2)[17].

In addition to actin-myosin cross bridge activation, cytoskeletal rearrangement is required for smooth muscle contraction[14]. The length of actin filaments is dynamically regulated in smooth muscle cells and polymerization of g-actin to form actin filaments is required for vascular smooth muscle contraction[14,18]. Myosin filaments can also assemble rapidly and thus enhance force development in smooth muscle[19].

Another mechanism by which cytoskeletal rearrangement increases tension development is increased stiffness of the cytoskeletal network. This stiffening potentiates the shortening action of myosin-actin interactions as the resultant increase in tension is more effectively transmitted to cell membrane adhesion complexes and thus to the extracellular matrix and adjacent cells[14]. Vascular smooth muscle cytoskeletal stiffness also contributes to altered vascular function by reducing the compliance of the blood vessel wall. This has been recently demonstrated in vascular smooth muscle cells isolated from the systemic circulation of spontaneously hypertensive rats in which cytoskeletal stiffness (measured by atomic force microscopy) is greater than that of vascular smooth muscle cells from normotensive controls. When these cells were cultured in collagen gel within a ring shaped mould, the stiffness of the reconstituted “vascular rings” was greater when cells isolated from spontaneously hypertensive rats were used than when cells from normotensive controls were used[20].

Stiffening of the cytoskeletal network is dynamically controlled and is triggered concurrently with the activation of the actin-myosin system (Figure 1). It involves both anchoring of newly generated actin filaments to cytoskeletal elements and cross-linking of cytoskeletal and/or contractile filaments enabling them to better transmit the contractile force[14,21]. Activation of the RhoA/ROCK pathway controls filamentous actin assembly by phosphorylating and thus activating the LIM kinases,

1 and 2. These kinases in turn phosphorylate and inactivate actin depolymerising factors, such as cofilin, resulting in net increases in cellular filamentous actin[22]. ROCK can also directly phosphorylate and inactivate cofillin[23]. These activities of the RhoA/ROCK pathway increase stiffness of the cytoskeleton and thus tension development through signaling pathways that are independent of changes in cytosolic calcium[21].

In summary, these studies have identified the key role of ROCK in vascular smooth muscle contraction. ROCK activity is required for the two essential processes underpinning contraction, actin-myosin interaction and the regulation of cytoskeletal function. Furthermore, it is the point of convergence of intracellular signalling pathways for membrane depolarisation induced contractions (ionotropic signalling) and receptor mediated contraction (metabotropic signalling). Because of this central role, inhibitors of ROCK are especially potent vasodilators.

Role of ROCK in the hypoxic pulmonary circulation

In hypoxic pulmonary hypertension ROCK inhibitors abruptly (within minutes) reduce pulmonary vascular resistance to nearly normal values in chronically hypoxic rats with established pulmonary hypertension[10,12,24,25]. Similar experiments in mice showed that ROCK inhibition reduced by approximately 50% the increase in resistance caused by prolonged hypoxic exposure[24-27]. Moreover, chelation of all Ca^{++} following maximal ROCK inhibition did not cause any further reduction in resistance[27]. These profound vasodilator effects were seen even though alveolar PO_2 had been restored to normal prior to inhibitor administration, and were much greater than those seen with previously used vasodilator agents, including inhibitors of voltage dependent calcium channel entry, IP_3 production, PKC and MLCK activity[12]. Recently, it has been shown that intravenous infusion of the rho kinase inhibitor fasudil acutely reduced the mean pulmonary artery pressure in bulls that were long term residents at high altitude (3300m) by over 50%, to values close to those in yaks, an adapted species that has a normal low pressure pulmonary circulation at high altitude. However, the measurements were made under hypoxic conditions, and cardiac output and pulmonary vascular resistance were not reported[28]. Overall, these reports demonstrated that acutely reversible

vasoconstriction played a very much greater role in maintaining established hypoxic pulmonary hypertension than had previously been appreciated. It is important to remember that these models exclude any contribution of the vascular changes caused by lung disease to pulmonary hypertension and focussed solely on the effects of hypoxia.

A further mechanism by which rho kinase induced increases in vessel wall stiffness could contribute to increased pulmonary vascular resistance is indirectly though an action in the endothelium[29]. Rho kinase action in vascular endothelial cells reduces nitric oxide synthase (NOS) activity, with a resultant increase in vascular smooth muscle tone, vascular resistance and stiffness. Inhibition of rho kinase activity, by restoring endothelial NOS activity, could potentially attenuate hypoxic pulmonary hypertension[29].

In addition to the effect of ROCK inhibition on pulmonary vascular smooth muscle contraction, it may also act to alleviate established hypoxic pulmonary hypertension by altering vascular compliance. In the normal pulmonary circulation, the vessels are thin walled and compliant when compared to the systemic circulation. This property contributes importantly to the normal low vascular resistance of this circulation as any increase in perfusion pressure markedly increases the lumen radius of the vessels thus reducing resistance. Additionally, the compliant nature of the larger conduit vessels serves an important Windekessel function. Disruption of the smooth muscle cytoskeleton by cytochalasin D increases the compliance of these conduit vessels from normal lungs demonstrating an important contribution of the cytoskeleton to wall stiffness[30].

In hypoxic pulmonary hypertension, increased stiffness of the walls of smaller intra-pulmonary arterioles hypertension contributes to a reduction in lumen radius at any given intravascular pressure thus increasing resistance to blood flow[25,31]. Chronic hypoxia also reduces compliance of isolated conduit pulmonary arteries[32,33] and conduit vessel compliance measured by imaging studies in intact whole lungs[25,34]. Stiffening of the walls of the larger conduit vessels increases pulmonary vascular impedance, which may contribute significantly to right ventricular over-loading[35].

Increased vessel wall stiffness in hypoxic pulmonary hypertension is due in part to changes in the extracellular matrix[36]. The more recent discovery that changes in vascular smooth muscle cytoskeletal stiffness also contribute importantly to the compliance of pulmonary conduit vessels, and that this is altered by ROCK actions on the cytoskeleton[14,21,30], suggests that rho kinase inhibitors might be used to reduce conduit vessel compliance. Jin et al found that stimulating nitric oxide production in the isolated chronically hypoxic lung restored the reduced arterial compliance towards normal and suggested that this might improve right ventricular function[37]. Vanderpool and colleagues investigated the effect of acute ROCK inhibition in hypoxic pulmonary hypertension *in vivo* and found an immediate reduction in vascular impedance[25]. However, their results suggested that this reduction was due to decreased resistance in the small vessels without any effect on conduit arteries. These reports suggest that an acutely reversible, ROCK dependent, increase in pulmonary vascular smooth muscle stiffness contributes to the altered haemodynamics of hypoxic pulmonary hypertension, although its potential role in regulating conduit vessel compliance remains to be fully elucidated.

Together these more recent studies provide evidence of a previously unrecognised, ROCK dependent, increase in pulmonary vascular resistance induced by chronic hypoxia. For convenience we term this chronic hypoxic pulmonary vasoconstriction (chronic HPV) to distinguish it from the well-known acute hypoxic pulmonary vasoconstriction (acute HPV). Chronic HPV differs importantly from acute HPV in that it is not immediately reversed by reoxygenation or conventional vasodilators, including L-type voltage gated calcium channel blockers (e.g. nifedipine), but can be abruptly reversed by inhibition of ROCK. In contrast, acute HPV is quickly reversed on restoration of normal alveolar P_aO_2, is very sensitive to blockade of L-type voltage sensitive calcium channels and does not contribute to chronic hypoxic pulmonary hypertension.

Changes in vascular structure: recent insights.

How can we reconcile recent results showing that acutely reversible, RhoA/ROCK mediated mechanisms contribute significantly to hypoxic pulmonary hypertension with the previous paradigm suggesting that the underlying mechanism was

structural? Many of the previous reports of remodelling and rarefaction in the hypertensive lung have been based on histological data collected in a conventional manner. However, images collected in such a way cannot provide reliable, quantitative estimates of the structural features within three dimensional organs, such as the lumen diameter of vessels and the total length of blood vessels (reviewed by Hsia et al[38]). Such three dimensional structures quantification within organs from two dimensional sections requires the use of design based stereological procedures[38]. A further difficulty is that measurement of lumen diameter in fixed specimens reflects not only structural features such as wall thickness but also the extent of vasoconstriction at the time of sampling. Finally, due to the normally compliant nature of the pulmonary vessels, the lumen diameter of the pulmonary vessels is markedly influenced by the transmural pressure at the time of fixation[38].

When standard intravascular transmural pressures were maintained in rodent studies and measures taken to ensure vascular smooth muscle relaxation during fixation (use of calcium free perfusate or vasodilators), it has been found that the lumen diameter of pulmonary vessels was not reduced, or that the reduction in lumen diameter was not sufficient to account for the increase in resistance[10,26,27], even though the walls of the pulmonary vessels had thickened[39,40]. This suggested that the wall thickening largely occurred in an outward direction, so-called compensatory hypertrophy[39]. To date no similarly conducted study of the effects of changes in vascular structure has been reported in larger animals. Further evidence that wall thickening is not directly related to the increased pulmonary vascular resistance was provided by experimental interventions that inhibited pulmonary vascular wall thickening in the chronically hypoxic mouse but did not prevent the increase in pulmonary arterial pressure e.g. heterozygous hypomorphic BMPR2 mutations[41]. Taken together, these reports demonstrate that there is no direct or inevitable link between thickening of the vessel walls and the hypoxia induced increase in vascular resistance.

Use of design based stereological techniques, has also shown that, in the absence of lung disease, chronic hypoxia does not cause loss of vessels from the pulmonary circulation; in contrast capillary angiogenesis is a consistent finding in the hypoxic hypertensive lung[10,27,39,42]. Cell lineage tracking techniques suggest that this

angiogensis results from resident pulmonary endothelial progenitor cell proliferation and not through recruitment from the bone marrow[43].

Taken together, these studies compel a paradigm shift in our understanding of the pathogenesis of chronic hypoxic pulmonary hypertension. Fixed structural changes in the pulmonary vasculature are a less important mechanism than previously thought, based on earlier vasodilator studies undertaken prior to the discovery of the central role of ROCK dependent pathways. Reversible vasoconstriction is a major contributor to the elevated pulmonary vascular resistance and offers important new potential targets for therapeutic intervention.

Current and future vasodilator targets.

The use of vasodilator agents to treat pulmonary hypertension in chronic hypoxic lung disease has been investigated in a number of trials. Two major concerns arise when considering the use of vasodilator agents in this context; first that such agents may reduce systemic vascular resistance and produce significant systemic hypotension, particularly in a group of patients with a limited capacity to increase left atrial filling and cardiac output due to pulmonary hypertension. The second potential problem is increased ventilation perfusion mismatch resulting in arterial hypoxaemia and reduced oxygen delivery to the systemic organs. It is well established that when lung disease is regionally localised (e.g. obstruction of a major bronchus or lobar pneumonia) hypoxic pulmonary vasoconstriction improves arterial oxygenation. However, when alveolar hypoxia occurs widely throughout the normal lung its effects on ventilation-perfusion matching may not be beneficial. Detailed assessments of pulmonary gas exchange and haemodynamics during five weeks of acclimatization of healthy sea level dwellers to hypoxia mimicing that at high altitude showed increased ventilation-perfusion mismatching[44]. In chronically hypoxic rats with established “fixed” hypoxic pulmonary hypertension, acute intravenous administration of a rho kinase inhibitor reduced pulmonary vascular resistance without reducing P_aO_2 or changing the alveolar-arterial PO_2 gap[10]. Similarly administration of sildenafil acutely to human subjects after three weeks at high altitude did not reduce arterial oxygen saturation at rest or during exercise, although

it reduced pulmonary arterial pressure, increased cardiac output and improved exercise performance[45]. Increases in P_aO_2, cardiac output and exercise performance were also observed with more sustained administration of sildenafil (six days) to native sea level dwellers sojourning at high altitude[46].

These findings all suggest that vasoconstriction does not improve gas exchange in the diffusely hypoxic lung in the absence of lung disease, but reduces cardiac output. This is particularly notable given recent evidence suggesting that when more severe pulmonary hypertension complicates COPD, it becomes a major factor limiting exercise[47].

In view of these findings, the question arises of whether there is a significant, hypoxia induced, vasoconstrictor component to the increased pulmonary vascular resistance and pulmonary hypertension of chronic lung diseases. A number of studies have reported the effect of a single dose or short term (maximum 16 weeks) vasodilator therapy on pulmonary haemodynamics and arterial oxygenation in patients with pulmonary hypertension (mean PAP>25mmHg) caused by such diseases. The agents investigated to date fall into three groups: (i) those that potentiate signalling via the nitric oxide-guanyl cyclase-cyclicGMP-protein kinase G cascade, (ii) prostacyclin and its analogue iloprost, and (iii) endothelin receptor blockers. Tables 1 and 2 summarise the outcomes of clinical studies of these agents in patients with chronic hypoxic lung diseases and pulmonary hypertension confirmed by pulmonary artery catheter studies (See text box for systematic literature review strategy). The chronic lung diseases studied fall broadly into two groups, chronic obstructive pulmonary disease and interstitial lung diseases (ATS/ERS Guidelines, 2002) including idiopathic pulmonary fibrosis[48].

Increased NO mediated signalling. Three different approaches have been taken to enhancing NO mediated signalling (Table 2): administration of inhaled nitric oxide[49-51], inhibition of phosphodiesterase–5 by sildenafil thus prolonging the half-life of cyclic GMP, the downstream second messenger of NO[50,52], and using riociguat to directly activate guanyl cyclase, the normal target of NO[53]. These trials, whether in patients with COPD or fibrotic interstitial lung diseases, have all reported a consistent finding of significant reduction in pulmonary vascular resistance. All reported an increase in oxygen delivery to the systemic organs, either without

supplemental inhaled oxygen or while background inhaled oxygen therapy remained unchanged (Tables 1 and 2). A single study reported a slight reduction in P_aO_2 during riociguat therapy but systemic oxygen delivery was increased due to the accompanying increase in cardiac output[53].

Increased prostacyclin receptor mediated signalling. The actions of prostacyclin on pulmonary haemodynamics in COPD patients with pulmonary hypertension have not been reported (Table 2). However, the inhaled prostacyclin analogue iloprost did not alter P_aO_2 while inhaled O_2 was maintained constant[54,55].

In patients with ILD, prostacyclin, whether inhaled or intravenous, acutely reduced pulmonary arterial pressure and pulmonary vascular resistance, and increased cardiac output (Table 2). In one trial that studied the effects of both inhaled and intravenous prostacyclin, S_aO_2 was unchanged, while a second trial in patients with IPF found a reduction in P_aO_2[50,56]. Importantly, systemic oxygen delivery was increased in all of these studies due to the accompanying increase in cardiac output, even though inhaled oxygen was unchanged (Table 2). No study of the effects of sustained treatment with prostacyclin or its analogues on pulmonary haemodynamics and gas exchange has been reported.

Endothelin receptor antagonists. Only one clinical trial has examined the effects of blocking the endothelin pathway in patients with chronic hypoxic lung disease (ILD) and pulmonary hypertension[57]. Following treatment with the non-selective, endothelin receptor antagonist bosentan for 16 weeks, pulmonary arterial pressure, pulmonary vascular resistance, cardiac output and arterial oxygenation were unchanged[57].

Taken together these trials (Table 1 and 2) give a number of important insights into the mechanisms underlying pulmonary hypertension complicating chronic hypoxic lung diseases. First, there is a significant vasoconstrictor component to the increased pulmonary vascular resistance, which is acutely reversible i.e. not due to fixed structural changes or loss of the vascular bed. It is probable that the vasoconstrictor component is not fully revealed in those studies as the doses used were limited by systemic hypotension. Furthermore, successful vasodilator treatment increased oxygen delivery to systemic tissues, in most trials without any reduction in P_aO_2 i.e. without an adverse effect on ventilation-perfusion matching. It

is important to note that these conclusions cannot be extended to patients with chronic lung diseases who do not have pulmonary hypertension.

Effect of vasodilator therapy on clinical outcomes

In a small number of trials, the effects of sustained oral vasodilator therapy (sildenafil or riociguat) on exercise tolerance have been examined. Hoeper and colleagues, who demonstrated effective reduction in pulmonary vascular resistance in patients with IPF treated with riociguat for 12 weeks, also found that six minute walk test distance was significantly increased[53]. In support of this finding, an earlier study of sildenafil given for three months to patients with IPF and PH, also showed improved exercise tolerance, although neither the effect on pulmonary haemodynamics nor on arterial oxygenation was reported[58].

Although extended trials of the effect of vasodilator therapy on survival in IPF have been completed, none of those specifically examined the effect on patients with concomitant pulmonary hypertension. In those trials, in which many patients did not have pulmonary hypertension, vasodilator therapy was of no benefit and vasodilators are not approved therapy. Importantly, one endothelin receptor antagonist, ambrisentan, adversely affected disease progression and should not be used in this condition[1,59].

Only one study has been completed in patients with COPD and pulmonary hypertension, which found that there was no improvement in exercise endurance time at constant workload, following 12 weeks of sildenafil treatment[52]. It is important to note that vasodilator therapy is not currently approved for use in the treatment of pulmonary hypertension in COPD. A number of prospective, multi-centre, randomized, clinical trials of oral vasodilator therapy are currently under way, designed to test the effect on exercise capacity (six minute walk test distance) and pulmonary vascular resistance or time to clinical worsening (See panel).

Regarding a potential beneficial effect of extended vasodilator therapy on survival, no trial of vasodilator treatment of patients with pulmonary hypertension in chronic hypoxic lung diseases has been longer than 16 weeks duration and thus none has been of adequate duration to address this issue.

Novel vasodilator targets in hypoxic pulmonary hypertension.

The doses of the currently available vasodilator therapies that can be used in the treatment of pulmonary hypertension are limited by their systemic hypotensive action, which significantly limits their therapeutic effects. Table 3 summarises recent publications that identify vasoconstrictor pathways in the chronically hypoxic lung *in vivo* that (i) are activated by hypoxia in the pulmonary circulation and (ii) in which targeted genetic manipulation of the pathway ameliorated hypoxic pulmonary hypertension without causing systemic hypotension.

The first group of lung selective vasoconstrictor pathways are those that permit calcium entry from the extracellular fluid (ionotropic pathways), which may be further subdivided into three subgroups, voltage dependent calcium channels, store operated and stretch activated calcium channels. Deletion of a specific voltage dependent calcium channel, Cav3.1, a member of the T-type voltage dependent calcium channel family, protects against the development of hypoxic pulmonary hypertension in mice[60] without affecting systemic blood pressure[61]. Furthermore, T-type voltage gated calcium channel blockers such as TTA-A2 and DHEA attenuate the development of hypoxic pulmonary hypertension in rats and mice, although these are not selective for the Cav3.1 subtype[60].

Store operated calcium channels (SOCC) are a group of plasma membrane channels that are opened by the depletion of sarcoplasmic reticulum stores of calcium during smooth muscle contraction. These include two members of the transient receptor potential channel family, TRPC1 and TRPC6, which are significantly elevated in pulmonary artery vascular smooth muscle cells *in vivo* following chronic hypoxic exposure[62,63]. Single gene deletion of either channel partially protects against the development of hypoxic pulmonary hypertension in mice without causing systemic hypotension, while double $TRPC6^{-/-}TRPC1^{-/-}$ mice exhibit a marked attenuation of hypoxic pulmonary hypertension[62,64]. Interestingly sodium tansinone IIA sulfonate (STS) inhibits the hypoxia-induced increase in TRPC1 and TRPC6 expression and attenuates hypoxic pulmonary hypertension[65]. A second therapeutic approach to inhibiting SOCC selectively in the lung has been identified. The enhanced calcium entry through the TRPC6 channel is mediated by

hypoxic upregulation of the integrin Notch3 and its ligands in the hypoxic hypertensive lung[66]. Mice in which the NOTCH3 gene has been deleted are protected against the development of hypoxic pulmonary hypertension and blockade of Notch signalling by the gamma secretase inhibitor DAPT reversed established hypoxic pulmonary hypertension *in vivo*[66,67].

Another mechanism causing increased cytosolic calcium in hypoxic pulmonary hypertension is increased calcium entry through stretch operated channels. TRPV4 is a stretch operated member of the TRPC family that is upregulated by hypoxia[68]. Specific TRPV4 knockout suppresses the development of hypoxic pulmonary hypertension in mice without affecting systemic blood pressure[69,70].

As discussed above the RhoA ROCK pathway plays an essential role in the development of hypoxic pulmonary hypertension. Normally it plays a greater role in the control of pulmonary vascular smooth muscle contraction than it does in the systemic circulation[71]. Recently, Kojonazarov and colleagues showed that acute intravenous administration of the non-selective ROCK inhibitor fasudil to native high altitude residents (3200-3600m) suffering from chronic mountain sickness immediately reduced pulmonary vascular resistance without changing systemic arterial oxygen saturation, and importantly did not reduce systemic arterial blood pressure[72]. This demonstrated that the chronic hypoxic vasoconstriction of sustained global alveolar hypoxia does not improve ventilation-perfusion matching or systemic oxygenation, and that it may be antagonised without adverse effects on gas exchange.

Of the two isoforms of ROCK, ROCK2 is predominantly responsible for smooth muscle contraction. Haploinsufficiency of ROCK2 in vascular smooth muscle in genetically manipulated mice attenuated the development of hypoxic pulmonary hypertension without altering systemic arterial blood pressure [73], providing further evidence for this pathway as a therapeutic target in hypoxic pulmonary hypertension[72].

In the normal pulmonary circulation tonic activation of heterodimeric bone morphogenetic protein receptors (BMPR1 and BMPR2) supresses ROCK activity. Loss of BMPR2 signalling in mice, induced by a dominant mutant of BMPR2, caused spontaneous pulmonary hypertension and increased ROCK activation, which was

relieved by the ROCK inhibitor fasudil[74]. This demonstrates a direct causal link of BMPR2 to the regulation of ROCK activity and is of particular interest given that inactivating mutations of the BMPR2 gene cause hereditary pulmonary arterial hypertension without affecting systemic blood pressure i.e. it is a lung selective pathway[75]. Reduced BMP signalling is also seen in hypoxic pulmonary hypertension, resulting both from reduced BMPR2 expression and increased expression of the endogenous BMP antagonist gremlin1[26,76]. Strategies that augment BMPR signaling, including administration of FK506 (tacrolimus) or blocking the action of the endogenous BMP antagonist Gremlin1, can ameliorate hypoxic pulmonary hypertension *in vivo*, identifying this as a further potential therapeutic approach in hypoxic pulmonary hypertension[26,77,78].

1.8 Conclusions

Chronic hypoxic pulmonary vasoconstriction is an important, recently recognised mechanism that increases pulmonary vascular resistance in chronic hypoxic pulmonary hypertension. Inhibition of this mechanism with rho kinase antagonists shows that it plays a much more important role in hypoxic pulmonary hypertension than previously thought, and that structural changes in the pulmonary circulation play a lesser role. Clinical studies of existing vasodilator agents show that pulmonary hypertension can be ameliorated and systemic oxygen delivery improved in carefully selected patients, demonstrating an important vasoconstrictor element in chronic hypoxic lung diseases. However, systemic vasodilator effects limit the doses that can be used and current vasodilators are not approved as standard therapy. More recently, vasoconstrictor pathways that act selectively on the hypoxic pulmonary circulation have been identified, which can be antagonised without causing arterial hypoxaemia or systemic vasodilatation. These provide potential targets for novel pharmacological interventions that, in the future, could effectively treat pulmonary hypertension complicating chronic lung diseases.

Contributors

SCR contributed to the concepts and design of the review, undertook systematic literature reviews and prepared the figures and tables, and contributed to drafting and writing the manuscript. SG and MK critically reviewed the concepts, and contributed to the drafting and design of the review. PMcL contributed to the concepts and design of the review, the literature reviews, and drafting and writing the review. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Declaration of interests

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References

1. Seeger W, Adir Y, Barbera JA, et al. Pulmonary hypertension in chronic lung diseases. *Journal of the American College of Cardiology* 2013; **62**(25 Suppl): D109-16.
2. Wells JM, Washko GR, Han MK, et al. Pulmonary arterial enlargement and acute exacerbations of COPD. *N Engl J Med* 2012; **367**(10): 913-21.
3. Chaouat A, Naeije R, Weitzenblum E. Pulmonary hypertension in COPD. *Eur Respir J* 2008; **32**(5): 1371-85.
4. Hayes D, Jr., Tobias JD, Mansour HM, et al. Pulmonary Hypertension in Cystic Fibrosis with Advanced Lung Disease. *Am J Respir Crit Care Med* 2014.
5. Carlsen J, Hasseriis Andersen K, Boesgaard S, Iversen M, Steinbruchel D, Bogelund Andersen C. Pulmonary arterial lesions in explanted lungs after transplantation correlate with severity of pulmonary hypertension in chronic obstructive pulmonary disease. *J Heart Lung Transplant* 2013; **32**(3): 347-54.
6. Sakao S, Voelkel NF, Tatsumi K. The vascular bed in COPD: pulmonary hypertension and pulmonary vascular alterations. *Eur Respir Rev* 2014; **23**(133): 350-5.
7. West JB. The physiologic basis of high-altitude diseases. *Ann Intern Med* 2004; **141**(10): 789-800.
8. Law MA, Grifka RG, Mullins CE, Nihill MR. Atrial septostomy improves survival in select patients with pulmonary hypertension. *Am Heart J* 2007; **153**(5): 779-84.
9. Groves BM, Reeves JT, Sutton JR, et al. Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen. *J Appl Physiol* 1987; **63**(2): 521-30.
10. Hyvelin JM, Howell K, Nichol A, Costello CM, Preston RJ, McLoughlin P. Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation. *Circ Res* 2005; **97**(2): 185-91.
11. Stenmark KR, Fagan KA, Frid MG. Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. *Circ Res* 2006; **99**(7): 675-91.
12. Nagaoka T, Morio Y, Casanova N, et al. Rho/Rho kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**(4): L665-72.

13. Somlyo AP, Somlyo AV. Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003; **83**(4): 1325-58.
14. Yamin R, Morgan KG. Deciphering actin cytoskeletal function in the contractile vascular smooth muscle cell. *The Journal of physiology* 2012; **590**(Pt 17): 4145-54.
15. Dimopoulos GJ, Semba S, Kitazawa K, Eto M, Kitazawa T. Ca2+-dependent rapid Ca2+ sensitization of contraction in arterial smooth muscle. *Circ Res* 2007; **100**(1): 121-9.
16. Burridge K, Wennerberg K. Rho and Rac take center stage. *Cell* 2004; **116**(2): 167-79.
17. Urena J, Lopez-Barneo J. Metabotropic regulation of RhoA/Rho-associated kinase by L-type Ca2+ channels. *Trends Cardiovasc Med* 2012; **22**(6): 155-60.
18. Moreno-Dominguez A, Colinas O, El-Yazbi A, et al. Ca2+ sensitization due to myosin light chain phosphatase inhibition and cytoskeletal reorganization in the myogenic response of skeletal muscle resistance arteries. *The Journal of physiology* 2013; **591**(Pt 5): 1235-50.
19. Seow CY. Myosin filament assembly in an ever-changing myofilament lattice of smooth muscle. *American journal of physiology Cell physiology* 2005; **289**(6): C1363-8.
20. Sehgel NL, Zhu Y, Sun Z, et al. Increased vascular smooth muscle cell stiffness: a novel mechanism for aortic stiffness in hypertension. *Am J Physiol Heart Circ Physiol* 2013; **305**(9): H1281-7.
21. Lan B, Wang L, Zhang J, et al. Rho-kinase mediated cytoskeletal stiffness in skinned smooth muscle. *J Appl Physiol (1985)* 2013; **115**(10): 1540-52.
22. Bernard O. Lim kinases, regulators of actin dynamics. *Int J Biochem Cell Biol* 2007; **39**(6): 1071-6.
23. Heidkamp MC, Bayer AL, Scully BT, Eble DM, Samarel AM. Activation of focal adhesion kinase by protein kinase C epsilon in neonatal rat ventricular myocytes. *Am J Physiol Heart Circ Physiol* 2003; **285**(4): H1684-96.
24. Fagan KA, Oka M, Bauer NR, et al. Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**(4): L656-64.

25. Vanderpool RR, Kim AR, Molthen R, Chesler NC. Effects of acute Rho kinase inhibition on chronic hypoxia-induced changes in proximal and distal pulmonary arterial structure and function. *J Appl Physiol* 2011; **110**(1): 188-98.
26. Cahill E, Costello CM, Rowan SC, et al. Gremlin plays a key role in the pathogenesis of pulmonary hypertension. *Circulation* 2012; **125**(7): 920-30.
27. Cahill E, Rowan SC, Sands M, et al. The pathophysiological basis of chronic hypoxic pulmonary hypertension in the mouse: vasoconstrictor and structural mechanisms contribute equally. *Exp Physiol* 2012.
28. Ishizaki T, Mizuno S, Sakai A, et al. Blunted activation of Rho-kinase in yak pulmonary circulation. *Biomed Res Int* 2015; **2015**: 720250.
29. Huveneers S, Daemen MJ, Hordijk PL. Between Rho(k) and a hard place: the relation between vessel wall stiffness, endothelial contractility, and cardiovascular disease. *Circ Res* 2015; **116**(5): 895-908.
30. Boer C, van Nieuw Amerongen GP, Groeneveld AB, et al. Smooth muscle F-actin disassembly and RhoA/Rho-kinase signaling during endotoxin-induced alterations in pulmonary arterial compliance. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**(4): L649-55.
31. Reeves JT, Linehan JH, Stenmark KR. Distensibility of the normal human lung circulation during exercise. *Am J Physiol Lung Cell Mol Physiol* 2005; **288**(3): L419-25.
32. Ferrer E, Peinado VI, Castaneda J, et al. Effects of cigarette smoke and hypoxia on pulmonary circulation in the guinea pig. *Eur Respir J* 2011; **38**(3): 617-27.
33. Kobs RW, Muvarak NE, Eickhoff JC, Chesler NC. Linked mechanical and biological aspects of remodeling in mouse pulmonary arteries with hypoxia-induced hypertension. *Am J Physiol Heart Circ Physiol* 2005; **288**(3): H1209-17.
34. Molthen RC, Karau KL, Dawson CA. Quantitative models of the rat pulmonary arterial tree morphometry applied to hypoxia-induced arterial remodeling. *J Appl Physiol* 2004; **97**(6): 2372-84; discussion 54.
35. Champion HC, Michelakis ED, Hassoun PM. Comprehensive invasive and noninvasive approach to the right ventricle-pulmonary circulation unit: state of the art and clinical and research implications. *Circulation* 2009; **120**(11): 992-1007.
36. Lammers SR, Kao PH, Qi HJ, et al. Changes in the structure-function relationship of elastin and its impact on the proximal pulmonary arterial mechanics of hypertensive calves. *Am J Physiol Heart Circ Physiol* 2008; **295**(4): H1451-9.

37. Jin Y, Chen B, Calvert TJ, Chicoine LG, Liu Y, Nelin LD. Chronic hypoxia decreases arterial and venous compliance in isolated perfused rat lungs: an effect that is reversed by exogenous L-arginine. *Am J Physiol Heart Circ Physiol* 2013; **304**(2): H195-205.
38. Hsia CC, Hyde DM, Ochs M, Weibel ER. An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. *Am J Respir Crit Care Med* 2010; **181**(4): 394-418.
39. Howell K, Preston RJ, McLoughlin P. Chronic hypoxia causes angiogenesis in addition to remodelling in the adult rat pulmonary circulation. *J Physiol* 2003; **547**(Pt 1): 133-45.
40. van Suylen RJ, Smits JF, Daemen MJ. Pulmonary artery remodeling differs in hypoxia- and monocrotaline-induced pulmonary hypertension. *Am J Respir Crit Care Med* 1998; **157**(5 Pt 1): 1423-8.
41. Frank DB, Lowery J, Anderson L, Brink M, Reese J, de Caestecker M. Increased susceptibility to hypoxic pulmonary hypertension in Bmpr2 mutant mice is associated with endothelial dysfunction in the pulmonary vasculature. *Am J Physiol Lung Cell Mol Physiol* 2008; **294**(1): L98-109.
42. Yilmaz C, Ravikumar P, Gyawali D, Iyer R, Unger RH, Hsia CC. Alveolar-capillary adaptation to chronic hypoxia in the fatty lung. *Acta Physiol (Oxf)* 2014.
43. Nishimura R, Nishiwaki T, Kawasaki T, et al. Hypoxia-induced proliferation of tissue-resident endothelial progenitor cells in the lung. *Am J Physiol Lung Cell Mol Physiol* 2015; **308**(8): L746-58.
44. Wagner PD, Sutton JR, Reeves JT, Cymerman A, Groves BM, Malconian MK. Operation Everest II: pulmonary gas exchange during a simulated ascent of Mt. Everest. *J Appl Physiol (1985)* 1987; **63**(6): 2348-59.
45. Ghofrani HA, Reichenberger F, Kohstall MG, et al. Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. *Ann Intern Med* 2004; **141**(3): 169-77.
46. Richalet JP, Gratadour P, Robach P, et al. Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. *Am J Respir Crit Care Med* 2005; **171**(3): 275-81.

47. Hilde JM, Skjorten I, Hansteen V, et al. Haemodynamic responses to exercise in patients with COPD. *Eur Respir J* 2012; **41**(5): 1031-41.
48. ATS/ERS CP. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002; **165**(2): 277-304.
49. Adnot S, Kouyoumdjian C, Defouilloy C, et al. Hemodynamic and gas exchange responses to infusion of acetylcholine and inhalation of nitric oxide in patients with chronic obstructive lung disease and pulmonary hypertension. *The American review of respiratory disease* 1993; **148**(2): 310-6.
50. Ghofrani HA, Wiedemann R, Rose F, et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. *Lancet* 2002; **360**(9337): 895-900.
51. Vonbank K, Ziesche R, Higenbottam TW, et al. Controlled prospective randomised trial on the effects on pulmonary haemodynamics of the ambulatory long term use of nitric oxide and oxygen in patients with severe COPD. *Thorax* 2003; **58**(4): 289-93.
52. Blanco I, Santos S, Gea J, et al. Sildenafil to improve respiratory rehabilitation outcomes in COPD: a controlled trial. *Eur Respir J* 2013; **42**(4): 982-92.
53. Hoeper MM, Halank M, Wilkens H, et al. Riociguat for interstitial lung disease and pulmonary hypertension: a pilot trial. *Eur Respir J* 2013; **41**(4): 853-60.
54. Boeck L, Tamm M, Grendelmeier P, Stolz D. Acute effects of aerosolized iloprost in COPD related pulmonary hypertension - a randomized controlled crossover trial. *PLoS One* 2013; **7**(12): e52248.
55. Dernaika TA, Beavin M, Kinasewitz GT. Iloprost improves gas exchange and exercise tolerance in patients with pulmonary hypertension and chronic obstructive pulmonary disease. *Respiration* 2010; **79**(5): 377-82.
56. Olschewski H, Ghofrani HA, Walmrath D, et al. Inhaled prostacyclin and iloprost in severe pulmonary hypertension secondary to lung fibrosis. *American journal of respiratory and critical care medicine* 1999; **160**(2): 600-7.

57. Corte TJ, Keir GJ, Dimopoulos K, et al. Bosentan in pulmonary hypertension associated with fibrotic idiopathic interstitial pneumonia. *American journal of respiratory and critical care medicine* 2014; **190**(2): 208-17.
58. Collard HR, Anstrom KJ, Schwarz MI, Zisman DA. Sildenafil improves walk distance in idiopathic pulmonary fibrosis. *Chest* 2007; **131**(3): 897-9.
59. Raghu G, Behr J, Brown KK, et al. Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. *Ann Intern Med* 2013; **158**(9): 641-9.
60. Chevalier M, Gilbert G, Roux E, et al. T-type calcium channels are involved in hypoxic pulmonary hypertension. *Cardiovasc Res* 2014; **103**(4): 597-606.
61. Le Quang K, Naud P, Qi XY, et al. Role of T-type calcium channel subunits in post-myocardial infarction remodelling probed with genetically engineered mice. *Cardiovasc Res* 2011; **91**(3): 420-8.
62. Malczyk M, Veith C, Fuchs B, et al. Classical transient receptor potential channel 1 in hypoxia-induced pulmonary hypertension. *Am J Respir Crit Care Med* 2014; **188**(12): 1451-9.
63. Wang J, Weigand L, Lu W, Sylvester JT, Semenza GL, Shimoda LA. Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca2+ in pulmonary arterial smooth muscle cells. *Circ Res* 2006; **98**(12): 1528-37.
64. Xia Y, Yang XR, Fu Z, et al. Classical transient receptor potential 1 and 6 contribute to hypoxic pulmonary hypertension through differential regulation of pulmonary vascular functions. *Hypertension* 2014; **63**(1): 173-80.
65. Wang J, Jiang Q, Wan L, et al. Sodium tanshinone IIA sulfonate inhibits canonical transient receptor potential expression in pulmonary arterial smooth muscle from pulmonary hypertensive rats. *Am J Respir Cell Mol Biol* 2013; **48**(1): 125-34.
66. Smith KA, Voiriot G, Tang H, et al. Notch Activation of Ca Signaling Mediates Hypoxic Pulmonary Vasoconstriction and Pulmonary Hypertension. *Am J Respir Cell Mol Biol* 2015.
67. Li X, Zhang X, Leathers R, et al. Notch3 signaling promotes the development of pulmonary arterial hypertension. *Nat Med* 2009; **15**(11): 1289-97.
68. Yang XR, Lin AH, Hughes JM, et al. Upregulation of osmo-mechanosensitive TRPV4 channel facilitates chronic hypoxia-induced myogenic tone and pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2012; **302**(6): L555-68.

69. Earley S, Pauyo T, Drapp R, Tavares MJ, Liedtke W, Brayden JE. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. *Am J Physiol Heart Circ Physiol* 2009; **297**(3): H1096-102.
70. Xia Y, Fu Z, Hu J, et al. TRPV4 channel contributes to serotonin-induced pulmonary vasoconstriction and the enhanced vascular reactivity in chronic hypoxic pulmonary hypertension. *Am J Physiol Cell Physiol* 2013; **305**(7): C704-15.
71. Hyvelin JM, O'Connor C, McLoughlin P. Effect of changes in pH on wall tension in isolated rat pulmonary artery: role of the RhoA/Rho-kinase pathway. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**(4): L673-84.
72. Kojonazarov B, Myrzaakhmatova A, Sooronbaev T, Ishizaki T, Aldashev A. Effects of fasudil in patients with high-altitude pulmonary hypertension. *Eur Respir J* 2013; **39**(2): 496-8.
73. Shimizu T, Fukumoto Y, Tanaka S, Satoh K, Ikeda S, Shimokawa H. Crucial role of ROCK2 in vascular smooth muscle cells for hypoxia-induced pulmonary hypertension in mice. *Arterioscler Thromb Vasc Biol* 2013; **33**(12): 2780-91.
74. Yasuda T, Tada Y, Tanabe N, Tatsumi K, West J. Rho-kinase inhibition alleviates pulmonary hypertension in transgenic mice expressing a dominant-negative type II bone morphogenetic protein receptor gene. *Am J Physiol Lung Cell Mol Physiol* 2011; **301**(5): L667-74.
75. Austin ED, Loyd JE. The genetics of pulmonary arterial hypertension. *Circulation research* 2014; **115**(1): 189-202.
76. Long L, Crosby A, Yang X, et al. Altered bone morphogenetic protein and transforming growth factor-beta signaling in rat models of pulmonary hypertension: potential for activin receptor-like kinase-5 inhibition in prevention and progression of disease. *Circulation* 2009; **119**(4): 566-76.
77. Ciuclan L, Sheppard K, Dong L, et al. Treatment with anti-gremlin 1 antibody ameliorates chronic hypoxia/SU5416-induced pulmonary arterial hypertension in mice. *Am J Pathol* 2013; **183**(5): 1461-73.
78. Spiekerkoetter E, Tian X, Cai J, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013; **123**(8): 3600-13.

Figure1. Cytoskeletal dynamics and contraction of smooth muscle contractile apparatus. The contractile apparatus of smooth muscle cells forms a network interlaced throughout the cell in multiple directions and connecting to the networks in adjacent cells. Consequently coordinated cell contraction increases tension along the entire chain of tensile structures. Smooth muscle tissue can rapidly adapt its structural and functional properties in response to a range of stimuli by: (A) increasing the number and stiffness of attachments of actin-myosin assemblies to membrane dense plaques and cytoplasmic dense bodies and (B) dynamically altering the sub cortical actin filament network to enhance the transmission of mechanical force generated by the actin-myosin system.

Figure 2. Schematic representation of the major pathways controlling myosin light chain phosphorylation, and thus contraction, in vascular smooth muscle cells. Vascular smooth muscle (VSM) tension development is caused by phosphorylation of myosin light chains which promotes the extent of actin-myosin interaction. MLK phosphorylation is determined at any time by the balance of myosin light chain (MLC) kinase and MLC phosphatase activities. ROCK activation plays a central role in increasing tension by phosphorylating CPI-17 and the MYPT1 subunit of MLCP, both of which then inhibit MLCP activity and increase MLC phosphorylation. ROCK can be activated by the small g-protein RhoA, reactive oxygen species and by IP_3 mediated Ca^{2+} release from the sacroplasmic reticulum. MLC phosphorylation and tension development is also increased by MLCK activity, which is regulated by cytosolic $[Ca^{2+}]_i$ and calmodulin.

Ca^{2+}, calcium; CaM, calcium calmodulin; DAG, diacylglycerol; ER, endoplamic reticulum; ERCC endoplamsic reticulum calcium channel; G, small monomeric g-protein; IP_3, inositol trisphosphate; Myosin LC, mysoin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; P, phorphorylation; PKC, protein kinase C; PLC, phospholipase C; ROCK, rho-kinase; ROS, reactive oxygen species; SAC, stretch activated calcium channel; SOCC, store operated calcium channel

Figure 1

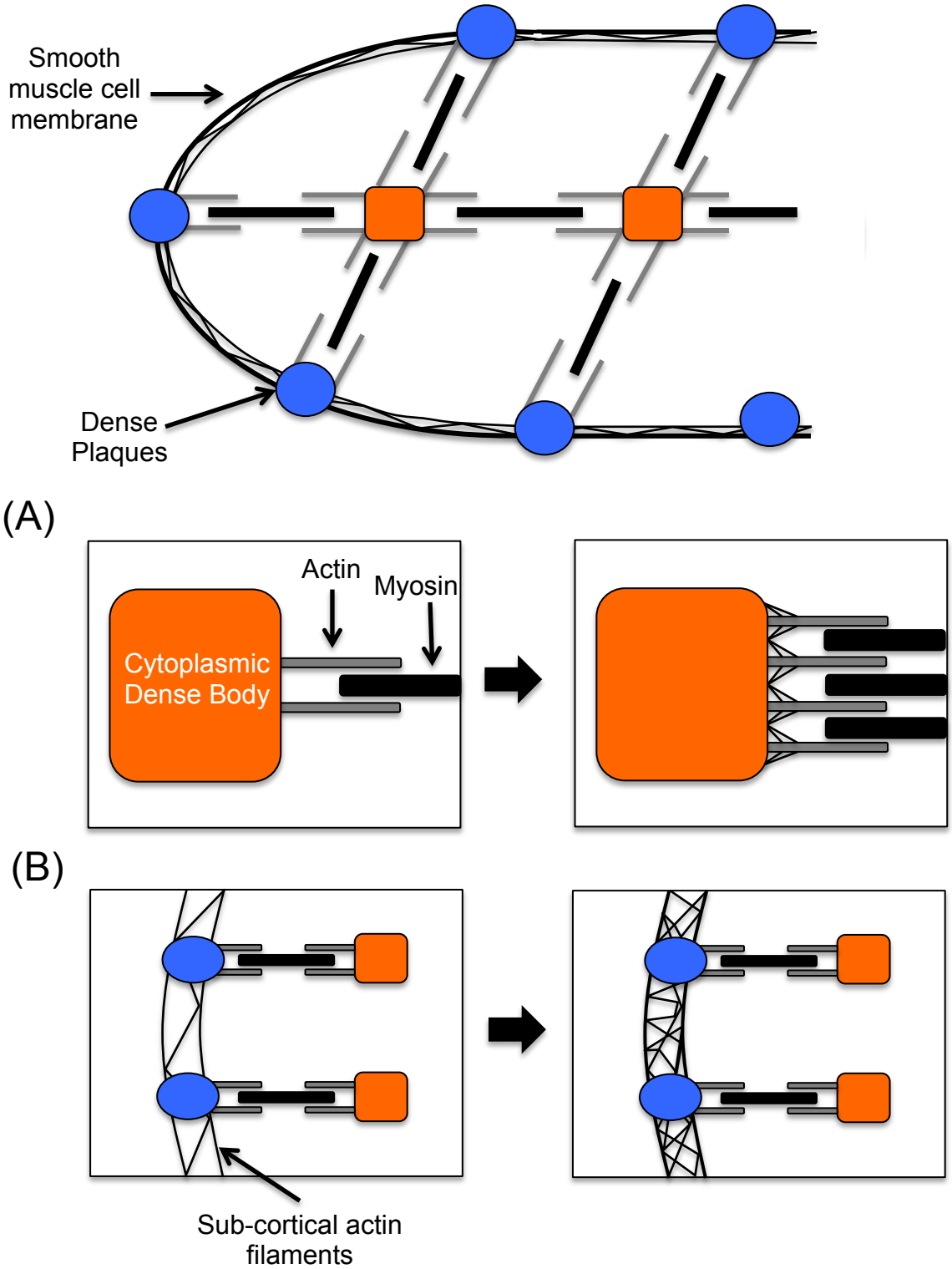
Smooth
muscle cell
membrane
Dense
Plaques
(A)
Actin
Myosin
Cytoplasmic
Dense Body
(B)
Sub-cortical actin
filaments

Figure 2

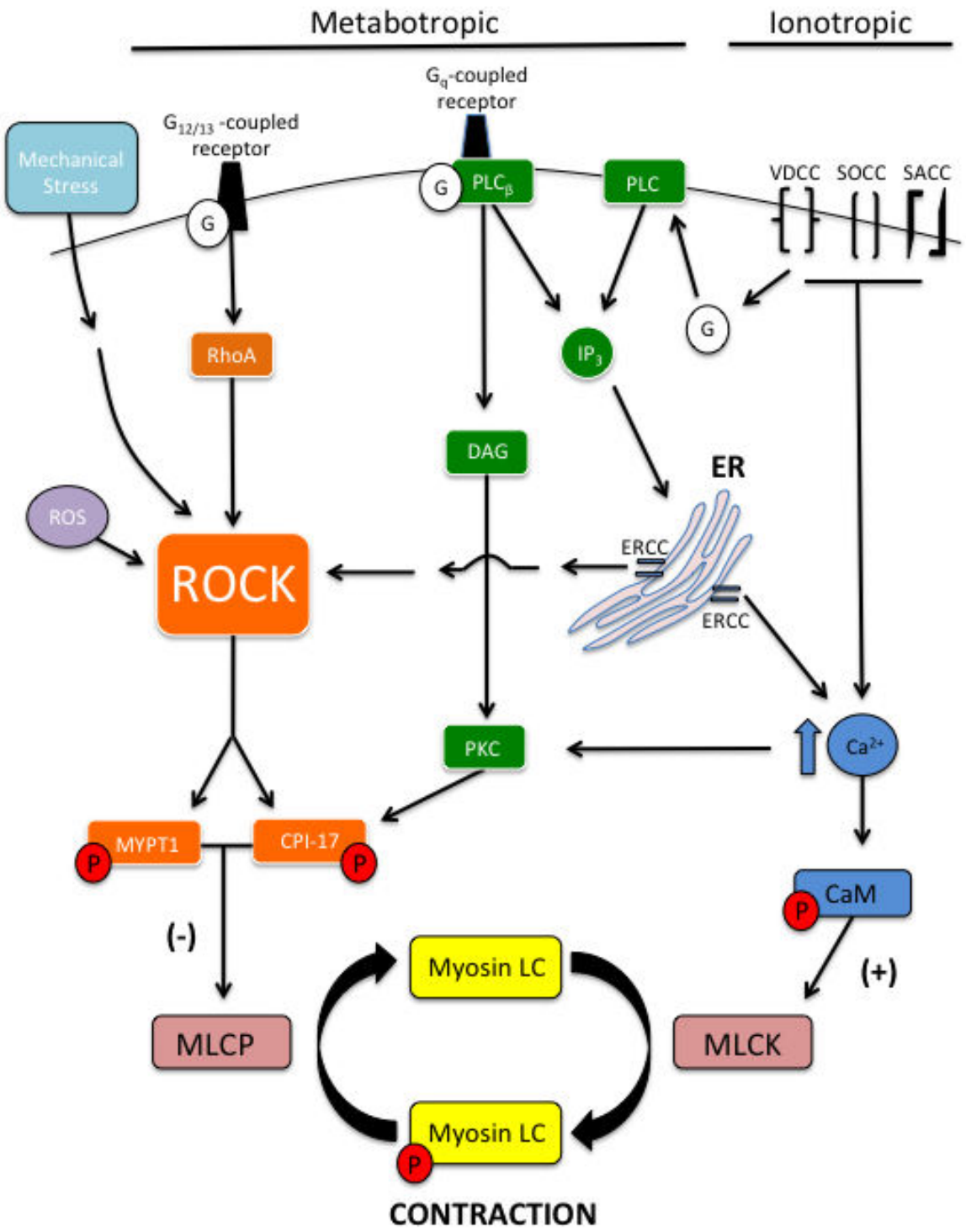
Metabotropic
Ionotropic
G_q-coupled receptor
$G_{12/13}$ -coupled receptor
Mechanical Stress
G
PLC_β
PLC
VDCC SOCC SACC
RhoA
IP_3
DAG
ER
ROS
ROCK
ERCC
ERCC
PKC
Ca^{2+}
MYPT1
CPI-17
P
P
CaM
P
(-)
(+)
Myosin LC
MLCP
MLCK
Myosin LC
P
CONTRACTION

Table 1. Effect of increased NO pathway signaling on pulmonary haemodynamics and arterial oxygenation in patients with chronic hypoxic lung disease complicated by pulmonary hypertension (mPAP>25mmHg).

Disease	mPAP (mmHg)	Agent	Route	Design	Duration (weeks)	Responses to treatment				
						mPAP	PVR	CO	Arterial O_2	DO_2
COPD[49]	>25	NO (+ O_2)	Inh	open label, within subject	Single dose	↓	↓(30%)	→	↑(P_aO_2)	↑
COPD[51]	>25	NO (+ O_2)	Inh	open label, randomised, two group	12	↓	↓(37%)	↑	→(P_aO_2)	↑
ILD[50]	>35	NO (+ O_2)	Inh	open label, within subject*	Single dose	↓	↓(22%)	→	↑(P_aO_2)	↑
ILD[50]	>35	Sildenafil	PO	open label, within subject*	Single dose	↓	↓(35%)	→	↑(P_aO_2)	↑
ILD[53]	>30	Riociguat	PO	open label, within subject	12	→	↓(18%)	↑	↓(P_aO_2)	↑

Abbreviations: CO, cardiac output. COPD, chronic obstructive lung disease. DO_2, systemic oxygen delivery. ILD, interstitial lung disease. Inh, inhaled. MIGET, multiple inert gas excretion technique. NO, nitric oxide. mPAP, mean pulmonary arterial pressure. PVR, pulmonary vascular resistance. RVSP, right ventricular systolic pressure estimated by echocardiography. ↓, reduced. →, unchanged. ↑, increased.

Table 2. Effect of prostacyclin receptor agonists on pulmonary haemodynamics and arterial oxygenation in patients with chronic hypoxic lung disease complicated by pulmonary hypertension (mPAP>25mmHg).

Disease	mPAP (mmHg)	Agent	Route	Design	Duration (months)	Responses to treatment				
						mPAP	PVR	CO	Arterial O_2	DO_2
COPD[54]	>30 (>45 on exercise)	Ilop	Inh	Double blind crossover, within subject	Single dose	NM	NM	NM	→(P_aO_2)	NM
ILD[56]	>30	PGI2	Inh	Open Label within subject	Single dose	↓	↓(52%)	↑	→(S_aO_2)	↑
ILD[56]	>30	PGI2	IV	Open Label within subject	Single dose	↓	↓(40%)	↑	→(S_aO_2)	↑
ILD[50]	>35	PGI2	IV	Open label within subject*	Single dose	↓	↓(37%)	↑	↓(P_aO_2)	↑

* Although the study was a two group, randomized, dose or drug comparison study, the conclusions tabulated regarding pulmonary haemodynamics and oxygenation are based on within subject comparisons.

Abbreviations: CO, cardiac output. COPD, chronic obstructive lung disease. DO_2, systemic oxygen delivery. ILD, interstitial lung disease. Inh, inhaled. MIGET, multiple inert gas excretion technique. NM, not measured. NO, nitric oxide. mPAP, mean pulmonary arterial pressure at rest. PVR, pulmonary vascular resistance. ↓, reduced. →, unchanged. ↑, increased.

Table 3. Lung selective vasoconstrictor pathways

Signalling category	Specific pathway	Gene Manipulation	HPH	Systemic BP	Potential therapeutic strategies
Cytosolic Ca^{2+}	T-type VDCC	$Ca_v3.1^{-/-}$	$\downarrow$[60]	$\rightarrow$[61]	TTA-A2[60]
	SOCC	$TRPC1^{-/-}$	$\downarrow$[62,64]	$\rightarrow$[64]	STS[65]
		$TRPC6^{-/-}$	$\downarrow$[64,66]	$\rightarrow$[65]	2-APB, Notch antagonism[66]
	SAC	$TRPV4^{-/-}$	$\downarrow$[70]	$\rightarrow$[69,70]	
RhoA/ROCK	Rho-kinase	$ROCK2^{+/-}$	$\downarrow$[73]	$\rightarrow$[73]	Y27632[10,27] Fasudil[74], FK506[77] Anti-Gremlin1 mAb[78]

Abbreviations: HPH, hypoxic pulmonary hypertension. BP, blood pressure. Ca^{2+}, calcium. VDCC, voltage dependent calcium channel. SOCC, store operated calcium channel. SAC, stretch activated calcium channel. $Ca_v3.1$, voltage dependent calcium channel 3.1. TRPC, transient receptor potential cation channel. ASIC, acid sensing ion channel. TRPV, transient receptor potential vanilloid channel. ROCK, rho-kinase. STS, Sodium tansinone IIA sulfonate. $\downarrow$, reduced. $\rightarrow$, unchanged. $\uparrow$, increased.

Key Messages

- Pulmonary hypertension in chronic hypoxic lung diseases increases morbidity and reduces life expectancy.

- Long term oxygen therapy ameliorates pulmonary hypertension and improves survival, but achieves only partial correction of pulmonary hypertension at best.

- Recent advances have identified a novel vasoconstrictor mechanism, here called chronic hypoxic pulmonary vasoconstriction, which plays a major role in hypoxic pulmonary hypertension.

- Signalling pathways that act selectively in the lung mediate chronic hypoxic vasoconstriction and provide novel targets for the future development of new therapeutic approaches.

Text panel: literature search strategy and selection criteria

Data for this review were identified by searches of Pubmed with the term “hypoxic pulmonary hypertension” combined with each of the following terms individually “Rho A”, “Rho kinase”, “remodelling” and “mouse or rat”, “rarefaction”, “angiogenesis”. These searches included the criteria “not review” to identify original research and only papers published from 2000 to the present were included. A separate search of Pubmed included the search terms “pulmonary circulation”, “distensibility” or “distensible” and the criteria previously mentioned. To identify human trials of vasodilator agents in patients with pulmonary hypertension and chronic lung diseases, we used the terms “pulmonary hypertension” and “COPD” or “interstitial lung disease” or “diffuse parenchymal lung disease” or “chronic lung disease” or “fibrotic lung disease” or “restrictive lung disease”or “hypoxic pulmonary hypertension” and “vasodilator” or “sildenafil” or “nitric oxide” or “riociguat” or “prostacyclin” or “iloprost” or “bosentan” or “ambrisentan” or “macitentan” or “fasudil” or “Y27632”. We restricted our search to the category “clinical trial”. We also reviewed articles identified in the reference list of those articles. We included in our review trials of vasodilator therapy in chronic lung diseases in which patient recruitment required arterial hypoxaemia while breathing room air, and pulmonary arterial pressure greater than 25mmHg at rest, measured by intravascular catheter measurements. Further, we reviewed reference lists of articles identified from these searches and selected those we judged especially relevant. We have also included relevant earlier reviews, highly regarded older publications or papers that reported seminal findings

Currently open trials of vasodilator therapy in WHO Group pulmonary hypertension.

NCT Identifier	Lung disease	mPAP (mmHg)	Agent	Reg Date	Duration (weeks)	Design	Primary outcome	Secondary Outcome	Status
01862536	COPD	>30	Tadalafil (oral)	May 2013	52	Randomised, double blind, placebo controlled	6MWTD	PVR	Recruiting
02138825	ILD	>25	Riociguat (oral)	April 2014	26	Randomised, double blind, placebo controlled	6MWTD	TTCW	Recruiting
02603068	IPF	>30	Treprostinil (oral)	Nov 2015	16	Randomised, Open label	PVR	6MWTD	Not yet recruiting

Trials were identified using the US National Institutes of Health ClinicalTrials.gov website. All are trials are Phase 2, multicentre trials, in which the diagnosis of pulmonary hypertension was confirmed by pulmonary arterial catheterisation studies (mPAP>25mmHg at rest). Only studies with an "Open recruitment status" are included.
Abbreviations: 6MWTD, six minute walk test distance. COPD, chronic obstructive lung disease. ILD, interstitial lung disease. IPF, idiopathic pulmonary fibrosis. mPAP, mean pulmonary arterial pressure at rest measured by right heart catheterisation study. NCT Identifier, unique identification number assigned by ClinicalTrials.gov. Reg date, date on which study details first received by Clinical Trials.gov. PVR, pulmonary vascular resistance. TTCW, time to clinical worsening.