Animal models of pulmonary hypertension: Getting to the heart of the problem

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Despite recent therapeutic advances, pulmonary hypertension (PH) remains a fatal disease due to the development of right ventricular (RV) failure. At present, no treatments targeted at the right ventricle are available, and RV function is not widely considered in the preclinical assessment of new therapeutics. Several small animal models are used in the study of PH, including the classic models of exposure to either hypoxia or monocrotaline, newer combinational and genetic models, and pulmonary artery banding, a surgical model of pure RV pressure overload. These models reproduce selected features of the structural remodelling and functional decline seen in patients and have provided valuable insight into the pathophysiology of RV failure. However, significant reversal of remodelling and improvement in RV function remains a therapeutic obstacle. Emerging animal models will provide a deeper understanding of the mechanisms governing the transition from adaptive remodelling to a failing right ventricle, aiding the hunt for druggable molecular targets.

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1 INTRODUCTION

Pulmonary hypertension (PH) is a multifactorial syndrome driven by remodelling of the pulmonary vascular bed (Humbert et al., 2019). The broad aetiology of PH is separated into five recognised groups (Table 1), each having a distinct pathophysiology and management strategy (Galiè et al., 2016; Simonneau et al., 2019). Common to all groups is progressive remodelling of the pulmonary vascular tree, leading to increased pulmonary vascular resistance (PVR) and mean pulmonary artery pressure (mPAP; Humbert et al., 2004).

Abbreviations: 5-LO, 5-lipoxygenase; CMR, cardiac magnetic resonance; CO, cardiac output; COPD, chronic obstructive pulmonary disease; CTEPH, Chronic thromboembolic PH; EF, ejection fraction; HF, high-fat diet; HFrEF, heart failure with reduced ejection fraction; HFD, high-fat diet; HFrEF, heart failure with preserved ejection fraction; IPF, idiopathic pulmonary fibrosis; LHD, left heart disease; MCT, monocrotaline; MCTP, MCT pyrrole; mPAP, mean pulmonary artery pressure; PAB, pulmonary arterial banding; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RAGE, receptor of advanced glycation end-products; RV, right ventricular; RVFE, RV ejection fraction; RVH, right ventricular hypertrophy; RVSP, RV systolic pressure; SV, stroke volume; TAPSE, tricuspid annular plane systolic excursion; WMSI, wall motion score index.

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Clinical classification of pulmonary hypertension

| Group 1 | Pulmonary arterial hypertension (PAH) |
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| Group 2 | Pulmonary hypertension due to left heart disease |
| Group 3 | Pulmonary hypertension due to lung diseases and/or hypoxia |
| Group 4 | Pulmonary hypertension due to pulmonary artery obstructions |
| Group 5 | Pulmonary hypertension with unclear multifactorial mechanisms |

Consequently, the afterload of the right ventricle is elevated, leading to right ventricular (RV) hypertrophy (RVH), RV failure and death (Humbert et al., 2019).

The approval of modern vasodilator therapies was a major milestone in the treatment of PH, markedly improving patient symptomatic status and reducing the rate of clinical deterioration (Hoepera et al., 2016; Rhodes et al., 2009). However, decreases in mPAP are ultimately minor and vascular remodelling remains unchecked (Pullamsetti et al., 2014; Rich et al., 2010). Interestingly, haemodynamic studies have illustrated that the capacity of the right ventricle to adapt to pressure overload, rather than pulmonary haemodynamics, determines prognosis in PH (Howard, 2011; Humbert et al., 2010). Consequently, cardioprotection is an attractive therapeutic avenue, particularly in the current absence of therapies able to reverse vascular remodelling. At present, optimisation of afterload is the mainstay for the management of RV dysfunction and the prevention of RV failure (Cassady & Ramani, 2020). Acute decompensation of RV function may require intensive care admission and pharmacological inotropic support. In these circumstances, the mortality rate exceeds 40% (Camp et al., 2011; Sztarym et al., 2010), eclipsing the 13–14% in-hospital mortality associated with acute uncompensated left ventricular (LV) failure (Abraham et al., 2005).

The development of novel cardioprotective therapies for use in PH has thus far been impeded by an incomplete understanding of the mechanisms driving RV failure. Translational experimental models will be key to unlocking the pathobiology of RV failure and developing right ventricle-targeted therapies for use in PH. Herein, we describe the principal experimental models of PH, with a particular focus on RV remodelling and dysfunction. We will also discuss the importance of a full assessment of RV structure and function in translational PH research. Current knowledge of the pathological mechanisms of RV failure is also summarised.

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2 | THE RIGHT VENTRICLE DETERMINES FUNCTIONAL STATUS IN PH

Early studies of the right ventricle suggested it was of minor functional importance. In dogs, destruction of the RV myocardium was found to have little impact on global heart function (Bakos, 1950; Donald & Essex, 1954; Starr et al., 1943), a finding that was seemingly confirmed decades later by the relative success of the Fontan procedure, which involves surgical exclusion of the right ventricle from the pulmonary circulation of patients with univentricular hearts (Fontan & Baudet, 1971). As such, for many years, the right ventricle was believed to be a passive conduit through which blood flows into the pulmonary circulation. More recently, the emergence of novel imaging techniques and advanced surgical approaches has reinvigorated the study of right-sided haemodynamics. Accumulating evidence now points to a clinically relevant role for the right ventricle, both in health and in numerous disease states, including congenital heart disease, myocardial infarction (MI), myocarditis and heart failure, in addition to PH (Konstam et al., 2018). In fact, RV function has emerged as a more robust indicator of outcome in PH than either mPAP or PVR (Howard, 2011; Humbert et al., 2010).

A growing body of evidence suggests that the efficaciousness of vasodilator drugs in PAH is determined by their effects on RV function. Indeed, after the initiation of pharmacotherapy, the 5-year survival of patients exhibiting reverse cardiac remodelling and a concomitant improvement in RV ejection fraction (RVEF) exceeds 90% (Badagliacca, Pocca, et al., 2018; van de Veerdonk et al., 2011). Unfortunately, this benefit is achieved only in individuals showing the greatest reductions in PVR, corresponding to <20% of the patient population (Badagliacca, Pocca, et al., 2018), though there is evidence of continued advantage with the addition of a second (Badagliacca, Raina, et al., 2018; van de Veerdonk et al., 2017) or third drug (D’Alto et al., 2020) to the treatment regimen. Furthermore, despite a therapeutic decrease in PVR, some 25% of patients experience continued deterioration in RV haemodynamics, ultimately leading to a poor outcome (van de Veerdonk et al., 2011). It is also critical to note that pulmonary arterial vasodilators are only approved for use in Group 1 and Group 4 PH; management of chronic RV failure in other PH groups is limited to optimisation of the underlying disease.

Collectively, these clinical findings and observations have important implications for experimental modelling of PH. To bridge the gap between preclinical and clinical research, a translational approach utilising clinically relevant parameters of RV structure and mechanics in animal models that faithfully recreate the clinical presentation of RV dysfunction will be vital.

3 | OVERVIEW OF SMALL ANIMAL MODELS OF PH

An ideal experimental model should manifest the key symptomatic and histopathological features of human disease. Due to the heterogeneous nature of PH, no single animal model is likely to be universally appropriate (Ryan et al., 2011). However, there are some key features that would pertain to a superior model of PH. Chief among these is a progressive increase in mPAP or RV systolic pressure (RVSP; often a surrogate for PAP in mice), driven by vascular remodelling (e.g., muscularisation and rarefaction of the pulmonary vascular tree) and increased PVR. Common histological findings in patients should be replicated, including stiffening and thickening of larger pulmonary arteries, underpinned by medial and adventitial hypertrophy, and muscularisation of smaller vessels (Tuder, 2017). For the study of
| Model of? | Condition | Species | Precapillary arteriopathy | Plexiform lesions | RV remodelling | RV function | Systemic effects | Mortality | Selected references |
|-----------|-----------|---------|--------------------------|------------------|--------------|-------------|----------------|-----------|-------------------|
| Monocrotaline | Inflammatory PAH | Rat | High dose (60–80 mg kg⁻¹) | Yes | No* | Maladaptive | Reduced | Yes | Yes | Gomez-Arroyo, Farkas, et al., 2012; Kay et al., 1976 |
| | | Rat | Low dose (20–40 mg kg⁻¹) | Yes | No | Adaptive | Maintained | Yes | No | Buermans et al., 2005; Ruiter, De Man, et al., 2013 |
| Chronic hypoxia | High-altitude, chronic lung disease | Rat | | Yes | No | Adaptive | Maintained | Yes | No | Meyrick & Reid, 1980; Sato et al., 1992 |
| | | Mouse | | Yes | No | Adaptive | Maintained | Yes | No | Hoshikawa et al., 2003; Voelkel et al., 1996 |
| Sugen + hypoxia | PAH | Rat | | Yes | Yes | Maladaptive | Reduced | Yes | Strain-dependentb | Jiang et al., 2016; Nicolls et al., 2012; Taraseviciene-Stewart et al., 2001 |
| | | Mouse | | Yes | No | Adaptive | Moderately reduced | Yes | No | Ciudan et al., 2011; Vitali et al., 2014 |
| Pulmonary artery banding | Isolated RV pressure overload | Rat/mouse | Mild constriction | No | No | Adaptive | Maintained | No | No | Bogaard, Natarajan, et al., 2009; Wang et al., 2018 |
| | | Rat/mouse | Severe constriction | No | No | Maladaptive | Reduced | No | Yes | Akazawa et al., 2020; Boehm et al., 2020; Borgdorff, Koop, et al., 2015 |

Abbreviations: PAH, pulmonary arterial hypertension; RV, right ventricular.

*Monocrotaline combinational models (pneumonectomy, chronic hypoxia and ETₐ receptor deficiency) develop plexiform lesions.

bSee pp. 12–13.
severe PH (particularly PAH), the presence of lumen-obliterating complex intimal (‘plexiform’) lesions, perivascular inflammation and pruning of peripheral vessels are desirable (Rabinovitch et al., 2014; Stacher et al., 2012). These vascular changes should give rise to RVH, with a gradual progression towards RV dilatation and functional decline, culminating in RV failure (Borgdorff, Dickinson, et al., 2015).

Finding experimental models that satisfy all these criteria is challenging indeed. At present, several animal models of PH are available (Table 2), and although none completely reproduce these features, they do at least reflect the clinical situation in that several diverse stimuli induce a somewhat comparable pulmonary vasculopathy (Gomez-Arroyo, Saleem, et al., 2012; Yeager & Colvin, 2014). The oldest and so-called classical models are those induced by chronic hypoxia (CH) or by administration of monocrotaline (MCT). Utilisation of these models led to the advent of modern vasodilator therapy, though the observed reversal of pulmonary vascular remodelling ultimately did not translate to the clinic (Schermuly, Kreisselmeier, Ghofrani, Samidurai, et al., 2004; Schermuly, Kreisselmeier, Ghofrani, Yilmaz, et al., 2004; Sebkhi et al., 2003).

FIGURE 1  In health, the adult right ventricle (RV) is a thin-walled crescent-shaped pocket, with a low systolic pressure (RVSP). In the context of pulmonary hypertension (PH) and pulmonary artery banding (PAB), RV function is initially preserved by hypertrophic remodelling and augmentation of contractility, at the expense of elevated RVSP. However, chronic RV pressure overload eventually leads to progressive RV dilatation and depression of RV function, marking the onset of RV failure. Typical traces were obtained from mice exposed to Sugen + hypoxia. RVP, right ventricular pressure; TAPSE, tricuspid annular plane systolic excursion. [Correction added on 27 May 2021, after first online publication: Figure 1 has been replaced with Figure 3 in this current version.]
To develop models more reflective of human PH, and with greater ability to predict clinical efficacy, models with multiple inciting stimuli were created. This development fitted more closely to the ‘multiple-hit’ paradigm of human PH, which posits that pathogenesis is the sum of multiple predisposing insults, or an amalgamation of aberrant cellular and molecular events (Yuan & Rubin, 2005). Examples of ‘two-hit’ models include injection of the vascular endothelial growth factor receptor (VEGFR)-2 antagonist Sugen (SU5416; semaxanib) followed by exposure to CH (Tarasевичiene-Stewart et al., 2001), and MCT combined with pneumonectomy (White et al., 2007). Pulmonary arterial banding (PAB) has also emerged as a useful model for the study of the consequences of RV pressure overload (Borgdorff, Dickinson, et al., 2015).

Although mice generally develop less severe PH and RV dysfunction than rats, their amenability to genetic manipulation has been frequently exploited in the development of transgenic PH models, examples being deletion of bone morphogenetic protein receptor type 2 (BMPR-II) (Beppu et al., 2004), IL-6 overexpression (Steiner et al., 2009) and, more recently, the Eglh1-Ind2 line (Dai et al., 2016). Other recent developments include the addition of transgenic rats to the repertoire of genetically modified models of PH, including BMPR-II (Hautefort et al., 2019) and KCNK3 mutant lines (Lambert et al., 2019).

4 | ASSESSMENT OF RV FUNCTIONAL STATUS IN SMALL ANIMALS

The RV capacity for adaptation is vast. In PH, the right ventricle may face a fivefold rise in afterload, far exceeding the 50% increase in LV afterload in systemic hypertension (Sanz et al., 2019). Although studies often classify RV remodelling as adaptive or maladaptive in phenotype, the remodelling process is best considered a progressive continuum (Vonk Noordegraaf et al., 2019; Figure 1). The right ventricle initially responds to chronic pressure overload with adaptive remodelling, characterised by hypertrophy and largely preserved RV volumes (Bogaard, Abe, et al., 2009). The increased mass-to-volume ratio decreases wall tension, leading to compensation of RV function (van de Veerdonk et al., 2016). Eventually, these adaptive mechanisms are exceeded, and RV contractile function is no longer able to counteract the increase in afterload. At this point, remodelling is considered maladaptive, characterised by progressive RV dilatation. In the first instance, dilatation leads to a maintenance in stroke volume (SV) through the Frank–Starling mechanism. However, this occurs at the expense of increased filling pressures, ultimately culminating in decompensation of RV function and predisposition to RV failure (Vonk Noordegraaf et al., 2017; Vonk-Noordegraaf et al., 2013).

RV failure is the inability of the right ventricle to adequately perfuse the lung circulation and maintain sufficient LV filling. Although many investigators limit their assessment of the right ventricle in small animal models to the Fulton index (the weight ratio of right ventricle/ left ventricle + septum [S]), RVH alone does not indicate a failing right ventricle. As such, RV function should be evaluated invasively by pressure–volume analysis or non-invasively (and longitudinally) by echocardiography or cardiac magnetic resonance (CMR) imaging, using clinically relevant parameters such as RV volumes, RVEF, tricuspid annular plane systolic excursion (TAPSE) and cardiac output (CO; Gomez-Arroyo, Saleem, et al., 2012; Borgdorff, Dickinson, et al., 2015; Lahm et al., 2018). In some cases, the failing right ventricle has reduced EF and diastolic dysfunction yet preserved CO, highlighting the importance of a multi-parameter approach (Rain et al., 2013).

The clinical manifestations of deteriorating RV function are exercise intolerance, fatigue, dyspnoea, poor peripheral circulation and fluid retention (evident in peripheral oedema, effusion and ascites). These symptomatic features are also recreated in animal models of RV failure (Borgdorff, Koop, et al., 2015) and are an important adjunct to functional RV parameters. In the clinic, exercise performance testing is frequently used to gauge the severity of heart failure and as a prognostic indicator. In animal models of RV failure, forced exercise capacity can be evaluated by a treadmill test (Fang et al., 2012; Sutendra et al., 2013) and voluntary exercise capacity by spontaneous activity in a running wheel (Bartelds et al., 2011; Borgdorff, Bartelds, Dickinson, Steendijk, de Vroomen, et al., 2013). Mortality is the final clinical endpoint of RV failure, but survival analysis in animal models is ostensibly not permitted for ethical reasons.

5 | MONOCROTALINE

MCT, an alkaloid compound derived from the plant Crotalaria spectabilis, was first noted to induce a pulmonary arteriopathy in rats nearly 60 years ago (Kay et al., 1967; Lalich & Merkow, 1961). In the intervening years, this model has been used extensively to study PH, due to its wide availability, technical simplicity and low expense (Gomez-Arroyo, Barkas, et al., 2012). Regardless, the mechanism by which MCT precipitates pulmonary vascular disease remains poorly understood, though it is known to rely on the conversion of MCT to a bioactive form, MCT pyrrole (MCTP), by the hepatic cytochrome P-450 system (Reid et al., 1998).

In rats, a single subcutaneous injection of MCT (60–80 mg·kg−1) is sufficient to induce the pathological features of PH (Gomez-Arroyo, Barkas, et al., 2012; Kay et al., 1976). Within hours, damage to pulmonary endothelial cells can be detected (Schultze et al., 1991). After a week, endothelial injury is well established and accompanied by perivascular inflammation (Meyrick et al., 1980). A rise in PVR is first noted in the second week, accompanied by extension of smooth muscle into normally non-muscularised pulmonary arteries (Rosenberg & Rabinovitch, 1988). By Week 3, substantial vascular remodelling is present, characterised by medial thickening of larger arteries and loss of peripheral vessels (Meyrick et al., 1980). RVH also becomes apparent at this point and progresses rapidly to RV failure and death. A mortality rate of almost 100% within 6–8 weeks was reported in one study (Urboniene et al., 2010). Lowering the dose of MCT (20–40 mg·kg−1) reduces the extent of pulmonary vascular remodelling (Buermans et al., 2005; Ruiter, de Man, et al., 2013), producing an adaptive RVH, which, contrary to the progressive nature of human PH, spontaneously reverses after 4 weeks (Ruiter, de Man, et al., 2013).
A more severe variation of the MCT model involves combination with pneumonectomy. In this model, MCT treatment is accompanied by surgical removal of one lung, causing the entire CO to flow through the residual lung. Compared with MCT administration alone, there is greater elevation of PAP, more advanced medial hypertrophy and an increased burden of plexiform-like lesions (Okada et al., 1997; White et al., 2007), presumably due to increased shear stress. Similar histological changes are seen when MCT is combined with CH (Morimatsu et al., 2011) or endotelin (ET)-1 receptor (ET$_B$ deficiency (Ivy et al., 2005). Attempts to establish a mouse model of MCT-PH have been met with limited success. Differences in hepatic metabolism necessitate weekly administration at a much greater dose ($\geq 300 \text{ mg kg}^{-1}$; Goto et al., 2002). Even with direct administration of MCTP, mice develop acute lung injury, but not overt PH (Dumitrascu et al., 2008).

The MCT model has been particularly useful in advancing our understanding of the pathophysiology of pulmonary vascular remodelling. For example, mirroring human PH, MCT rats display increased expression of ET-1 and blunted NO-dependent relaxation in pulmonary arteries (Mathew et al., 1995), as well as reduced BMPR-II signalling in the lung (Ramos et al., 2008). This model has also revealed a pivotal role for inflammatory cells in the initiation of vascular remodelling (Kimura et al., 1998). However, it is important to note that the pathological consequences of MCT administration include some clear divergences from the clinical condition. There is a greater involvement of the lung parenchyma (fibrosis, hypoxaemia, alveolar oedema and septal cell hyperplasia), a greater perivascular inflammatory component and a complete absence of complex lesions (Carman et al., 2019; Wilson et al., 1992).

The broad toxicity of MCT also gives rise to numerous extrapulmonary features that may contribute to mortality, including hepatic veno-occlusive disease, renal insufficiency and biventricular myocarditis (Gomez-Arroyo, Farkas, et al., 2012). The direct cardiac effects of MCT are a particular problem and, further to myocarditis, coronary arteriolar thickening has also been noted (Akhvein et al., 2007; Miyauchi et al., 1993), complicating the study of RV failure in this model. Indeed, the rapid progression to RV failure and death may preclude the development of compensatory mechanisms that manifest over many years in human PH (Ryan et al., 2011). This may explain why dozens of new therapeutic agents have shown a robust prevention and reversal of PH in MCT-treated rats that has not translated into the clinic (Hill et al., 2017; Maarman et al., 2013).

### 6 | CHRONIC HYPOXIA

The link between low atmospheric oxygen levels and PH has been long established in communities living at high altitude (Arias-Stella & Saldana, 1963). CH has also been linked to the development of PH in patients with chronic lung conditions, including idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD) and sleep apnoea (Zhao, 2010). In fact, many species develop PH in response to alveolar hypoxia, though the severity of the phenotype varies considerably (Grover, 1965; Hoshikawa et al., 2003).

From an experimental standpoint, the exposure of rats to CH is a commonly used approach to study PH, due to the reproducibility of the response, for a given strain (Stenmark et al., 2009). Rats housed in hypoxic conditions (10% O$_2$) develop increased RVSP, pulmonary vascular remodelling and RVH, which stabilise within 3–4 weeks (West & Hemnes, 2011). Histological changes include stiffening of larger pulmonary arteries (due to medial hypertrophy and adventitial fibrosis), small vessel muscularisation and perivascular inflammation (Danielle et al., 2007; Drexler et al., 2008; Meyrick & Reid, 1980; Stenmark et al., 2009).

In general, rats tolerate CH remarkably well, despite exposure to a level of hypoxia exceeding that of human disease states. Indeed, a major limitation of the CH rat model is the modest phenotype, particularly when compared with human PAH (Maarman et al., 2013). Interestingly, there are significant strain differences in the response to hypoxia. Of the two most common strains, Sprague–Dawley (SD) rats demonstrate greater increases in RV mass and PVR than Wistar rats (Jin et al., 1990). In contrast, the Fischer strain is largely resistant to hypoxic PH (He et al., 1991), whereas Fawn-hooded rats are notable for developing a spontaneous, severe PH that is accelerated by hypoxia (Sato et al., 1992). Regardless of strain, pulmonary endothelial architecture is largely unaltered and obstructive intimal remodelling is correspondingly absent (Voelkel & Tuder, 2000). RV failure, which is inevitable in the clinic without therapeutic intervention, is also lacking (Stenmark et al., 2009), and there is no evidence of mortality (Zhao, 2010).

Despite an increase in RVSP, mice exposed to CH develop mild pulmonary vascular remodelling, even compared with that induced in rats (Voelkel et al., 1996). This species difference may be driven by distinct gene expression profiles. Microarray gene analysis suggests that the hypoxic rat lung is primarily characterised by increased expression of genes controlling endothelial cell proliferation, whereas the predominant change in the mouse lung is a down-regulation of genes responsible for vascular smooth muscle proliferation and vasodilation (Hoshikawa et al., 2003). Irrespective of these differences, the PH phenotype induced by CH is reversible upon return to normoxia in both species (Voelkel & Tuder, 2000).

In sum, despite its frequent employment in the study of PAH, the caveats of the CH model render its best use in the investigation of less severe forms of PH, namely, those associated with chronic lung diseases (Group 3).

### 7 | SUGEN + HYPOXIA

Attempting to recreate the severe pathology of human PAH, Taraseviciene-Stewart et al. combined CH exposure with administration of the VEGF receptor-2 (VEGFR-2) antagonist, Sugen 5416. Although VEGF inhibition produces only mild PH in normoxic rats, administration of Sugen to chronically hypoxic rats results in severe
and progressive pulmonary vascular remodelling that is irreversible, even with return to normoxia (Taraseviciene-Stewart et al., 2001).

A common protocol for the Sugen + hypoxia (SuHx) model involves a single subcutaneous injection of Sugen (20 mg·kg⁻¹), followed by 3–4 weeks of hypoxic exposure (10% oxygen), after which animals are returned to normoxia for 2–3 weeks (de Raaf et al., 2014; Nicolls et al., 2012). However, as VEGFR-2 plays a pro-survival role in endothelial cells, selective death of pulmonary artery endothelial cells is thought to be the initial trigger for vascular remodelling (Voelkel et al., 2002). This enriches a subpopulation of apoptosis-resistant endothelial cells, which begin to proliferate extensively (Sakao et al., 2005), forming complex ‘angio-obliterative’ vascular lesions comparable with the plexiform lesions seen in PAH (Abe et al., 2010; Vitali et al., 2014). This common histological pattern of intimal thickening may underpin the shared lack of response to vasodilators, such as iloprost and NO, in SuHx rats and PAH patients (Oka et al., 2007).

In addition to developing extensive vascular disease, RV maladaptive remodelling is evident in SuHx rats within 6 weeks, demarcated by RVH, increased RV diastolic diameter and concomitant reductions in TAPSE and CO (Bogaard et al., 2010; Bogaard, Natarajan, et al., 2009). Notably, background strain has a profound influence on the RV adaptation to overload in the SuHx model. Despite comparable elevations in RVSP, occlusive pulmonary vascular lesion burden and RVH, Fischer rats exposed to SuHx develop rapid RV failure leading to near 100% mortality by 7–8 weeks, whereas SD rats show complete survival up to 13 weeks (Jiang et al., 2016; Suen et al., 2019). The propensity of Sugen to exacerbate experimental PH led to combination with several insults other than hypoxia, including T-cell deficiency (Taraseviciene-Stewart et al., 2007), ovalbumin sensitisation (Mizuno et al., 2012), pneumonectomy (Happé et al., 2016) and morphine administration (Agarwal et al., 2020).

Naturally, attempts have been made to recreate the SuHx model in mice, though results have been mixed. Administration of three consecutive weekly doses of Sugen is required for the development of PH in mice (Ciucan et al., 2011), which may be due to greater systemic and renal clearance of Sugen when compared with the rat (Ye et al., 2006). Although mice treated with such a regimen do exhibit arteriolar muscularisation (Ciucan et al., 2011), significant angio-obliterative lesions do not occur, and SV and CO are maintained, indicating that RV dysfunction does not derail into failure (Vitali et al., 2014; Wang et al., 2018). Unlike the rat, the pathological changes seen in mice are also reversible upon return to normoxia (Ciucan et al., 2011).

8 | PULMONARY ARTERY BANDING

Pulmonary artery banding (PAB) was initially described for the surgical palliation of infants born with cardiac defects characterised by significant left-to-right shunting and pulmonary overcirculation (Muller & Dammann, 1952). This surgery has since been replicated in animals to create a model of pure RV pressure overload (LekanneDeprez et al., 1998). PAB involves mechanical constriction of the pulmonary trunk with a suture tie or metal clip (Figure 2), leading to increased RV afterload in the absence of pulmonary vascular remodelling (Guihaire et al., 2013).

The PAB model is ideal for evaluating potential cardioprotective molecules as an adjunct to traditional therapies. Although rats

![Figure 2](https://example.com/figure2.png)
exposed to MCT or SuHx develop RV failure, direct cardiac effects of potential therapies cannot be distinguished from PVR-driven afterload modification using these models, due to the functional coupling of the right ventricle and pulmonary vasculature (Andersen et al., 2018). The fixed afterload produced by PAB nullifies this coupling, permitting RV remodelling and its potential for reversal, to be studied independently. PAB also avoids potential confounding effects of hypoxia, VEGF inhibition and MCT toxicity on RV function (Borgdorff, Dickinson, et al., 2015).

PAB is an extremely versatile model as, by changing the diameter of constriction, severity can be altered as desired, from adaptive RVH to decompensated RV failure (Andersen et al., 2018). Modest banding leads to increased RVSP and RVH, limited RV dilatation and fibrosis, and preservation of TAPSE and CO (Bogaard, Natarajan, et al., 2009; Faber et al., 2006; Schäfer et al., 2009), consistent with a chronically compensated pressure overload. In support of this notion, survival has been noted up to 22 weeks, despite substantially elevated RVSP and a doubling of RV weight (Bogaard, Natarajan, et al., 2009). In contrast, restrictive banding leads to overt RV fibrosis, dilatation and diastolic dysfunction, with concomitant reductions in TAPSE and CO, and clinical symptoms of RV failure (Akazawa et al., 2020; Borgdorff, Koop, et al., 2015; Hirata et al., 2015). In addition to reductions in forced high intensity exercise (Piao et al., 2012) and voluntary low-intensity exercise (Borgdorff et al., 2012; Borgdorff, Bartelds, Dickinson, Steendjik, de Vroemen, et al., 2013), inactivity, reduced grooming, dyspnoea/tachypnoea, poor peripheral circulation, ascites and pleural/peri-cardial effusions have been reported, culminating in a high mortality rate (Borgdorff, Bartelds, Dickinson, Steendjik, de Vroemen, et al., 2013; Borgdorff, Koop, et al., 2015; LekanneDeprez et al., 1998; Schou et al., 2007). These pathological changes have also been replicated in mice (Boehm et al., 2017, 2020; Egemnazarov et al., 2015; Kapur et al., 2013; Urashima et al., 2008).

One limitation of this model is the sudden nature of afterload elevation, which does not reflect the gradual increase in PVR observed in PH. This can be offset with the use of juvenile animals, as growth of the animal around a mildly constricted band produces a progressive increase in afterload (Andersens, 2018; Bogaard, Natarajan, et al., 2009). Nevertheless, the proximal occlusion around the pulmonary trunk is more representative of pulmonary valve stenosis than the small vessel remodelling that drives PH. These issues are pertinent, as distinct loading patterns producing varying patterns of RV adaptation to pressure overload (Borgdorff, Bartelds, Dickinson, Steendjik, de Vroemen, et al., 2013). Accordingly, PAB requires great technical skill, as the positioning of the constriction on the pulmonary trunk must be carefully replicated to ensure reproducibility.

9 | SCHISTOSOMIASIS

Owing to the large number of Schistosoma infections in resource-poor areas, schistosomiasis is a leading cause of PAH worldwide (Fernandes et al., 2011). Schistosoma-associated PAH (Sch-PAH) is likely to result from a combination of several factors. Obstruction of the portal system and pre-hepatic vasculature by Schistosoma eggs opens portosystemic shunts, increasing shear stress on the pulmonary vasculature and facilitating egg embolisation into the lung (Andrade & Andrade, 1970). Although rats are largely resistant to Schistosoma infection (Khalife et al., 2000), the susceptibility of mice to schistosomiasis and subsequent development of models of Sch-PAH has greatly expanded understanding of the underlying mechanisms, particularly the role of systemic type 2 inflammation (Sibomana et al., 2020).

Crosby et al. characterised the development of PH in an established model of transcutaneous infection with *Schistosoma mansoni* larvae (cercariae; Smithers & Terry, 1965). After 12 weeks, several features of the human disease were present, including marked pulmonary vascular remodelling, elevated plasma Th1 and Th2 cytokines, perivascular inflammation and, in some cases, plexiform-like lesions (Crosby et al., 2010). Overall, there was no change in RVSP or in Fulton index up to 20 weeks after infection, though a later study found significant increases in both parameters after 25 weeks of infection (Crosby et al., 2011).

To generate a more robust PH phenotype, Graham et al. employed the immunological procedure of antigen sensitisation followed by antigen challenge. In their model, mice were sensitised by transcutaneous infection with *S. mansoni* cercariae and then challenged 55 days later with an intravenous injection of *S. mansoni* eggs (Graham et al., 2010). The resultant pathology included pulmonary arterial remodelling, Th2 inflammation and significantly increased RVSP, though plexiform-like lesions and RVH were notably absent in wild-type mice. This model has since been refined by intraperitoneal sensitisation with an optimised dose of *S. mansoni* eggs, improving the homogeneity of egg deposition in the lung and reducing the sensitisation window to 14 days (Chabon et al., 2014; Graham, Chabon, Gebreab, et al., 2013; Graham, Chabon, Kumar, et al., 2013; Kassa et al., 2019; Kumar et al., 2015, 2017, 2019).

The role of type 2 inflammation in PH has been fortified by the characterisation of pulmonary vascular remodelling in models of allergic asthma (ovalbumin, *Aspergillus fumigatus* and house dust mite exposure). Although these models develop increased RVSP, medial thickening, muscularisation of small vessels (Daley et al., 2008; Rydell-Törnänen et al., 2008; Törnänen et al., 2005) and in some cases occlusive neointimal lesions (Steffes et al., 2020), robust morphological changes to the right ventricle are lacking.

10 | LEFT HEART DISEASE

Due to a passive backward transmission of increased filling pressures into the pulmonary vasculature (Vachíery et al., 2013), PH is a frequent complication of left heart disease (LHD), considerably worsening outcome (Guazzi & Borlaug, 2012). The prognostic value of TAPSE in patients with heart failure with preserved ejection fraction (HFrEF) and heart failure with reduced ejection fraction (HFrEF; Damy et al., 2012) suggests that therapeutic modulation of the right ventricle would be beneficial in PH-LHD (Group 2).
PH-HFpEF is increasingly recognised as a clinical complication of metabolic syndrome, a cluster of abnormalities including obesity, dyslipidaemia, hyperglycaemia, insulin resistance and systemic hypertension (Ussavarungsi et al., 2017). Consequently, Lai et al. developed a two-hit PH-HFpEF model in which obese ZSF1 (double-leptin receptor defect) rats were administered SU5416. Fourteen weeks later, ZSF1 rats had elevated RVSP. LV end-diastolic pressure and mean arterial BP, preserved LV ejection fraction, pulmonary vascular remodelling and hypertrophy (Lai et al., 2016). The same group subsequently developed a murine model of metabolic syndrome-associated PH-HFpEF, with comparable haemodynamic disturbances, by administering high-fat diet (HFD) to AKR/J mice (Meng et al., 2017). The functional state of the right ventricle in these models remains undocumented. Recent efforts have seen metabolic syndrome and PH induced in supracoronary aortic banded rats by a combination of HFD and the anti-psychotic agent olanzapine. RVH has not been comprehensively assessed in this model, but decreased TAPSE is evident by echocardiography, consistent with RV mechanical decline (Ranchoux et al., 2019).

Left anterior coronary artery ligation, a common method of inducing MI, has been widely used to study PH-HFpEF in rats (Jasmin et al., 2003; Jiang et al., 2010; Kemi et al., 2013; Rossconi et al., 2007; Yin et al., 2011). Recently, Phillip et al. sought to characterise the development of PH in the murine equivalent of this model. Following coronary artery ligation, mice demonstrated a moderate increase in RVSP and right ventricle/body weight ratio (Philip et al., 2019). The Fulton index was unchanged in infarcted mice, indicating that RVH developed in proportion to LV remodelling. Indeed, LV wall motion score index (WMSI) correlates positively with Fulton index and negatively with TAPSE in this model, signalling that the degree of RV dysfunction is closely linked to infarct size (Dayeh et al., 2018). As such, the magnitude of MI-induced LV dysfunction in the study of Phillip et al. may have been insufficient to induce RV mechanical insufficiency, as RVEF and stroke work (assessed by pressure–volume analysis) were preserved after MI.

11 | BLEOMYCIN

The clinical use of the anti-cancer agent bleomycin is limited by the development of pulmonary inflammation and fibrosis. Consequently, intratracheal instillation of bleomycin in rodents is commonly used as an experimental model of pulmonary fibrosis (Moore et al., 2013), which is also characterised by the development of secondary PH. The degree of RVSP elevation and RVH are comparable with the CH mouse model (Bubb et al., 2014), and medial thickening and vessel muscularisation are also evident in the pulmonary vasculature (Hemnes et al., 2008). A study in male mice reported a reduction in CO, concomitant with RV fibrosis (Hemnes et al., 2008), though this may be due to direct cardiac fibrogenic effects of bleomycin rather than pressure overload. In contrast, a further study found that CO was unchanged in multiple strains of female mice following bleomycin administration (Ortiz et al., 2002).

The bleomycin model has high translational potential for PH associated with parenchymal lung disease (Group 3), but may be hampered by early mortality (Hemnes et al., 2008) or spontaneous, progressive resolution of PH (Jarman et al., 2014), depending on the dose of bleomycin used. The recent profiling of pulmonary inflammation and fibrosis in a novel mouse model of PH combining administration of pristane (a mineral oil) with CH (Mori et al., 2020) may present an alternative model without the shortcomings of bleomycin administration.

12 | CIGARETTE SMOKE EXPOSURE

Over 90% of patients with COPD, the most common cause of Group 3 PH, have elevated mPAP (Chaouat et al., 2005). Cigarette smoke exposure is regarded as the gold standard model of COPD, sharing the same aetiology and much of the pathology of the human disease, including emphysema, airways remodelling and inflammation, and secondary PH (Wright et al., 2008).

Guinea pigs have been widely used in COPD due to comparable pulmonary anatomy and physiology to humans, though usage has declined recently in favour of transgenic mice (Ghorani et al., 2017). Guinea pigs exposed to cigarette smoke for 3 months develop elevated PAP, pulmonary vascular remodelling and RVH (Dominguez-Fandos et al., 2015; Weissmann et al., 2014). Three months of smoke exposure is also sufficient to induce elevated RVSP, medial hypertrophy and pruning of small vessels in mice, though RVH develops later, after 6 months of exposure (Seimetz et al., 2011). Although there is some evidence of decreased RVEF and diastolic dysfunction in mice (Sussan et al., 2009), RV failure has not been reported after smoke exposure. This is not surprising given the mild nature of the ensuing PH in these models (Gredic et al., 2021) and, importantly, the patient population (Chaouat et al., 2005; Thabut et al., 2005).

Although it is hypothesised that PH in COPD is secondary to emphysematous loss of the pulmonary vascular bed and/or hypoxia (Churg & Wright, 2009), Seimetz et al. (2011) reported no change in the lung function or alveolar structure of mice after 3 months of smoke exposure, indicating that PH precedes, and is not triggered by, emphysema. These investigators went further, demonstrating that cigarette smoke did not induce alveolar hypoxia or hypoxaemia. In a direct comparison of models, the loss of vessels seen in smoke-induced COPD was not mirrored in CH-induced PH. This disparity may result from significant differences in gene regulation of small pulmonary vessels—smoke exposure induces the expression of pro-apoptotic genes, whereas CH exposure leads to an increase of genes related to oxidative stress (Seimetz et al., 2011). A study in guinea pigs provides evidence of an alternative mechanism by which cigarette smoke triggers PH: direct and rapid effects on the expression of vasoconstrictive, vasodilatory and vasoproliferative mediators in the pulmonary vasculature (Ferrer et al., 2009).
13 | PULMONARY EMBOLISM

Chronic thromboembolic PH (CTEPH; Group 4) is characterised by obstruction of the major pulmonary vessels with unresolved, organised fibrotic thrombi and subsequent distal arteriopathy (Kim et al., 2019). CTEPH is difficult to model in vivo because of the vast adaptive and fibroinotic capacity of the pulmonary circulation and the inability of the right ventricle to overcome steep, abrupt increases in afterload (Mercier et al., 2013). To date, CTEPH studies have primarily been conducted in large animals, mostly piglets, using common techniques for studying pulmonary embolism, such as surgical pulmonary artery ligation (Fadel et al., 2000; Mercier et al., 2013) and microsphere injections (Mulchrone et al., 2019; Sato et al., 2008). However, in many cases, RV failure was not replicated, or RV function was not assessed. Rat models of CTEPH have also been created, using microspheres in conjunction with thrombin (Arias-Loza et al., 2016) or SU5416 (Neto-Neves et al., 2017) to induce PH and RVH. In the study of Neto-Neves et al., pulmonary embolism + VEGFR inhibition was associated with RV fibrosis, reduced CO and exercise intolerance, though only one animal was followed to the point of RV failure.

14 | GENETIC MODELS

Genetic manipulation of rodents has allowed investigation of the effects of down-regulation or overexpression of specific genes on the development of PH. Notably, studies in PH patients have identified associated gene mutations (e.g., BMPR-II and KCNK3), which have been recreated in animal models. Although this approach has proven incredibly useful for discerning the cellular and molecular signalling pathways involved in the pathogenesis of PH, the highly specific nature of transgenic models means that the complex pathology is rarely satisfactorily reproduced by manipulation of a single gene (Voelkel et al., 2012) and a secondary stimulus (such as hypoxia) is often required to demonstrate a robust phenotype. This does at least parallel the clinical scenario: mutations in the BMPR-II gene are present in ~80% of patients with a family history of PAH and up to 20% of supposedly sporadic cases (Evans et al., 2016), yet 80% of carriers never develop significant disease (Hamid et al., 2009).

14.1 | BMPR-II

BMPR-II is a serine/threonine receptor kinase that binds members of the TGF-β superfamily of ligands (Fessell et al., 2011). Reduced levels of BMPR-II have been described in a variety of non-genetic experimental models of PH (Long et al., 2009; Morty et al., 2007; Takahashi et al., 2006). Attempts to generate globally BMPR-II-deficient mice were initially hampered by developmental issues (Beppu et al., 2000; Liu et al., 2007). Mice heterozygous for BMPR-II mutant alleles are viable but exhibit only a slight increase in RVSP, mild pulmonary arteriolar muscularisation and no RVH (Beppu et al., 2004). This phenotype can be exacerbated by administration of 5-HT (Long et al., 2006) or overexpression of 5-lipoxygenase (5-LO; Song et al., 2005).

To elicit a robust (yet viable) phenotype, mouse lines expressing inducible, smooth muscle-specific, dominant negative alleles for the BMPR-II gene have been generated. One such line, SM22-tet-BMPR2R899X, demonstrates increased RVSP and RVH, albeit in the absence of significant pulmonary arterial remodelling (West et al., 2004). On the other hand, SM22-rtTA x TetO7-BMPR2R899X mice exhibit substantial pulmonary vascular pruning, remodelling and perivascular inflammation (West et al., 2008). Conditional deletion of BMPR-II in endothelial cells also leads to increased RVSP and RVH and an increase in the number and thickness of distal muscularised arteries, though only in a subset of mice (Hong et al., 2008). Despite the modest phenotype, the development of PH following restricted deletion of BMPR-II in two distinct cell types exemplifies the pathophysiological importance of this signalling pathway.

A more recent advance has been the inception of BMPR-II mutant rats, which were developed on the basis that rats are more sensitive to PH than mice. The first line carrying a monoallelic mutation in BMPR-II was created by Hautefort et al. In parallel to human familial PH, a subset of these rats develop age-dependent, spontaneous PH. Other findings include a susceptibility to CH-induced PH and a predisposition to contraction in isolated pulmonary arteries. Notably, BMPR-II mutation also has direct right ventricle-specific cardiac effects, including a reduction in cardiomyocyte diameter, altered calcium handling, decreased calcium sensitivity and a shortening of action potential duration (Hautefort et al., 2019). Two further strains of monoallelic BMPR-II mutant rats have since been generated (Tian et al., 2019) though they do not develop age-dependent PH (in the first year of life) nor do they show exaggerated responses to CH, SU5416 alone, SuHx or MCT. Pulmonary 5-LO overexpression, however, elicits severe PAH complete with angio-occlusive lesions in both mutant strains, but not in wild-type rats.

14.2 | S100A4/Mst1

S100A4/Mst1 (Mst1) belongs to a family of calcium-binding proteins that have been implicated in cellular proliferation, migration and angiogenesis (Donato et al., 2012). Consequently, mice overexpressing Mst1 were initially developed with the aim of elucidating a role in metastatic cancer (Ambartsouman et al., 1999; Ambartsouman et al., 2005). Greenway et al. (2004) later reported that Mst1 overexpression leads to a mild increase in RVSP and pulmonary vascular remodelling, including the development of plexiform-like lesions in a subset of mice. These changes occur almost exclusively in females, consistent with greater pulmonary arterial expression of Mst1 in female mice (Dempsie et al., 2011). There are discrepancies as to the presence of RVH in Mst1 overexpressing mice. Although Merklinger et al. (2005) demonstrated increased RVH, two further studies failed to show a difference to control mice (Dempsie et al., 2011; Spiekerkoetter et al., 2008). Both Mst1 and receptor of advanced glycation end-products (RAGE), a receptor for which Mst1 is a ligand,
have been implicated in the pathogenesis of human PH (Greenway et al., 2004; Meloche et al., 2013; Moser et al., 2014).

### 14.3 | IL-6

Inflammatory cells can be found within the lungs of humans and animals with PH, both in vascular lesions, and organised into perivascular lymphoid aggregates (Perros et al., 2012; Rabinovitch et al., 2014). The pro-inflammatory cytokine IL-6 is elevated in human and experimental PH, in correlation with disease severity (Humbert et al., 1995; Miyata et al., 1995; Soon et al., 2010). Other pro-inflammatory conditions associated with PH are also characterised by increased levels of IL-6, including systemic lupus erythematosus (Yoshio et al., 1997) and scleroderma (Gourh et al., 2009).

Steiner et al. investigated the development of PH in a mouse model of IL-6 overexpression. These mice demonstrated increased RVSP, pulmonary vascular remodelling and RVH, all of which were greatly worsened by exposure to hypoxia. The pulmonary vasculopathy was characterised by an increased muscularisation throughout the pulmonary vascular bed and the formation of occlusive neointimal angio proliferative lesions composed of endothelial cells and T lymphocytes. These changes were accompanied by the activation of pro-angiogenic, pro-proliferative and anti-apoptotic pathways (Steiner et al., 2009). Consistent with these findings, IL-6 knockout mice are resistant to the development of CH-induced PH (Savale et al., 2009). However, a Phase 2 study of anti-IL-6 therapy in PAH (TRANSFORM-UK) ultimately reported no overall efficacy in PAH patients (although the approach may benefit certain subpopulations; EudraCT number: 2015-002799-26, EU Clinical Trials Register). As such, immunomodulation may only be beneficial in subpopulations of PH patients with strong inflammatory phenotypes.

### 14.4 | KCNK3

Beyond BMPR-II, numerous loss-of-function mutations have been identified in the KCNK3 (K2P3.1 channel) gene, which encodes an outward rectifier K⁺ channel, in PAH patients (Ma et al., 2013; Navas Tejedor et al., 2017). The KCNK3 channel has a role in the regulation of resting membrane potential in several cell types, including pulmonary arterial smooth muscle cells (Lambert et al., 2018). Although KCNK3 does not form a functional channel in the mouse (Manoury et al., 2011), expression is evident in the pulmonary vasculature of rats (Antigny et al., 2016). Recently, Lambert et al. described a KCNK3 mutated rat with mild elevations in RVSP (that could be potentiated by MCT or CH) and pulmonary vascular remodelling, including distal vessel muscularisation and perivascular collagen deposition. In addition, isolated pulmonary arteries from these rats demonstrated a blunted response to vasodilators. Notably, RVH was absent and CO was maintained, though on a cellular level, RV cardiomyocyte excitability was curtailed (Lambert et al., 2019).

### 14.5 | HIF/PHD2

The hypoxia-inducible factor (HIF) prolyl hydroxylase domain enzymes (PHDs), namely, PHD2, regulate the stability of HIF-α subunits in an oxygen-dependent manner (Berra et al., 2003; Takeda et al., 2007). Under hypoxic conditions, inhibited PHD activity results in stabilisation and accumulation of HIF-1α and HIF-2α in the nucleus (Shimoda & Semenza, 2011), leading to the expression of target genes that regulate angiogenesis, erythropoiesis, metabolism, inflammation and vascular responses (Majmundar et al., 2010). The development of PH is delayed in HIF-1α−/− mice exposed to hypoxia (Yu et al., 1999), and HIF-2α−/− mice are completely protected against hypoxic PH (Brusselmans et al., 2003). Taken together, these findings suggest that HIF-α activation plays a key role in the pathogenesis of PH. In support of this notion, mice carrying gain of function mutations in HIF-2α display erythrocytosis and PH, characterised by elevated RVSP, increased RV wall thickness and a small degree of pulmonary vascular remodelling (Tan et al., 2013).

Although global deficiency in PHD2 is lethal during development (Takeda et al., 2006), patients with PAH demonstrate reduced expression of PHD2 in endothelial cells of obliterator pulmonary vessels (Dai et al., 2016), validating the use of endothelial-specific Egln1 (encoding PHD2) knockout mice. Two such strains have been developed. Egln1CdhsCre and Egln1Tie2, both of which develop spontaneous PH. Kapitsinou et al. first described Egln1CdhsCre mice, reporting markedly elevated RVSP and RVH compared with controls, accompanied by a greater number of muscularised peripheral pulmonary arteries. Despite the large haemodynamic changes, no lumen-obliterating lesions were found (Kapitsinou et al., 2016). Egln1Tie2 mice exhibit even greater increases in RVSP and RVH, and extensive pulmonary vascular remodelling, including vascular occlusion and plexiform-like lesions. Echocardiography reveals a threefold increase in RV wall thickness, marked chamber dilatation and a decrease in RV fractional area change, implying that RV failure underlies the progressive mortality observed in these mice (Dai et al., 2016). This striking phenotype is perhaps the best murine representation of clinical PAH to date.

### 15 | LARGE ANIMAL MODELS

Large animal models, such as CH in calves (Wohrley et al., 1995), chronic embolism in pigs (Mercier et al., 2013) and simian immunodeficiency virus-associated PAH in primates (George et al., 2011), share common features of human PH, including the development of complex pulmonary vascular lesions and RV failure, and have significantly advanced our understanding of this condition. However, the large size and cost of these species are prohibitive in target
validation. As such, they are typically reserved for confirmatory safety and efficacy studies prior to translating novel drug candidates to clinical trials (van der Velden & Snibson, 2011).

16 | MECHANISMS OF RV FAILURE: LESSONS FROM ANIMAL MODELS

In PH, the chronic, progressive increase in afterload is the principal driver of RV failure, exemplified by the near total recovery of RV homeostasis just weeks after lung transplantation (Kasimir et al., 2004) or pulmonary endarterectomy (Iino et al., 2008). Still, there is much heterogeneity in the response of the RV to pressure overload. Despite comparable mPAP, patients with Eisenmenger’s syndrome exhibit RVH from birth yet remain free from RV failure for decades (Hopkins et al., 1996), whereas scleroderma–PAH patients rapidly develop severe RV failure (Overbeek et al., 2008). Indeed, there are also disparities in the degree of RVH, CO reduction and likelihood of progression to RV failure between different models of RV pressure overload that cannot be solely explained by differences in afterload (Bogaard, Natarajan, et al., 2009). The cellular and molecular events dictating the transition of RV remodelling into a state of maladaptation are largely unknown. Emerging evidence from experimental models suggests that an interplay of factors such as metabolic alterations, inflammation and oxidative damage may contribute to the development of RV failure (Figure 3).

16.1 | Initial adaptation

Cardiomyocytes detect pressure overload through integrins and stretch-sensitive ion channels, which act as ‘mechanosensors’, converting mechanical stress into chemical signals by activating numerous intracellular messengers (Mann, 2004; Ross et al., 1998). Evidence from experimental models suggests that distinct integrin-dependent pathways are stimulated under different loading conditions. Umar et al. report that integrin-dependent activation of focal adhesion kinase (FAK) drives the adaptive hypertrophic response of the myocardium in rats treated with low-dose MCT. In contrast, rats treated with high-dose MCT develop RV failure that is predominantly driven by integrin-dependent induction of neuronal NOS, sustained NO production and subsequent up-regulation of matrix metalloproteinases (MMPs; Umar et al., 2007). Downstream signalling by MAPKKK-2 (MEKK-2) is thought to be important in mechanosensation, as MEKK-2 null mice are protected from hypoxia-induced RVH and exhibit diminished myocardial expression of inflammatory genes (Dale Brown et al., 2013).

The influx of calcium through stretch-activated ion channels leads to the activation of PKC and modulation of gene expression (Hu & Sachs, 1997; Ruwhof & van der Laarse, 2000; Tatsukawa et al., 1997). Sustained increases in cytosolic calcium also serve to activate calcineurin, leading to activation of the transcription factor, GATA4. GATA4 is well described as a mediator of LV hypertrophy (Crabtree, 1999; Molkentin, 2004), yet its role in RVH remains to be further elucidated. However, GATA4 expression is known to be

**FIGURE 3** Putative mechanisms of right ventricular (RV) maladaptation. [Correction added on 27 May 2021, after first online publication: Figure 3 has been replaced with Figure 2 in this current version.]
enhanced in the rat right ventricle following PAB or exposure to CH (Park et al., 2010). Modulatory calcineurin-interacting protein-1 (MCIP1) expression is also increased in the right ventricle of mice subjected to PAB, indicating calcineurin activation (Bartelds et al., 2011).

The signalling pathways activated by mechanosensation terminate with the increased synthesis of contractile proteins. The assembly of new sarcomeres leads to increased cardiomyocyte size, the cellular basis for RVH (Bogaard, Abe, et al., 2009). Elevated protein synthesis is accompanied by the re-emergence of a fetal gene expression pattern (Friedberg & Redington, 2014), hallmarked by a transition from α-myosin heavy chain (MHC) expression to the slower and less active β-MHC, which has lower energy requirements (Korte et al., 2005; Lowes et al., 1997).

16.2 Angiogenesis and ischaemia

RV myocardial underperfusion is evident in severe PH (van Wolferen et al., 2008), with some patients experiencing ischaemia (Go et al., 2001; Torbicki et al., 2003). This may be explained, in part, by increased cellular oxygen consumption and diffusion distance secondary to RVH (Rain et al., 2014; Ruitter, Ying Wong, et al., 2013). Studies in experimental models of PH suggest that a decreased capillary density (due to impaired angiogenesis and/or loss of small vessels) may also contribute to macrovascular ischaemia in the right ventricle.

In a comparison of the PAB and MCT rat models, Sutendra et al. demonstrated an up-regulation of the HIF-1α/VEGF axis in adaptive RVH, leading to angiogenesis and greater blood supply. However, RV failure was accompanied by a marked reduction in HIF-1α expression and ultimately a suppression of angiogenesis (Sutendra et al., 2013). The findings of Sutendra et al. are supported by a study reporting RV myocardial capillary rarefaction in SuHx rats, but not PAB rats. The authors attributed this finding to the presence of RV failure in the SuHx model (indicated by a fall in CO) versus adaptive RVH in the PAB model (Bogaard, Natarajan, et al., 2009). RV capillary density was also unaffected in a murine model of LHD-PH and adaptive RVH (Philip et al., 2019). Of course, it could be argued that the reduced capillary density in the SuHx model is directly linked to VEGFR blockade, though SuHx exposure does not affect LV capillary density, and S5416 treatment does not decrease RV myocardial capillary density in normoxic or PAB rats (Bogaard, Natarajan, et al., 2009). Interestingly, microRNA-126, an angiogenic mediator that disinhibits the VEGF pathway, is down-regulated in RV tissue from patients with decompensated, but not compensated, RVH. Likewise, up-regulation of microRNA-126 in MCT rats increases RV capillary density to near control levels and improves RV function (Potus et al., 2015).

Recently, assessment of the RV vasculature by stereology in human PAH and SuHx rats revealed that augmentation of the vascular network was in proportion with RVH (Graham et al., 2018; Naing et al., 2017), calling in to question the conclusions previously drawn from analysis of 2D sections. Still, several other mechanisms may contribute to ill-perfusion of the failing right ventricle, including systemic (aortic) hypotension, a reduction in the perfusion gradient of the right coronary artery and compression of the left coronary artery by the pulmonary artery (Galiè et al., 2017; van Wolferen et al., 2008).

16.3 Metabolic adaptations

LV dysfunction is associated with a switch in cardiomyocyte metabolism, from predominantly mitochondrial-based fatty acid oxidation (FAO) to glycolysis (the ‘Warburg effect’; Dávila-Román et al., 2002). Reduced FAO (Kim et al., 1997) and increased glucose uptake (Oikawa et al., 2005) have also been noted in the right ventricle of PH patients. The high rate of glycolysis may be an adaptive mechanism to acutely reduce O₂ demand, albeit at the expense of ATP yield. Comparison of the RV metabolic profiles of PAB rats and MCT rats indicates that maladaptation is ultimately accompanied by a decline in glucose uptake and glycolysis, favouring the development of RV ischaemia (Sutendra et al., 2013).

Kim et al. (1997) report that FAO depression was present only in patients with severe RVH. In parallel, Gomez-Arroyo et al. (2013) demonstrated that expression of key genes required for FAO was diminished in SuHx-RVH with reduced TAPSE but unchanged in PAB-RVH. However, there are conflicting reports of the metabolic profile of PAB rats, as a further study reported increased FAO (Fang et al., 2012), whereas another reported a decrease (Faber et al., 2005). This discrepancy may be related to the variable severity of this model.

In vivo, uncoupling of glycolysis from the Krebs cycle has been linked to increased activity of pyruvate dehydrogenase kinase (PDK), an inhibitor of the mitochondrial enzyme pyruvate dehydrogenase (PDH), the gatekeeping enzyme of glucose oxidation, and results in reduced RV function (Piao et al., 2010; Sutendra et al., 2013). Although the PDK inhibitor dichloroacetate (DCA) partly restored CO in PAB rats, even greater improvement in RV function is achieved in the MCT model (Piao et al., 2010), presumably due to a previously reported reversal of pulmonary vascular remodelling (McMurty et al., 2004). Indeed, a first-in-human trial in patients with idiopathic PAH (IPAH) reported that DCA can reduce mPAP and PVR and increase RVF. However, the clinical response to DCA was limited by common single-nucleotide polymorphisms associated with deficiencies in sirtuin 3 (SIRT3; the main mitochondrial deacetylase) and uncoupling protein 2 (UCP2; increases mitochondrial calcium), both of which activate PDH in a PDK-independent manner (Michelakis et al., 2017).

16.4 Oxidative stress and mitochondrial dysfunction

Mitochondria regulate numerous processes in the cellular response to stress, including inflammation, induction of apoptosis and formation of ROS (Dromparis & Michelakis, 2013). RV mitochondrial membrane potential (a marker of overall mitochondrial function) is lower than that of the left ventricle in health but is increased in patients with
PH. Mitochondrial hyperpolarisation is also evident in the RV myocardium of MCT rats, where it has been linked to activation of the transcription factor NFAT; Nagendran et al., 2008).

In the left ventricle, ROS contribute to maladaptive remodelling through processes of apoptosis, myofibroblast stimulation and activation of MMPs, culminating in reduced contractile function (Tsutsui et al., 2011). NADPH oxidase and mitochondrial complex II are significant sources of ROS production in the right ventricle of MCT rats (Redout et al., 2007). Mitochondrial ROS production is also stimulated by ischaemia (Zelt et al., 2019), providing a putative link to altered angiogenesis and metabolism in the pressure-overloaded right ventricle. In support of this notion, HIF-1α activation has been linked to complex II-mediated ROS production in MCT-RVH (Redout et al., 2007; Sutendra et al., 2013). Similarly, reduced expression of PGC-1α in SuHx rats has been linked to decreased mitochondrial biogenesis and increased mitochondrial ROS production, as well as decreased FAO (Gomez-Arroyo et al., 2013).

In vivo evidence indicates that maladaptive RVH is associated with impaired anti-oxidant defence. In comparison with PAB, rats with SuHx-induced PH exhibit reduced RV expression of the anti-oxidant transcription factor Nrf2 and its target gene haem oxygenase (HO)-1. In this model, oxidative stress is associated with RV apoptosis, increased fibrotic burden and poorer RV function (Bogaard, Natarajan, et al., 2009). Studies in MCT rats suggest that failure of anti-oxidant defences is an early event in RV pressure overload. In the adaptive RVH phase, SOD and GSH peroxidase are inactive (Ecarnot-Laubriet et al., 2003; Pachard et al., 1999), predisposing to ROS-induced damage. This damage can be therapeutically targeted: administration of EUK-134, a SOD/catalase mimetic, reduces RV oxidative stress, leading to diminished proapoptotic signalling, decreased interstitial fibrosis and improvements in RVEF and TAPSE (Redout et al., 2010).

16.5 | Neurohormonal signalling

Akin to LV failure, elevated levels of circulating catecholamines and sympathetic nerve activation correlate with disease severity in patients with RVH and PAH (Ma et al., 2012; Nootens et al., 1995; Velez-Roa et al., 2004). Sympathetic stimulation also activates the renin–angiotensin–aldosterone system (RAAS), and increased levels of angiotensin (Ang)-I, Ang-II and renin have also been associated with disease progression and mortality in PAH patients (de Man, Tu, et al., 2012). In the context of heart failure, the activation of the sympathetic nervous system (SNS) and RAAS is a compensatory response that initially preserves CO through mechanisms of increased inotropy, peripheral vasoconstriction, and salt and water retention. However, persistent activation of the SNS and RAAS produces a detrimental phenotype, characterised by receptor down-regulation, altered metabolism and apoptosis (Kimura et al., 2007).

Aberrant adrenergic signalling in the right ventricle is evident in animal models of PH, with a broad down-regulation of α1- and β1-adrenoceptors, plus dopamine D1 receptors, linked to impairment of inotropic reserve (Piao et al., 2012). Changes in receptor expression were found to be more profound in maladaptive RVH (SuHx and MCT) than adaptive RVH (PAB), in fitting with earlier findings in PAH patients (Bristow et al., 1992). However, robust evidence supporting the use of β-adrenoceptor blockers in PAH is lacking (Perros et al., 2017). Elevated expression of RAAS components has also been reported in the myocardium of MCT rats (Park et al., 2001). However, losartan, an AT1 receptor antagonist, failed to improve RV function in MCT rats, despite reducing afterload (de Man, Tu, et al., 2012). Furthermore, combination of losartan with an aldosterone blocker or adrenocortical blocker, mainstay treatments for LV failure, failed to improve RV structure and function in PAB rats with RV failure (Andersen et al., 2014; Borgdorff, Bartelds, Dickinson, Steendijk, & Berger, 2013). Another strategy is to combine an AT1 receptor antagonist (valsartan) with a neprilysin inhibitor (sacubitril), thereby augmenting endogenous natriuretic peptide levels. In SuHx rats, this approach was found to reduce pulmonary vascular remodelling, RVH and fibrosis, yet no effects on TAPSE, CO, exercise capacity or mortality were observed (Clements et al., 2019). Consistent results were found in PAB rats, whereby prophylactic treatment with sacubitril/valsartan attenuated the development of RVH, but did not affect fibrosis or SV (Sharifi Kia et al., 2020). Combining neprilysin inhibitors with conventional PAH therapy (PDE5 inhibition) shows promise in early clinical studies (Hobb et al., 2019).

16.6 | Inflammation

Although inflammation is known to play a prominent roles in LV failure and the vascular component of PAH (Poels et al., 2015; Rabinovitch et al., 2014), comparatively less is known about its role in RV remodelling. Histological analyses of RV tissue from patients with acute pressure overload induced by fatal pulmonary embolism demonstrate an accumulation of monocytes and macrophages (Iwadate et al., 2003; Watts et al., 2006). Widespread investigations have not yet been conducted in chronic pressure overload, but patients with scleroderma–PAH were found to have extensive RV inflammatory infiltration post-mortem, in comparison with IPAH patients (Overbeek et al., 2010). This is noteworthy given that RV systolic function and survival are also significantly worse with scleroderma–PAH, despite a comparable afterload to IPAH (Badesch et al., 2010; Tedford et al., 2013).

In experimental models, RV inflammation appears to coincide with maladaptive RVH; increased RV expression of the chemokine CCL2 and IL-6 has been reported in SuHx rats (Frump et al., 2015), yet myocardial inflammation was absent in rats treated with low-dose MCT (Brown et al., 2017). It was recently reported that RV failure-prone Fischer rats exposed to SuHx have a profound deficiency of NK cells in the RV myocardium, compared with SD rats (Suen et al., 2019). NK cells have previously been linked to angiogenesis in the placenta (Hanna et al., 2006) and may support RV adaptation to pressure overload by orchestrating vascularisation of the hypertrophied myocardium.
16.7 | Fibrosis

RV pressure overload is associated with fibroblast expansion, myofibroblast trans-differentiation and secretion of extracellular matrix (ECM) proteins (Frangogiannis, 2017). Fibrogenesis is likely to be secondary to cardiomyocyte death and sustained by pro-inflammatory signalling and neurohumoral influences (Baicu et al., 2012). Growth factor-mediated pathways may also play a role; for example, cardiomyocytes respond to hypertrophic stimuli by induction of TGF-β (Takahashi et al., 1994).

CMR imaging suggests the presence of RV fibrosis, linked to outcome, in patients with PH (Freed et al., 2012; Ozawa et al., 2017). Indeed, histological analysis of hearts explanted from PAH patients undergoing heart-lung transplantation has found modest increases in RV collagen deposition (Rain et al., 2013; van der Bruggen et al., 2016). Significant perivascular and interstitial RV fibrosis has also been noted post-mortem in end-stage RV failure (Gomez-Arroyo et al., 2014). Still, these studies are limited by relatively small sample sizes and provide little insight into earlier and compensated stages of pressure overload. Furthermore, it should be noted that the fibrotic burden of the right ventricle is much lower than that of the left ventricle in the setting of pressure overload (Vonk-Noordegraaf et al., 2013), which may underpin the substantial recovery of RV function in patients with severe RV failure following mechanical unloading (Kasimir et al., 2004).

In vivo studies may elucidate the fibrotic response of the compensated as well as the decompensated pressure-overloaded right ventricle. Interstitial and perivascular RV fibrosis are commonly observed in SuHx and MCT rats and to a lesser extent in PAB rats with adaptive RVH (Bogaard, Natarajan, et al., 2009; Borgdorff, Bartelds, Dickinson, Steendijk, de Vroomen, et al., 2013; de Man, Handoko, et al., 2012), suggesting that fibrotic remodelling is associated with decompensation of RV function. In support of this notion, RV fibrosis was absent in a mouse model of LHD-PH and adaptive RVH (Philip et al., 2019) but strongly correlated with measures of depressed systolic and diastolic function in murine PAB (Egemnazarov et al., 2013; de Man, Handoko, et al., 2012), suggesting a protective role for oestrogen in the right ventricle. This clinically relevant sexual dimorphism has been further explored in vivo. In rats and mice, ovariectomy is detrimental to RV function in SuHx-induced PH (Frump et al., 2015; Liu et al., 2014). Moreover, administration of 17β-oestradiol to ovariectomised and male SuHx rats improves cardiac index and exercise capacity, also exerting beneficial effects on RVH, oxidative stress, metabolic dysfunction, autophagic flux, proapoptotic signalling and activation of pro-inflammatory cytokines (Frump et al., 2015; Lahm et al., 2016). Similar findings were made in male MCT rats, where 17β-oestradiol also had anti-fibrotic and proangiogenic effects in the right ventricle and profoundly improved survival (Umar et al., 2011). Of note, Frump et al. found that RV expression of oestrogen receptor-α (ERα) was tightly correlated with elevated CO in female SuHx rats.

As oestrogen signalling also confers protection against pulmonary vascular remodelling (Frump et al., 2015; Lahm et al., 2016; Liu et al., 2014; Umar et al., 2011), the primary trigger for RV remodelling is tempered. As such, Cheng et al. used the PAB model to investigate the role of ERα in RV morphology and function. Following pressure overload, loss-of-function ERα mutant rats of both sexes developed RVH, yet deficient right ventricle–vascular coupling (end-systolic elastance/effective arterial elastance: Ees/Ea), diastolic dysfunction and fibrosis developed in female rats alone, suggesting that ERα signalling protects against maladaptive remodelling. In addition, studies in castrated PAB mice found that testosterone did not affect RV haemodynamics but was associated with RVH, fibrosis and decreased

16.9 | Sex hormones

Although PAH is more prevalent in women, female sex carries a survival advantage attributed to superior RV function (Jacobs et al., 2014), suggesting a protective role for oestrogen in the right ventricle. This clinically relevant sexual dimorphism has been further explored in vivo. In rats and mice, ovariectomy is detrimental to RV function in SuHx-induced PH (Frump et al., 2015; Liu et al., 2014). Moreover, administration of 17β-oestradiol to ovariectomised and male SuHx rats improves cardiac index and exercise capacity, also exerting beneficial effects on RVH, oxidative stress, metabolic dysfunction, autophagic flux, proapoptotic signalling and activation of pro-inflammatory cytokines (Frump et al., 2015; Lahm et al., 2016). Similar findings were made in male MCT rats, where 17β-oestradiol also had anti-fibrotic and proangiogenic effects in the right ventricle and profoundly improved survival (Umar et al., 2011). Of note, Frump et al. found that RV expression of oestrogen receptor-α (ERα) was tightly correlated with elevated CO in female SuHx rats.

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16.8 | Apoptosis

Apoptosis is the culmination of multiple mechanisms pathologically relevant to pressure overload (mechanical stress, ischaemia, mitochondrial/metabolic dysfunction, oxidative stress, neurohumoral activation and inflammation) and leads to fibrosis, contractile and diastolic dysfunction (Bogaard, Natarajan, et al., 2009; Vonk-Noordegraaf et al., 2013). Nevertheless, the vast capacity of the right ventricle for recovery following normalisation of afterload suggests that cardiomyocyte loss in the pressure-overloaded right ventricle is not extensive.

Regardless, in vivo evidence suggests that apoptosis can be indirectly therapeutically targeted in the pressure-overloaded right ventricle, by addressing the underlying pathogenic processes. Serial non-invasive assessment of MCT rats using 99mTc-annexin V scintigraphy has revealed that apoptosis peaks early after the onset of pressure overload, before declining to a sustained level that is nevertheless elevated compared with baseline. Inhibition of the early peak using an AT1 receptor antagonist delays the onset of RVH and RV dilatation and attenuates the development of fibrosis (Campian et al., 2009). In addition, elevated levels of the pro-apoptotic signalling factors Bax and caspase-3 in PAB rats are restored to control levels by chronic inhibition of angiotensin-converting enzyme (ACE) (Braun et al., 2003). A further study in MCT rats evaluated nicorandil, a mitochondrial ATP-dependent K+ channel opener. Nicorandil treatment restored the expression ratio of mitochondrial proapoptotic/anti-apoptotic factors to control levels, leading to diminished interstitial and perivascular fibrosis (Zuo et al., 2012).
survival following pressure overload (Hemnes et al., 2012). Collectively, these findings indicate that the transition from adaptive to maladaptive RV remodelling is sexually dimorphic.

17 | FUTURE DIRECTIONS

At its core, PH is a cardiopulmonary condition with a high, right ventricle-driven mortality rate. A thorough understanding of the pathophysiology of both the vascular and cardiac components is essential, for although the pulmonary vasculopathy is characterised by angiogenesis, proliferation and apoptosis resistance, the right ventricle may suffer from ischaemia, fibrosis and apoptosis (Klinke et al., 2020). The prospect of inadvertent cardiotoxicity underscores the importance of testing new therapeutic agents designed to address pulmonary vascular remodelling in the pressure-overloaded right ventricle.

Supressing the RAAS is the crux of left heart failure management and continues to be explored within the context of RV dysfunction in patients with PH (Cassady & Ramani, 2020). In fact, the right and left ventricles share many common features in their response to pressure overload and failure (Friedberg & Redington, 2014) that rationalise the investigation of LV-targeted therapies in the right ventricle. Although such an approach may be particularly beneficial in the context of Group 2 PH, shared ventricular pathways could lead to unwanted LV-directed effects in forms of PH, which otherwise have minimal LV involvement. Auspiciously, several divergences between the RV and LV response to adverse loading have been documented at the molecular level, including differential gene expression and responses to hormonal and pharmacological effectors (Friedberg & Redington, 2014). Employing animal models to further understand these differential responses will provide a platform to develop targeted therapies.

The lessons learned from animal models of PH are starting to bear fruit as emerging pharmacotherapies (reviewed elsewhere: Klinke et al., 2020), particularly those targeting RV metabolism, oxidative stress or inflammation, show success in preclinical and early clinical studies. Recent advances in small animal models of PH offer the prospect of inadvertent cardiotoxicity underscores the importance of testing new therapeutic agents designed to address pulmonary vascular remodelling in the pressure-overloaded right ventricle.

CONCLUSION

The capacity of the right ventricle to adapt to pressure overload is a key determinant of survival in PH. Several small animal models of PH are available to the interested investigator, each with a distinct pattern of RV (mal)adaptation to persistent afterload elevation. The use of these models has provided great insight into the role of secondary cardiac insults (myocardial capillary rarefaction, metabolic remodelling, oxidative stress, neurohormonal signalling, inflammation and fibrosis) in the transition from compensated hypertrophy to a state of maladaptation. Although it remains unclear whether these events are the cause or consequence of deteriorating RV function, preclinical evidence suggests they are amenable to pharmacological targeting, which may lead to improvement in RV functional status. Further investigations using complementary models of both adaptive and maladaptive RV remodelling will be essential to discern the molecular mechanisms underlying RV failure, to elaborate novel right ventricle-specific therapeutic strategies and to enhance patient outcomes in PH.

18.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Cidlowski et al., 2019; Alexander, Kelly et al., 2019a,b; Alexander, Mathie et al., 2019).

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AUTHOR CONTRIBUTIONS

J.P.D. prepared the manuscript. A.J.H., T.E.S. and B.K.K.H. proofed and advised on the content of the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

A.J.H. is a consultant/advisory board member for Palatin Technologies Inc., Novo Nordisk and PharmaIN Corp.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this review.
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