The pathophysiology of pulmonary hypertension in left heart disease

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Breitling S, Ravindran K, Goldenberg NM, Kuebler WM. The pathophysiology of pulmonary hypertension in left heart disease. Am J Physiol Lung Cell Mol Physiol 309: L924–L941, 2015. First published August 21, 2015; doi:10.1152/ajplung.00146.2015.—Pulmonary hypertension (PH) is characterized by elevated pulmonary arterial pressure leading to right-sided heart failure and can arise from a wide range of etiologies. The most common cause of PH, termed Group 2 PH, is left-sided heart failure and is commonly known as pulmonary hypertension with left heart disease (PH-LHD). Importantly, while sharing many clinical features with pulmonary arterial hypertension (PAH), PH-LHD differs significantly at the cellular and physiological levels. These fundamental pathophysiological differences largely account for the poor response to PAH therapies experienced by PH-LHD patients. The relatively high prevalence of this disease, coupled with its unique features compared with PAH, signal the importance of an in-depth understanding of the mechanistic details of PH-LHD. The present review will focus on the current state of knowledge regarding the pathomechanisms of PH-LHD, highlighting work carried out both in human trials and in preclinical animal models. Adaptive processes at the alveolocapillary barrier and in the pulmonary circulation, including alterations in alveolar fluid transport, endothelial junctional integrity, and vasoactive mediator secretion will be discussed in detail, highlighting the aspects that impact the response to, and development of, novel therapeutics.

Although broadly defined as a persistent elevation in pulmonary artery pressure (PAP) above 25 mmHg at rest (42), pulmonary hypertension (PH) has been subclassified into five categories by the World Health Organization (WHO) at the 1998 World Congress for Pulmonary Hypertension in Evian (126). The goal of this subclassification was to separate different clinical presentations of PH based on underlying cause, to facilitate treatment. This classification scheme has subsequently been revised in 2003, 2008, and 2013 at World Congresses for Pulmonary Hypertension in Venice, Dana Point, and Nice, respectively (140–142). Despite these revisions, the five major categories have largely remained unchanged since their inception in 1998. PH owing to left heart disease (LHD), which under the 1998 classification was referred to as pulmonary venous hypertension, is classified under Group 2 and comprises the largest set of PH patients (53). In this group, LHDs such as heart failure with preserved ejection fraction (HFpEF), systolic heart failure, or mitral valve disease result in elevated left atrial filling pressures and subsequent retrograde increases in pulmonary venous, capillary, and arterial pressures. Consequently, patients with group 2 PH uniquely display both elevated PAP and pulmonary capillary wedge pressure (PCWP) (49), and PAP and PCWP correlate closely, with the latter being a predictor of the former (43). Accordingly, pulmonary hypertension with left heart disease (PH-LHD) is defined as a subset of PH where PCWP > 15 mmHg and is therefore sometimes referred to as postcapillary PH, in contrast to precapillary PH where PCWP is normal (128). In addition, PH-LHD patients can be further subcategorized on the basis of their transpulmonary pressure gradient (TPG), defined by the difference between mean PAP and left atrial pressure or, more commonly, PCWP, with a TPG > 12 mmHg indicating the presence of “out of proportion” PH (109). This important classification indicates vascular changes in the pulmonary circulation that lead to increased pulmonary pressures that cannot be fully explained by venous congestion due to left-sided heart failure alone (110). To avoid potential confounding effects of stroke volume and arterial compliance, Naeije and colleagues (109) recently further modified this approach by using the diastolic pulmonary arterial pressure/PCWP gradient (DPG). In combination with systemic blood pressure and cardiac output, the DPG can be used in a step-by-step algorithm that allows for a rather precise differential diagnosis between pulmonary vascular disease, high output or high left-heart filling pressure state, and sepsis (109). The consequence of PH-LHD for the right heart is an increase in right
ventricular (RV) afterload, which can cause RV hypertrophy and ultimately right heart failure; although distensible, the right ventricle is unable to cope with the stress placed on it and ultimately succumbs, leading to systemic edema and death (105). Thus both increased RV afterload and left ventricular (LV) preload are observed in PH-LHD, and RV failure more than doubles the mortality risk in patients with LHD (48, 105).

Patients that present with PH secondary to LHD have classically been placed into two broad categories, on the basis of underlying etiology: those with mitral valve disease, and those with end-stage heart failure. This notion has recently been extended, now recognizing that heart failure (HF) patients with HFpEF also often present with PH (49). In the past, the predominant cause of PH-LHD, associated with PAP pressures frequently exceeding 35 mmHg, was mitral stenosis (50, 160). With the advent of suitable therapeutic interventions such as valve replacement and valvuloplasty, and a decrease in rheumatic heart disease, the focus has moved to myocardial ischemia and systemic hypertension as underlying causes of LHD. Heart failure results from either loss of functional myocardium, pressure overload, or volume overload, leading to decreased cardiac output that is insufficient to meet metabolic demand. This decrease in cardiac output leads to a compensatory increase in LV end-diastolic pressure that, in turn, leads to the development of PH in a manner similar to that described above.

Epidemiological data on PH due to LHD are scant; however, results from community-based studies show that a high percentage of patients with left heart failure develop PH (50). A 3-yr-long community-based study by Lam and colleagues in 2009 (88) showed that 83% of patients with HFpEF displayed PH (Fig. 1A). It was also shown that PH was exacerbated in HFpEF patients compared with a control cohort of patients with systemic hypertension, with median PAP values of 28 and 48 mmHg, respectively (88). Even after correction for elevated PCWP, patients with HFpEF had a higher pulmonary artery systolic pressure than those with systemic hypertension (Fig. 1B), demonstrating that PH-LHD in HFpEF patients could not be explained by passive elevation of pulmonary venous pressures alone but also involves an active component in the form of vascular remodeling and/or vasoconstriction (88). In 2010, Leung and coworkers (91) showed that 52.5% of patients with left heart failure and preserved LV systolic function had a mean PAP above 25 mmHg, substantiating the strong correlation between LHD and PH. This is especially relevant in view of the high incidence of LHD in the general population, which ranges from 0.2–12.4/1,000 people (63). In a 2012 study by Bursi and colleagues (17), the percentage of congestive heart failure (CHF) patients that had a systolic PAP above 35 mmHg was reported even higher at 79%. This study also demonstrated the poor outcome for patients with LHD that had secondary PH: mortality was severely increased in these patients compared with patients without PH, with a 45% increase in death at PA pressures between 41 and 54 mmHg compared with patients with PA pressures <25 mmHg (17). Similarly, mortality is also increased by near twofold in LHD patients that also develop subsequent RV failure (43). Presently, the reasons why certain LHD patients are more prone to PH development than others remain unclear.

PH-LHD differs significantly from pulmonary arterial hypertension (PAH) not only in its pathogenesis, but, more importantly, with regards to the resulting changes that occur in the pulmonary circulation, therefore requiring different therapeutic options and interventional strategies. Unlike PAH, there are relatively few studies that have examined the pathophysiology of PH-LHD in patients or animal models, and no established therapies for PH-LHD have been approved. The goal of this review is, therefore, to summarize what is known about PH-LHD from the perspective of cellular and molecular physiology and, on the basis of these considerations, to discuss new ways in which this condition might be managed clinically.

Animal Models

Unlike the wealth of literature that exists on PAH, there are perhaps only 50 or so animal studies looking at PH-LHD. Typically, the approach to investigating PH-LHD in the laboratory has consisted of heart failure induction and subsequent monitoring of hemodynamic variables, followed by in vivo functional tests and harvesting of tissue for biochemical and microscopic analyses.

Canonical animal models of heart failure have included myocardial infarction (MI), ventricular pressure/volume overload, and pacing-induced cardiomyopathy (34). For surgically induced PH-LHD, rat models have been the most successful of the small animal models, owing to the rat heart being ~10 times larger than that of the mouse. In rats, several methods of heart failure induction have been used, such as coronary artery banding (120) or ligation (35). By decreasing the blood supply to the heart itself, a MI is induced, resulting in pump failure and CHF (32a). Both of these models result in animals displaying clinical characteristics similar to human CHF.

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**Fig. 1.** Clinical relevance of pulmonary hypertension (PH) due to left heart disease. A: prevalence of PH in patients with hypertension (HTN) without heart failure compared with patients with heart failure and preserved ejection fraction (HFpEF). B: correlation of pulmonary artery systolic pressure (PASP) with pulmonary capillary wedge pressure (PCWP) in HTN patients without heart failure and in HFpEF patients. Notably, PASP is higher for any given PCWP value in HFpEF patients compared with HTN patients. Data from Lam et al. (88).
vantages of these techniques include high initial mortality and a rather lengthy postoperative period (58). Another commonly used model of CHF in rats is the aortocaval fistula, created between the inferior vena cava and abdominal aorta to produce LV volume overload (1). Its main advantages are its effectiveness at mirroring human CHF through activation of the neurohumoral axis and its ability to rapidly induce CHF: 7 days after surgery animals display hemodynamic signs of PH-LHD. However, the surgery required is more technically challenging than other methods, and also more time consuming. Furthermore, it is near impossible to determine exactly how much blood gets through the aortocaval shunt, making it difficult to control and diminishing the power of the model. In PH-LHD, the utility of the aortocaval fistula model is further limited by the fact that volume overload is not the predominant manner in which patients typically develop PH-LHD. Based on the typical clinical scenario, whereby patients develop PH-LHD in the context of pressure overload or ischemia, PH-LHD is better simulated by arterial banding models, specifically of the left anterior descending artery (LAD) and the aorta.

Although coronary artery ligation/banding and aortocaval fistulas are frequently applied animal models of heart failure, the most commonly used model for preclinical studies of PH-LHD has in the past been the aortic banding model. In this model, a ligature is placed around the ascending aorta of 3- to 4-wk-old rats (120). Normal growth of the animal results in the development of LV hypertension due to impeded aortic outflow, with compensatory LV hypertrophy and subsequent development of HFpEF (157). Our group has extensively used this model to study both cellular and molecular pathophysiology and to evaluate new treatment options in PH-LHD. In brief, a titanium clip of 0.8-mm diameter is placed around the ascending aorta, just distal to the coronary orifices, in juvenile rats of 80–100 g, and hemodynamic changes and lung vascular adaptations characteristic of PH-LHD are typically assessed 9 wk postbanding (165). The power of this model lies in its clinical relevance; the gradual onset in which the aortic restriction first stimulates cardiac compensation, and subsequent decompensation leading to heart failure, closely mirrors the scenario in human patients.

Bandaging of the ascending aorta has also been used in guinea pigs, with the main drawback being the substantial amount of time required for the development of chronic heart failure in this species, usually around 150 days (65). A common theme among these models is therefore a convergence upon mimicking the main consequences of heart failure, namely an inability of the heart to provide for metabolic demands, yet the timeline of heart failure induction is highly variable depending on the method used.

Mouse surgical models for studying heart failure have increased in popularity, largely because of the rapid gestation and relatively low handling and housing costs of mice. Commonly used models have been those of pressure overload, since tachycardic pacing is not feasible in mice. Most notably, the transverse aortic constriction (TAC) model is used, in which a ligature is placed around the aortic arch, between the brachiocephalic trunk and the left common carotid artery (127). This model has been primarily used for investigating LV compensation to pressure overload, specifically hypertrophy and remodeling, and has not been extensively used to study the decompensated state or for studies of PH-LHD. Drawbacks with this model include the high level of surgical expertise required compared with coronary ligation and the fact that the resultant acute onset of severe hypertension is rather far removed from the clinical picture (120). Furthermore, there appears to be a high degree of variability among mouse strains in the responses of the left ventricle to pressure overload. Another popular model is coronary artery occlusion, generally performed by clipping the LAD; this model has the advantage of being technically simpler but is hampered by high perioperative mortality (14).

In addition to these surgical models, there are also genetic models of heart failure, including cardiac muscle LIM knock-out mice, and the cardiomycocyte-specific TNF-α-overexpressing mouse (120). These models are created in mice owing to ease of genetic manipulation, and both of these models induce dilated cardiomyopathy leading to CHF. The advantage of being a nonsurgical model, however, is offset by their high cost and low availability. Again these models have most of all been used to study CHF rather than PH-LHD, and pulmonary vascular data arising from these models are scant.

Small animal models are effective for generating large data sets, since they are fairly inexpensive and breed quite quickly. Limitations to their use arise from underlying physiological differences, such as an absence of a plateau phase in the action potential of rat cardiac myocytes (58). Furthermore, there appear to be differences in the excitation-contraction coupling process, specifically with regards to differing myosin isoforms (α vs. β) that hinder extrapolations to human cardiac function (34). These differences are, however, likely of limited relevance for the study of PH-LHD where the induction of heart failure is merely utilized as a trigger of the disease rather than as the target parameter itself. That notwithstanding, large animal models, whose physiology and anatomy more closely mirror those of humans, have been increasingly used in translational bench-to-bedside research (34), and heart failure models have been created in larger mammals such as dogs, pigs, and sheep (65, 150). Disadvantages with their use include greater housing and feeding costs, longer gestational periods, and ethical considerations. In dogs, a pacing model has been used, whereby a pacing lead is introduced into the right ventricle and set to 245 beats per minute (bpm); the heart is thus paced until a decrease in LV systolic function is observed (150). Once cardiac function is sufficiently impaired, the rate is brought down to 225 bpm (150). The advantage with this approach is that the time required for heart failure induction is shorter; the average pacing period (including the stabilization phase) was 46.6 ± 1.0 days. (150). In porcine MI models, occlusion of a coronary artery has been demonstrated to be most effective at heart failure induction (34). The anatomy of the pig myocardium and coronary arterial tree is very similar to that of humans, giving power to porcine heart failure models in translational research.

Rabbit models of heart failure provide an intermediary between murine and large animal models; not only are they far less expensive than dogs, but in their myocardium the β-myosin isoform predominates, as in humans. Thus rabbits provide a cheaper option for a more accurate heart failure model. Species-specific models of heart failure are further discussed extensively in previously published reviews (58).

In conclusion, it needs to be pointed out that although there are a multitude of different heart failure models, only very few
of them have been specifically utilized in the scenario of PH-LHD; the vast majority of data gleaned from these models has focused on cardiac function per se. Among them are the aortic banding model in rat and guinea pig and the pacing model in dogs. To obtain better insights into the underlying pathobiological mechanisms and to establish novel treatment strategies, further attention should be given to the development of models specific to PH-LHD, or to evaluating existing models specifically for pulmonary vascular effects.

**Endothelial Dysfunction**

The development of PH in left heart failure is not solely caused by a passive increase in pulmonary vascular pressure, but is commonly aggravated by a concomitant rise in pulmonary vascular resistance (PVR) (20, 88) that is attributable to increased pulmonary vascular tone (57) and extensive lung vascular remodeling (30). Both of these phenomena have been proposed to be triggered by lung endothelial dysfunction (105), which is characterized by an imbalance in the secretion of vasodilative and vasoconstrictive factors, especially nitric oxide (NO) and endothelin-1 (ET-1), respectively. Additionally, a reduction in vasodilator responsiveness is seen in PH-LHD (35, 116, 136).

Nitric oxide production by the endothelium plays a key role as a trigger of vasodilatory as well as anti-inflammatory and antiproliferative pathways (149). NO is continuously synthesized from the amino acid L-arginine by the constitutively expressed endothelial NO-synthase (eNOS) (104) and is instantly released (144). NO has a half-life of only 6–30 s and rapidly diffuses to the adjacent smooth muscle cells, causing dilatation mainly in response to stimulation by endogenous factors such as bradykinin, acetylcholine, and catecholamines or mechanical stimuli, including shear stress (97, 149) or stretch (82).

Several studies have reported a diminished dilative response of the pulmonary artery, as well as impaired NO synthesis in the pulmonary endothelium in PH-LHD. In 1991, Ontkean and colleagues (116) showed an impaired vasodilative response of the pulmonary artery to acetylcholine (ACh), mediated by the endothelium-derived relaxing factor (EDRF, which later was identified as NO), in experimental heart failure. The response was, however, unimpaired, and thus comparable to the control, when treated with the Ca\(^{2+}\) ionophore A23187. As a possible explanation, changes in receptor properties or in downstream signal transduction in chronic heart failure were postulated. Nearly 20 years later, Kerem and colleagues (76), using real-time imaging techniques, provided evidence for an almost complete lack of both a Ca\(^{2+}\) response and NO synthesis, toward mechanical stress (such as, e.g., an acute elevation in hydrostatic pressure), ACh, or histamine, in an experimental model of PH-LHD (Fig. 2). Similar to what had been previously reported by Ontkean, the NO response could be rescued by treatment with a Ca\(^{2+}\) ionophore. Notably, a lack or downregulation of eNOS, as is thought to occur in PAH, could be excluded (46, 161). Instead, Kerem and colleagues gave mechanistic evidence, showing remodeling of the endothelial cytoskeleton in response to the increase in mechanical forces as a main reason for the impairment in Ca\(^{2+}\) homeostasis and
signaling and the subsequent impairment in NO synthesis (Fig. 3) (76). In line with these findings, in humans, Porter and colleagues (124) observed a vasodilatory response in the pulmonary artery of heart failure patients with normal PAP, after intrapulmonary infusion of Ach. However, they failed to show a similar vasodilation in patients with PH-LHD. Interestingly, pulmonary vasodilation was observed in both groups after infusion with the endothelium-independent vasodilator nitroglycerin, confirming the presence of endothelial dysfunction in patients with PH-LHD.

In contrast to NO, the 21-residue vasoactive peptide ET-1 acts as a potent vasoconstrictor and promotes vascular remodeling by triggering smooth muscle growth and collagen production (33, 77). ET-1 was first isolated in 1988 by Yanagisawa and colleagues (162) in the supernatant of vascular endothelial cells. Under normal physiological conditions, ET-1 is predominantly produced by endothelial cells; under pathophysiological conditions, however, it is also produced by many different cell types including vascular smooth muscle cells, cardiac myocytes (67), and inflammatory cells (37). ET-1 is primarily expressed in lung tissue with ET-1 mRNA levels in lungs exceeding those in any other organ by five times (98). In mammals, ET-1 exerts its actions via two different receptors, ET\(_A\) and ET\(_B\). In the cardiovascular system, ET\(_A\) receptors are located on vascular smooth muscle cells (SMCs) and cardiac myocytes, and their activation causes vasoconstriction. ET\(_B\) receptors are located on SMCs and endothelial cells (ECs) and mediate either vasoconstrictive or vasodilative effects, depending on their respective location (137). Binding of ET-1 to ET\(_A\) and ET\(_B\) receptors located on SMCs activates phospholipase C (PLC), leading to the generation of the second messengers inositol triphosphate (IP\(_3\)) and diacylglycerol (DAG). Both of these second messengers are capable of triggering the release of calcium from intracellular stores, the outcome of which is a sustained vasoconstrictive response through activation of myosin light chain kinase, and subsequent phosphorylation of the myosin light chain (123). By contrast, binding of ET-1 to ET\(_B\) expressed on the EC membrane stimulates the release of NO and prostacyclin (62), displays antiapoptotic properties (138), and plays a role in the clearance of circulating ET-1 (36). However, the expression ratio in human resistance and conduit pulmonary arteries between ET\(_A\) and ET\(_B\) receptors is about 9:1 and, overall, vasoconstrictive and mitogenic effects dominate (41). Moreover, in heart failure there seems to be a shift toward a reduced responsiveness of the ET\(_B\) receptor on the one hand and an increase in responsiveness toward the ET\(_A\).

Fig. 3. Disruption of the actin cytoskeleton rescues both the pulmonary endothelial [Ca\(^{2+}\)]\(_i\) and NO response. Data were obtained in rats following induction of CHF by supracoronary aortic banding and in situ disruption of the actin cytoskeleton by cytochalasin D. A: representative images show endothelial cells in the intact lung loaded with either fura 2 (for Ca\(^{2+}\) imaging) or DAF-FM (for NO imaging) and color-coded for [Ca\(^{2+}\)]\(_i\) or NO, respectively. In CHF rats, disruption of the actin cytoskeleton by cytochalasin D reconstitutes both the endothelial [Ca\(^{2+}\)]\(_i\), and NO response to acute hydrostatic pressure stress (induced by an increase of left atrial pressure, P\(_{LA}\), from 5 to 15 cmH\(_2\)O). B: in lungs of CHF rats treated with cytochalasin D, both the endothelial [Ca\(^{2+}\)]\(_i\), (top panel) and NO (bottom panel) response to acute hydrostatic pressure stress (P\(_{LA}\) increase from 5 to 15 cmH\(_2\)O) are restored, while spontaneous and induced [Ca\(^{2+}\)]\(_i\) oscillations remain absent. Reproduced from Kerem et al. (76) with permission from Wolters Kluwer Health.
receptor on the other hand (147). Furthermore, increased levels of ET-1 and an increased pulmonary vasoconstrictive response to ET-1 have been reported (33, 152, 167).

**Vascular Remodeling**

Pulmonary vascular remodeling is one of the main characteristics of all forms of PH, independent of etiology. Involving extensive structural changes in the pulmonary vascular wall, the changes seen in PH-LHD arise secondary to a sustained increase in intravascular pressure. As an adaptation to the chronic increase in intraluminal pressure, the vessel wall becomes thickened and stronger. This leads to a decrease in lumen diameter, a reduced capacity for vasodilation, and subsequently an increase in pulmonary vascular resistance, tone and sustained PH (69).

Vascular remodeling in PH-LHD has only been described in a few studies, and even fewer studies have been dedicated to assessing the specific structural and functional changes seen in the pulmonary veins, partly because of the difficulty in effectively distinguishing between pulmonary arteries and veins. The main histological features seen in the pulmonary vasculature in PH-LHD include intimal fibrosis and pronounced medial hypertrophy of muscular pulmonary arteries. In pulmonary veins, medial hypertrophy, arteriolization, and moderate intimal fibrosis were described (Fig. 4A) (30, 31). Hunt and colleagues (66) reported significant vascular remodeling in all vessel layers in a PH-LHD rat model and in patients, with a similar degree in arteries and veins (Fig. 4B). Moreover, thickening of the alveolar-capillary barrier, constituting an increase in the alveolar and the capillary basal laminae, as well as a thickening of the interstitium, with pericyte and collagen infiltration, were reported. In some rare cases, the appearance of chondroid metaplasia in close proximity to the pulmonary vasculature has been identified (78, 165). Delgado and colleagues (30) showed a correlation between vascular remodeling and the severity of PH in systolic heart failure (Fig. 4C). Notably, no correlation between vascular remodeling and the extent of PH has been found in human mitral stenosis (47).

The structural changes of the pulmonary vasculature observed in PH-LHD are often accompanied by impaired vasodilator responsiveness. It is then commonly referred to as “fixed” and is not rapidly reversible by pharmacological treatments (105). For end-stage heart failure patients, cardiac transplantation can be an effective treatment. However, fixed PH is a high risk factor for mortality after cardiac transplantation and therefore these patients usually are not candidates for transplantation. Heart failure patients with reversible PH also have increased mortality after transplantation, compared with heart failure patients without PH, generally because of a higher risk for RV failure (18). An elevated PVR often sustains after cardiac transplantation; thus ~50% of all complications and ~20% of early deaths following cardiac transplantation are due to RV dysfunction (146). Importantly, in patients with PH-LHD, an implanted left ventricular assist device (LVAD) may represent a valuable bridge to transplantation. In fact, LVAD insertion in patients with refractory heart failure and PH was shown to improve TPG, PAP, and PVR and enabled transplantation of patients who would otherwise not have been eligible candidates without LVAD therapy. Notably, these

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Fig. 4. Lung vascular remodeling in pulmonary hypertension due to left heart disease. A: histological sections (van Gieson staining, ×100) show muscular pulmonary arteries from patients with preoperative CHF who died shortly after heart transplantation. Images show examples of mild (left) and severe (right) medial thickening. Reproduced from Delgado et al. (30) with permission from John Wiley and Sons. B: histological sections (Russel-Movat pentachrome stains, scale bars 100 μm) show pulmonary arteries (arrows) with normal intima and media from a control subject (left), and arterial intima (arrowhead) and media (arrow) thickening with luminal narrowing in a PH-LHD patient (right). Reproduced from Hunt et al. (66), with permission from the American Physiological Society. C: medial wall thickness of pulmonary arteries in 17 patients with CHF correlate with pulmonary vascular resistance (PVR). Based on data from Delgado et al. (30).
patients had a similar peritransplant risk as conventional transplant recipients, pointing toward the feasibility and potential of this innovative strategy (87).

In previous work, we utilized the supracoronal aortic banding model in rats to study the underlying mechanisms of vascular remodeling and PH in LHD. In a whole genome microarray analysis, we detected differential regulation of genes involved in inflammation and immune responses, vascular regulation, extracellular matrix regulation, and developmental pathways including the canonical WNT signaling pathway, which has recently also been implicated in the pathogenesis of PH (28a, 28b, 168). However, the most prominently upregulated genes in banded compared with sham-operated rats related to mast cells. An increase in mast cells was confirmed on the genomic, proteomic, and histological level, indicating an important role for mast cells in the development of PH-LHD (64). Interestingly the concept of mast cell accumulation in PH-LHD is not new. Paul Ehrlich, who first described this cell type in his 1878 doctoral thesis, noted that mast cells were abundant in “brown induration of the lung” (37a), i.e., in hemosiderosis following mitral stenosis. Since then, the accumulation of mast cells has been reported in several studies in lungs of patients with PH secondary to congenital cardiac septal defects or mitral stenosis, as well as in patients with idiopathic PAH (59, 60) and in experimental animal models (153).

Mast cells release a wide range of mediators, including serotonin, known to exert vasoconstrictive effects and stimulate smooth muscle cell proliferation (29, 94, 121); histamine, which has vasoconstrictive effects on pulmonary veins; cytokines such as interleukin-6 (an important mediator in PH) (134); and proteases such as chymase and tryptase. It has been reported that the constituents of mast cell granules, once released, stimulate endothelial cell proliferation (96). In a more recent study, Cho and colleagues (24) demonstrated that activated mast cells stimulate airway smooth muscle cell proliferation. In line with a potential role of mast cells in lung vascular remodeling in PH-LHD, a critical contribution of mast cells has also been hypothesized in several systemic vascular diseases such as arteriosclerosis or restenosis (75). To study the role of mast cells in PH-LHD, rats genetically deficient of mast cells, the so-called Ws/Ws rats, were utilized. Ws/Ws rats harbor a mutation in c-kit, previously known as the mast cell growth factor receptor, that is essential for the development of mast cells (151). In parallel pharmacological approaches, the mast cell stabilizers ketotifen or sodium cromolyn, respectively, were used. Both the genetic and pharmacological approach reduced 1) PH; 2) the increase in PVR; 3) lung vascular remodeling; 4) RV hypertrophy; and 5) end-diastolic RV dilation in aortic banding and monocrotaline-treated rats, giving proof of concept for a crucial role of mast cells in the pathophysiology of PH-LHD and PAH, respectively (26, 64).

Similarly, in an independent study in experimental PAH, treatment with the mast cell stabilizer cromolyn markedly reduced vascular remodeling in rats (9). These preclinical data are consistent with clinical data from a pilot study with the tyrosine kinase inhibitor imatinib, which yielded beneficial results in PAH patients (45). Although the positive effects of imatinib in this study were primarily attributed to its inhibitory effects on the platelet-derived growth factor (PDGF) receptor, imatinib also inhibits c-kit and, thus, mast cells, suggesting that pharmacological interventions targeted against mast cells might be a promising strategy for targeting lung vascular remodeling in various forms of PH including, at least on the basis of the demonstrated role of mast cells in rats with aortic banding, PH-LHD (38).

Vasoactive Drugs in the Treatment of PH-LHD

Mainstays of treatment for PH-LHD have included pharmacological agents for afterload reduction and rhythm modulators, as well as surgical and electrical interventions (all discussed extensively in clinical reviews elsewhere) (56, 81). However, although there has been considerable progress in the treatment of PAH in recent years, effective pharmacological treatment strategies in PH-LHD are still lacking.

Drugs that have been approved for the treatment of PAH belong to one of three classes: phosphodiesterase type 5 (PDE5) inhibitors, prostacyclin analogs, or endothelin receptor antagonists. All three strategies target vasoactive pathways; however, the same drugs used for PAH can have fatal consequences in PH-LHD. Moreover, the use of vasodilators in heart failure can be accompanied by serious side effects, which include edema formation (93, 115), LV volume overload (113), or systemic hypotension (169). In previous clinical trials, intravenous or oral administration of vasodilators such as epoprostenol, or inodilators, such as the phosphodiesterase-3 inhibitor milrinone, even increased mortality (19, 117).

One reason for the occurrence of pulmonary edema after the administration of vasodilators might be found in the opening of what has been proposed to be precapillary sphincters that contract in response to elevated lung microvascular pressures, known as the “Kitajew reflex.” This purported reflex responds to a sustained increase in pulmonary venous and microvascular pressures by constricting precapillary sphincters. This vasoconstriction becomes evident during the vasodilatory response to inhaled or systemic vasodilators such as NO, iloprost, or other vasodilators (16, 61, 131) in subjects with increased pulmonary pressure. Similar interventions have little or no effect in healthy patients/animals, because the naïve pulmonary vasculature is fully dilated. This precapillary vasoconstriction has been considered to protect the pulmonary capillary bed from excessive pressure increases in pulmonary venous hypertension and therefore to prevent fluid leak and edema formation (115, 135). It should be pointed out that although there is clear evidence for a vasoconstrictive response to sustained pressure elevation in the pulmonary circulation, the term Kitajew reflex is actually a misnomer. First of all, it is not a nervous system-modulated response; rather it seems to represent an example of autoregulation, in a fashion similar to the myogenic response observed in peripheral arterioles. Secondly, the time course of events would indicate that this adaptation of the capillary bed occurs on the span of days or weeks, rather than in the short term, as would be implied by the term reflex. Third, there are no precapillary sphincters in the pulmonary circulation as initially proposed by Kitajew; at least, no one has found an anatomic correlate. Rather, it seems to be a general vasoconstrictive response of upstream arterioles in the pulmonary circulation.

Despite several discouraging clinical studies in past years, vasodilators might still be suitable drug candidates in the treatment of PH-LHD, under the appropriate conditions. A key
factor is targeting the pulmonary vasculature more specifically. This can be achieved by applying more direct forms of administration, such as via the inhaled route as opposed to systemic administration, or alternatively, by using lung-specific drugs. A recent series of smaller double-blind, placebo-controlled, randomized trials have indicated that treatment with selective pulmonary vasodilators, such as PDE5 inhibitors, may improve hemodynamic as well as functional parameters including exercise capacity in PH-LHD patients (10, 51, 53, 92).

One promising vasodilator in the treatment of PH-LHD is the PDE5 inhibitor sildenafil. Sildenafil is already an established drug in the treatment of PAH, and since PDE5 is especially abundant in the pulmonary circulation, it also demonstrates relative lung selectivity (4). One of the main features in the pathophysiology of PH is a decrease in endothelial NO production (46) and an increase in PDE5 expression in pulmonary artery smooth muscle cells (107, 170). Whereas NO activates soluble guanylate cyclase, which subsequently synthesizes cyclic guanosine monophosphate (cGMP) (86), PDE5 hydrolyzes cGMP, which leads to an increase in intracellular calcium (156) and potassium (3) and subsequently to vasoconstriction, proliferation of smooth muscle cells, and resistance to apoptosis (159). Sildenafil inhibits PDE5, thereby increasing cGMP availability and enhancing the beneficial vasodilatory effects of NO. In several animal as well as human trials, sildenafil has been tested with encouraging outcomes. In an aortic banding rat model of PH-LHD, the long-term oral administration of sildenafil reduced PH, lung vascular resistance, lung vascular remodeling, and RV hypertrophy and dysfunction, without causing edema formation (165). In clinical studies, acute sildenafil administration improved PH, PAP, and PVR, as well as overall exercise performance (52, 92). Similar beneficial results were reported in clinical studies of chronic sildenafil administration (51). Furthermore, in a study of 45 patients with systolic dysfunction, sildenafil therapy resulted in improvements in several indexes of LV function, as well as in overall exercise capacity and quality of life (54). In a trial in over 200 patients with HFpEF, sildenafil therapy failed, however, to improve overall oxygen consumption, echocardiographic parameters, or exercise performance significantly (125). Consistent with this result, in the recently published multicenter RELAXED trial, sildenafil treatment had no effect on exercise capacity, LV remodeling, diastolic function parameters, or overall quality of life in HFpEF patients. Moreover, sildenafil treatment was associated with an increased mortality compared with placebo-treated patients. Importantly, it needs to be considered that PH was not a requirement for this study and a specific subgroup analysis for PH patients was not performed (125). It thus remains unclear to which degree the beneficial or detrimental results in these studies actually relate to PH-LHD as therapeutic target for sildenafil. Clearly more work is required to define the potential benefits of sildenafil therapy for Group 2 PH, and to precisely define the optimal patient population for this treatment, as it seems possible that, e.g., a beneficial effect seen in patients with LV systolic dysfunction does not translate to those with HFpEF.

Another strategy for enhancing cGMP signaling involves stimulating its production by soluble guanylate cyclase (sGC) (85). Riociguat is a pharmacological sGC activator that is currently approved for chronic thromboembolic PH and is under extensive study for Groups 1 and 3 PH (44, 139). Specifically regarding PH-LHD, the Phase 2b Left Ventricular Systolic Dysfunction Associated With PH Riociguat Trial (LEPHIT) was recently reported (13). In this placebo-controlled study, 201 patients with PH-LHD and systolic dysfunction were randomized to one of three doses of riociguat for 16 wk. Although no significant decrease in mean PAP was reported, several secondary outcomes, including improved LV functional indexes and decreased PVR, reached statistical significance. Additionally, the drug was well tolerated at all three doses. As in the sildenafil literature, further investigation is required to determine the best population and regimen for this novel therapy.

An additional class of drugs with both direct cardiac and vasoactive effects is the class of β-adrenergic receptor antagonists (beta blockers). There are several rationales for the use of beta blockade in patients with PH-LHD. First, beta blockers are first line therapy for left-sided heart failure, improving myocardial oxygen balance, increasing ventricular filling time, lowering myocardial work, and countering the strong neurohormonal activation seen in patients with CHF (101). Second, beta blocker therapy can potentially address the problem of biventricular functional interdependence; that is, that dysfunction of one ventricle negatively impacts function of the other (80, 114). Indeed, in monocrotaline-treated rats, carvedilol therapy resulted in improved biventricular function, decreased TGF-β signaling, and decreased myocardial fibrosis compared with controls (114). Third, by decreasing heart rate in PH-LHD patients, diastolic filling time will increase, which should allow for more complete filling of the RV and, therefore, improved cardiac output. However, in a propensity-matched analysis of patients with idiopathic PAH or connective tissue disease-associated PAH, use of beta blockers (any type) was not associated with any improvement in mortality, disease progression, or 6-min walk distance (8).

A newer beta blocker, nebulivolol, has garnered special interest as a therapy. While being a cardioselective β1-antagonist, nebulivolol also possesses β2- and β3-agonist function (129). In a rodent model of PAH, and in cells isolated from patients with PAH, nebulivolol improved NO production and limited vascular remodeling and RV systolic pressure (122). Much of the interest in nebulivolol as a therapy for PAH stems from the β3-mediated enhancement of NO production. However, it must again be highlighted that 1) this work has not been carried out in patients or models of PH-LHD and therefore may not translate to this population, and that 2) vasodilator therapies as a whole remain disappointing in this population.

In the light of the role of Rho and Rho-kinase (ROCK), in smooth muscle cell contraction and proliferation, several groups sought to investigate the role of this pathway in experimental PAH. Although none of these models specifically examined PH-LHD, some insights can be gained from this work, nonetheless. In hypoxia-induced PAH in rats, inhalation of two different ROCK inhibitors, Y-27632 and fasudil, resulted in improved PAP without any effects on systemic hemodynamics (111). In a small human study of nine patients with severe PAH, fasudil administration resulted in a substantial decrease in PVR, with only a modest decrease in PAP (39). A larger study of 22 patients with PAH arising from various etiologies, however, failed to show any significant improvement in pulmonary hemodynamics or exercise capacity after fasudil treatment, although cardiac index was improved in the
treatment arm (40). These results have not been encouraging thus far, and currently no further registered clinical trials involving fasinulid for PAH are underway. In view of these results, it might be fair to speculate that the effect of ROCK inhibitors in PH-LHD may be equally limited, although direct evidence from preclinical or clinical trials is presently lacking.

Prostaglandins are another class of potent vasodilators already successfully used in the treatment of PAH (65a), and with promising results in vivo and in small clinical pilot studies of PH-LHD. Prostaglandin I$_2$ (prostacyclin) and its analogs inhibit platelet aggregation and have predominantly vasodilatory effects, via increasing intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP) (79). In an aortic banding model in rats, inhaled administration of the prostacyclin analog iloprost reduced PH without any detectable side effects (166). In CHF patients prior to heart transplantation, prostacyclin inhalation induced pulmonary vasodilation, along with a decrease in PVR, PAP, and TPG, with no significant effect on systemic vascular resistance (57). These findings are in stark contrast to the earlier results of the Flolan International Randomized Survival Trial (FIRST) in chronic heart failure patients wherein chronic infusion with epoprostenol increased mortality and did not show any improvement in quality of life, resulting in the trial being ended prematurely (19). The main notable difference between the FIRST trial (19) and the subsequent preclinical and clinical trials with prostacyclin analogs in PH-LHD (57, 166) lies in the route of administration, suggesting that inhaled, and thus pulmonary-selective, delivery may provide beneficial effects, whereas systemic administration yields detrimental results.

The third class of vasodilators currently used in PAH are the endothelin-1 receptor antagonists. Despite the success of ET-1 receptor antagonists in the treatment of PAH (21, 130) and in animal models of heart failure (25), their performance in clinical trials for PH-LHD has been largely disappointing. One of the first larger pilot studies (REACH-1) that aimed to test the long-term effects of the dual endothelin receptor antagonist bosentan in patients with advanced heart failure had to be stopped due to liver toxicity. Moreover, in the entire study group no difference in the efficacy between treatment and placebo group was observed (108). The subsequent large-scale ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) trial used lower doses of bosentan than the REACH-1 study; however, the treatment with bosentan increased the risk of worsening heart failure, as a result of fluid retention (74). Subsequent studies with the ETA receptor antagonist darusentan (EARTH) (2) or the nonselective ET receptor antagonist tezosentan (VERITAS and TACTICS) (32, 100) also failed to improve outcomes in patients with chronic heart failure.

In conclusion, the use of vasodilators for the treatment of PH-LHD still remains problematic since many vasodilators result in serious systemic side effects. On the surface, this finding is in conflict with the known vasodilator deficiency in this patient population. As discussed elsewhere in this review, molecular phenotypes in this patient population suggest that current vasodilator therapies, while causing advantageous decreases in PVR, also block several beneficial adaptations of the lung vasculature in PH-LHD. Furthermore, the deleterious (or lack of advantageous) effects of vasodilators in PH-LHD may reflect issues with maintenance of systemic blood pressure, rather than strictly negative effects with respect to the PH. The future seems to be in the use of more lung-specific vasodilators, such as the PDE5 inhibitors, which at the moment seem to be the most promising therapeutic strategy, and/or lung-specific administration of drugs via inhalation. It should be noted, however, that even with lung-specific strategies, the potential risk of increased lung edema formation due to precapillary pulmonary vasodilation resulting in increased lung capillary hydrostatic pressures will persist. Although existing preclinical and smaller clinical trials did not find evidence for an increased tendency for pulmonary edema formation in such interventions as of yet, inhibition of the Kitajew reflex by vasoactive drugs remains an important concern.

**Endothelial Permeability**

The maintenance of a partially selective barrier between the capillary, the interstitial space of the alveolocapillary membrane, and ultimately the alveolus is a key role of the pulmonary endothelium. The bulk of this integrity is provided by interendothelial junctions (IEJs), which link cells via cytoskeletal attachments and include adherens junctions, gap junctions, and tight junctions, with VE-cadherin, occludin, and connexin-40 and -43 as the principal components, respectively (102). Movement of molecules occurs paracellularly between IEJs for small molecules, or transcellularly for large plasma proteins such as albumin (103, 155). As quantitatively described by the Starling equation, oncotic pressure exerted by plasma proteins draws fluid into the capillary, whereas hydrostatic pressure forces fluid out into the interstitial and, ultimately, the alveolar space (12).

Barrier permeability can be regulated by mechanical forces such as shear stress, or by molecular factors that can essentially modulate endothelial barrier integrity via three distinct pathways: the assembly/disassembly and reorganization of IEJs, an increase in the intracellular Ca$^{2+}$ concentration that triggers myosin-light chain kinase-dependent endothelial contraction and gap formation, and the Rho-kinase dependent formation of actin stress fibers, which in turn causes endothelial contraction and IEJ disassembly. The canonical view of endothelial permeability places the IEJ as the primary source of barrier integrity and Rho-mediated cytoskeletal changes as the prime mechanism by which barrier function is compromised. However, the vast majority of the work on IEJs and their role in regulating endothelial permeability was performed in vitro. Emerging evidence points to a large discrepancy between the responses of endothelial cells, generally cultured on a stiff substrate, and their behavior in vivo. The intact lung differs significantly in responsiveness to mediators of increased permeability, and despite overwhelming in vitro work supporting the Rho pathway for barrier dysfunction, it seems to play no or only a minor role in the intact lung (154). In vivo, increased vascular permeability leading to the development of pulmonary edema is primarily a calcium-dependent process, whereby increased cytosolic calcium leads to endothelial contraction (154). Most notably, the large majority of barrier protection observed in PH-LHD has been attributed to the endothelial calcium response (vide infra).

Fluid flux across the capillary endothelium is determined by two principal forces leading to either fluid filtration or absorption, namely the hydrostatic pressure difference and the oncotic
pressure differential, i.e., the so-called “Starling forces.” The capillary hydrostatic pressure ($P_{c}$), exerted by the fluid against the endothelial capillary wall, and the oncotic pressure of proteins normally present in the interstitial space ($P_{i}$) tend to force fluid out of the capillary. Similarly, the hydrostatic pressure of the interstitial fluid ($P_{i}$) and the oncotic pressure due to plasma proteins such as albumin ($I_{i}c$) tend to draw fluid into the capillary. The Starling equation describes the volume of fluid flux ($J_{c}$) in terms of these pressures, the osmotic reflection coefficient ($\sigma$, a measure of the protein permeability of the barrier), and the filtration coefficient ($K_{f}$), which reflects the product of endothelial surface area and hydraulic conductivity ($L_{p}$, a measure of the water permeability of the barrier): $J_{c} = K_{f} [(P_{c} - P_{i}) - \sigma(I_{i}c - I_{i})]$ (145). Disruption of the structural integrity of the endothelial monolayer, resulting from endothelial cell contraction in the context of inflammation, causes plasma proteins to leak into the extravascular space and subsequent formation of permeability-type edema, which is protein rich. Conversely, an imbalance in the Starling forces, via either an increase in capillary hydrostatic pressure or a decrease in oncotic pressure, results in the formation of hydrostatic edema, with edema fluid that is relatively protein poor. The latter scenario is a characteristic of left heart failure, as elevated pulmonary vascular pressures resulting from pulmonary vasocongestion lead to the development of cardiogenic (hydrostatic) pulmonary edema.

Traditionally, it was believed that hydrostatic-type pulmonary edema was simply due to passive filtration, and resulted in a protein-free transudate. However, initial data from Ivey and colleagues (27) showed, by indirect measurement using radio-labeled transferrin, that chronic heart failure patients with elevated pulmonary venous pressure are protected from pulmonary edema, through an adaptive decrease in pulmonary microvascular permeability (Fig. 5A). Notably, the reduction in microvascular permeability correlated with the severity of heart failure. Additionally, they showed that there was no correlation between the accumulation of radio-labeled transferrin in the lung and PCWP in heart failure patients (28). In rats, resistance to thapsigargin-induced endothelial leak has been demonstrated in CHF models, via assessment of the filtration coefficient $K_{f}$ (1). Furthermore, Townsley et al. (150) demonstrated that $K_{f}$ at physiological hydrostatic pressures was no different in dogs with pulmonary venous hypertension secondary to CHF, compared with controls, yet $K_{f}$ measured at pulmonary vascular pressures commonly seen in heart failure (20–50 cmH$_2$O) were significantly less in this group compared with control. Regression analysis showed that in vivo this difference in $K_{f}$ would result in a 50% reduction in the amount of water and protein cleared across the pulmonary capillary endothelial barrier in the CHF group. On the other hand, De Pasquale and colleagues (32a) reported increased protein transport across the endothelium and epithelium of CHF lungs. Although this finding may initially seem counterintuitive in

![Fig. 5. Lung vascular permeability in left heart failure. A: plasma protein accumulation (PPA) index as a measurement of pulmonary microvascular permeability is reduced in mitral stenosis patients compared with control patients with mild coronary artery disease. Reproduced from Davies et al. (27), with permission from BMJ Publishing Group. B: fluid accumulation is decreased in lungs from guinea pigs with congestive heart failure induced by banding of the ascending aorta (banded) compared with sham controls (top). Capillary filtration coefficient ($K_{f}$) is reduced in lungs of heart failure guinea pigs compared with sham controls (bottom). *$P < 0.05$, ***$P < 0.001$ vs. Sham. Reproduced from Huang et al. (65), with permission from Oxford University Press.](http://ajplung.physiology.org/DownloadedFrom)
conjunction with the notion of barrier protection in PH-LHD, recent evidence showing an inverse relationship between paracellular permeability and transcellular protein transport across the microvascular endothelium (5) suggests that transcellular transport (as measured by De Pasquale and colleagues in 2003) may thus be increased as a result of reduced paracellular permeability.

Decreased levels of cytosolic Ca\(^{2+}\) have been postulated to be responsible for the apparent lack of endothelial permeability responsiveness and, hence, barrier protection in PH-LHD (68). Ca\(^{2+}\) is a second messenger with myriad functions in cellular physiology and plays a large role in the regulation of endothelial permeability. Increased cytosolic Ca\(^{2+}\) results in phosphorylation of the myosin light chain via MLCK and in dissociation of VE-cadherin-mediated binding of adherens junctions (133). Both of these events induce the uncoupling of IEJs and result in increased paracellular permeability. Using direct real-time imaging of endothelial Ca\(^{2+}\) in lungs of rats with previously induced CHF, Kerem et al. (76) detected a 15% reduction in the mean intracellular Ca\(^{2+}\) concentration in CHF rats compared with control, which is notably also associated with a loss of the spontaneous Ca\(^{2+}\) oscillations that are normally characteristic of the intact lung endothelium (Fig. 2B). More importantly, the pulmonary endothelium of CHF rats also displayed a complete lack of endothelial Ca\(^{2+}\) responsiveness to many established stimuli, including hydrostatic stress, histamine, or acetylcholine (Fig. 2A).

Decreased Ca\(^{2+}\) signaling was also implicated by Ivey and colleagues (68) as a reason for the lack of a physiological endothelial permeability response to angiotensin II (AngII) in a canine model of CHF. The AngII receptor 1 (AT1) has been implicated as a reason for the lack of physiological responsiveness and, hence, barrier protection in PH-LHD (68). Notably, the lack of endothelial Ca\(^{2+}\) responsiveness to pharmacological agents in CHF such as histamine or acetylcholine is not attributable to a loss of the respective endothelial receptors, which are expressed in equal abundance in lung endothelial cells of CHF rats compared with sham-operated controls (76). At another level, there is also an impairment in basal endothelial Ca\(^{2+}\) levels and oscillations, and the effects of endosomal Ca\(^{2+}\) release are markedly attenuated in CHF lungs (76). It has thus been hypothesized that the mechanism linking intracellular Ca\(^{2+}\) release to membrane Ca\(^{2+}\) influx via store-operated Ca\(^{2+}\) channels is decoupled, because thapsigargin did not induce the same increase in cytosolic Ca\(^{2+}\) in CHF rats compared with controls (68, 76). Cytoskeletal reorganization is additionally hypothesized as a cause of this decreased intracellular Ca\(^{2+}\) (76). F-actin is a Ca\(^{2+}\) store and can similarly act as a Ca\(^{2+}\) sink at the level of the submembrane cytoskeleton, thereby counteracting excessive Ca\(^{2+}\) increases (89). Indeed, β-actin expression in lung microvascular endothelial cells of CHF rats was found to be increased more than tenfold, which accords well in the context of PH-LHD where the endothelium is continuously exposed to increased mechanical forces (76). More importantly, disruption of the endothelial actin cytoskeleton by cytochalasin D restores endothelial Ca\(^{2+}\) responsiveness, and lung microvascular barrier protection that is likely to constitute an important adaptive mechanism by which PH-LHD lungs are protected from chronic alveolar flooding.

It has also been shown that the pulmonary endothelium undergoes structural changes in PH-LHD, leading to thickening of the endothelial barrier and thus further protecting against alveolar leak (90). In dogs with CHF, morphometric analyses have shown significant thickening of endothelial and epithelial basement membranes (150). The point at which stress injures the alveolocapillary interface is inversely related to the strength of the capillary endothelial basement membrane, and hence a thickened basement membrane acts as a functional barrier to limit mechanical stress failure of lung capillaries and subsequent alveolar flooding (158). Although the mechanisms behind this thickening remain unclear, with potential candidates including increased surface adhesion molecule expression and collagen deposition, its purpose is clear: to stabilize a membrane that is continuously exposed to increased mechanical stress due to hydrostatic pressure. This remodeling does not only occur at the level of the basement membrane; after 150 days of aortic banding, Huang et al. (65) reported significant increases in pulmonary microvessel wall thicknesses in guinea pigs. Not only were wall thickness-to-lumen ratios increased, but also the capillary filtration coefficient was reduced in lungs from animals with isolated heart failure compared with control (Fig. 5B), again reiterating the barrier-protective adaptation of the pulmonary microvasculature in CHF.

Taken together, the pulmonary vasculature exhibits profound adaptation at the structural, functional, and molecular level, with the aim of protecting the lung vascular barrier from leakage and edema formation, in a setting of increased hydro-
static forces. The combination of the aforementioned phenomena constitutes an important protective mechanism that is likely essential for the survival of patients with CHF by preventing or limiting excessive hydrostatic edema formation.

**Alveolar Fluid Clearance**

Although the primary role of the alveolar epithelium, and specifically the type I alveolar epithelial cells, is the diffusive exchange of O$_2$ and CO$_2$ between the alveoli and the pulmonary capillaries, this cell layer provides a tight barrier that also serves a similar buttressing function to that of the vascular endothelium. Increased hydrostatic stress in the capillaries, through the mechanisms discussed above, results in the egress of fluid into the extra-alveolar interstitium, which, if persistent, leads to the deposition of fluid into the alveolar air spaces. Removal of this fluid from the alveolar space is imperative for the resolution of edema and is primarily brought about via active sodium ion transport in the surfactant-producing alveolar type II epithelial cells (11, 15). Na\(^+/\)H\(^+\) transport at the apical side via the epithelial sodium channel ENaC and is then pumped out basolaterally by the Na\(^+/\)H\(^+\)-K\(^+/\)H\(^+\)-ATPase (99). This generates an osmotic gradient across the epithelial barrier, which drives fluid reabsorption back into the interstitium and vasculature.

Although alveolar fluid clearance constitutes an important rescue mechanism, it is unfortunately acutely inhibited by hydrostatic stress, thereby promoting rather than alleviating the formation of hydrostatic lung edema. Exposure of rat lungs to left atrial pressures of 15 cmH$_2$O significantly diminished their fluid-clearing ability, by decreasing active Na\(^+/\)H\(^+\) transport (132). At these moderately elevated vascular pressures, inhibition of alveolar fluid clearance is purported to be the primary mechanism for the development of hydrostatic edema, mediated by NO, via cGMP-dependent inhibition of ENaC (73). Furthermore, through use of a double-indicator dilution technique that can differentiate between pressure-dependent and alveolar-dependent changes in fluid flux, our group has shown that acute hydrostatic stress not only blocks fluid clearance but also induces active alveolar fluid secretion that is driven by Cl$^-$ transport through cystic fibrosis transmembrane conductance regulator (CFTR) channels and Na$^+$-K$^+$-Cl$^-$ cotransporters (NKCC) (143). The connection between the underlying hydrostatic forces and the switch from alveolar fluid clearance to alveolar fluid secretion relies on intricate signaling between the pulmonary endothelium and the alveolar epithelium through endothelial-derived NO. Elevated PCWP has been shown to result in eNOS phosphorylation, via activation of PI3 kinase and its downstream effector, Akt (82). After being produced in the endothelium, NO is free to diffuse from the capillary into the type II alveolar cell, where it has been shown to inhibit Na\(^+/\)H\(^+\) transport via both cGMP-dependent protein kinase I (cGKI)-dependent and -independent processes (55), and to concomitantly activate CFTR via cGMP-dependent protein kinase II (cGKII) (22). Notably, both pharmacological inhibition of NO and genetic knockout of eNOS attenuate hydrostatic edema in isolated rat lungs, as determined by lung wet-to-dry weight ratios (73). Whereas increased endothelial NO production in acute hydrostatic stress therefore promotes edema formation, the endothelial dysfunction in chronic PH-LHD improves Na\(^+/\)H\(^+\) transport-mediated fluid clearance, via attenuating NO production, and thus constitutes again an adaptive response that protects the CHF lung from chronic alveolar edema formation (Fig. 6).

Furthermore, Azzam and colleagues (6) have shown that expression of the Na$^+/\)K$^+/\)H\(^+\)-ATPase at the basolateral membrane is increased in CHF rats by almost 2.5-fold, compared with controls. CHF induction would therefore appear to further increase active Na\(^+\) transport, and thus fluid absorption across the alveolar epithelium, again with the aim to limit alveolar fluid accumulation.
fluid accumulation and protect the CHF lung from edema formation.

**Novel Therapeutics and Ongoing Clinical Trials**

Several clinical trials are currently underway to investigate some of the agents discussed above, as well as novel compounds, for the treatment of PH-LHD. Although trials using endothelin receptor antagonists in PH-LHD have been disappointing thus far, a safety study is presently underway using the nonselective ET receptor antagonist macitentan in patients with PH-LHD (MELODY-1 Trial, NCT02070991). Building on the enthusiasm surrounding nebivolol for PH treatment, recruitment is concurrently underway for a study of the effect of nebivolol on PAP in patients with HFpEF (NCT02053246). Although not specifically assessing PA pressures, the SilHF trial aims to study sildenafil in patients with NYHA Class II-III CHF and elevated pulmonary pressure (NCT01616381). The primary outcome is a global functional assessment, and this study will provide important evidence for the role of sildenafil in patients with PH and LV systolic dysfunction, to bolster the existing work done in patients with HFpEF. A parallel study in a similar population is also currently recruiting patients (NCT01913847). Finally, building on previous human data (23), the PADN-5 trial is recruiting patients to investigate the effects for pulmonary arterial denervation using a surgical approach as a possible strategy for reducing PVR and PAP in PH-LHD patients (NCT02220335).

**Conclusion**

The current state of the art indicates that not all PH is created equal. This concept holds true from the cell biology through to clinical treatment strategies. Although it is tempting to transfer therapeutic notions from the realm of PAH into patients with PH-LHD, an understanding of the biology underpinning this unique disease entity illustrates the potential pitfalls of such a course of action. As discussed in this review, in PH-LHD, the lung vasculature has undergone a series of adaptations in an attempt to protect the small vessels and capillaries from exposure to injurious pressures. These adaptations include the structural thickening of the alveolocapillary interface, the loss of the endothelial Ca^{2+} and permeability response to characteristic triggers, the increase in alveolar fluid clearance and inhibition of alveolar fluid secretion to prevent edema development, the Kitajew reflex and loss of vascular reactivity to limit hydrostatic pressure-induced capillary damage, and changes in the balance of NO and ET-1 signaling. As with many systems in cardiovascular physiology, these initially beneficial changes eventually become maladaptive and worsen the initial insult (in this case, further elevation of pulmonary vascular pressure) (Fig. 7). As such, any potential therapy that seeks to limit these changes must strive to find a balance between improving hemodynamics without disrupting the beneficial effect manifested by limitation of alveolar fluid accumulation. With this in mind, PDE5 inhibition may represent a promising strategy for the PH-LHD population: by combining pulmonary vaso-dilatation with endothelial barrier protection, it is possible that such therapies may combat PVR without inducing collateral damage to alveolar fluid homeostasis. Despite this promising physiological profile, it is important to note that sildenafil has not been an effective therapy for HFpEF patients. The SilHF trial and others aim to provide important knowledge as to the potential efficacy of sildenafil for PH-LHD with systolic function. As with most other conditions, a detailed understanding of the cell physiology of PH-LHD is critical if we are to make gains in the treatment of this unique patient group. Further intensive research will deepen this knowledge and will

![Fig. 7. Schematic representation of the pulmonary pathophysiology in left heart disease.](http://ajplung.physiology.org/)
hopefully translate into new therapies and improved patient outcomes.

DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES

20. Deucher G, Docherty A, MacLean MR, Hicks MN. Pulmonary hypertension secondary to left ventricular dysfunction: the role of nitric
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PULMONARY HYPERTENSION IN LEFT HEART DISEASE


