Handbook of Experimental Pharmacology 218

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Pharmacotherapy of Pulmonary Hypertension

Springer

Handbook of Experimental Pharmacology

Volume 218

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Pharmacotherapy of Pulmonary Hypertension

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ISSN 0171-2004 ISSN 1865-0325 (electronic)
ISBN 978-3-642-38663-3 ISBN 978-3-642-38664-0 (eBook)
DOI 10.1007/978-3-642-38664-0
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013948127

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Over the past decade, we have witnessed major advancements in the understanding of the molecular mechanisms and pathophysiology of pulmonary hypertension (PH) that occurred in parallel with the discovery and development of new therapies. Pharmacological agents that modulate the main pathophysiological pathways of pulmonary arterial hypertension, including endothelin-1, nitric oxide, and prostacyclin, have changed the course of this devastating disease by relieving symptoms and improving and prolonging patients' lives. Moreover, molecular biological tools have supplied a wealth of knowledge about the roles of established and emerging signaling pathways in PH. This book is an example of how pharmacotherapy can further expand our understanding of such a heterogeneous group of diseases as PH.

Written by leading experts in the field, this volume focuses on current, evidence-based pharmacological treatments of various forms of PH and also provides a comprehensive review of most recent developments in this area. The first part of the book covers definition, classification, pathophysiology, pathology, biomarkers, and animal models of the disease, thus laying the conceptual basis for what follows. The middle section provides an overview of the established therapies such as calcium channel blockers, prostanoids, endothelin receptor antagonists, phosphodiesterase-5 inhibitors, and inhaled nitric oxide. The last section explores novel pathways and emerging therapeutic approaches including soluble guanylate cyclase stimulators, Rho-kinase inhibitors, inhibitors of serotonin receptors and transporters, peptide growth factors, vasoactive peptides, modulators of redox equilibrium and cyclic nucleotides homeostasis, as well as immunosuppressive and anti-proliferative agents. Particular attention is given to clinical applications of these experimental therapies, which are on the horizon, thus spanning the continuum from basic science to clinical applications. The catalogue of signaling cascades in PH is rapidly growing and with it the wish to modulate these pathways for therapeutic reasons. We can expect to see further progress and achievements in developing novel therapeutics over the next decade, with new improved agents in established drug classes, new drug classes in established pathways, and novel drugs aimed at new molecular targets.

We hope that this book will help the reader appreciate the importance of elucidating the pathophysiology of PH and the research on new signaling pathways and their biochemical and genetic background. It also showcases the commitment of academia and industry to drug discovery and development focusing on this devastating condition. Unfortunately, despite recent advances in the understanding of the molecular mechanisms and pathology of PH, this disease remains incurable. There is still a significant need for identifying new therapeutic targets and developing new treatment options. Undoubtedly, exciting times lie ahead of us, for both the science and the patients. If this book helps to stimulate further research and improve patient care, the wishes of the authors, the editors, and the editorial manager, Susanne Dathe, will be fulfilled.

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Part I
Pulmonary Hypertension: Conceptual Bases of the Disease

Definition and Classification of Pulmonary Hypertension

Marc Humbert, David Montani, Oleg V. Evgenov, and Gérald Simonneau

Abstract Pulmonary hypertension is defined as an increase of mean pulmonary arterial pressure $\geq$25 mmHg at rest as assessed by right heart catheterization. According to different combinations of values of pulmonary wedge pressure, pulmonary vascular resistance and cardiac output, a hemodynamic classification of pulmonary hypertension has been proposed. Of major importance is the pulmonary wedge pressure which allows to distinguish pre-capillary (pulmonary wedge pressure $\leq$15 mmHg) and post-capillary (pulmonary wedge pressure $>$15 mmHg) pulmonary hypertension. Pre-capillary pulmonary hypertension includes the clinical groups 1 (pulmonary arterial hypertension), 3 (pulmonary hypertension due to lung diseases and/or hypoxia), 4 (chronic thrombo-embolic pulmonary hypertension) and 5 (pulmonary hypertension with unclear and/or multifactorial mechanisms). Post-capillary pulmonary hypertension corresponds to the clinical group 2 (pulmonary hypertension due to left heart diseases).

Keywords Pulmonary arterial hypertension • Pulmonary artery pressure • Pulmonary hypertension • Right heart catheterization

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M. Humbert et al. (eds.), *Pharmacotherapy of Pulmonary Hypertension*,
Handbook of Experimental Pharmacology 218, DOI 10.1007/978-3-642-38664-0_1,

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1 Introduction

Pulmonary hypertension (PH) has previously been called an orphan disease, that is, a condition which affects a few individuals and is overlooked by the medical profession and pharmaceutical industry (Humbert et al. 2007). Although undoubtedly rare, the concept that PH is overlooked cannot be considered to be the case today. Indeed, there have been a number of important discoveries in recent years that have significantly improved our understanding of the disease, helped guide patient management, and laid foundations for future research (Simonneau et al. 2009; Galiè et al. 2009). The fifth world symposium on PH has taken place in 2013 in Nice, France, 40 years after the World Health Organization sponsored the first international conference in Geneva, Switzerland, on a mysterious condition named primary pulmonary hypertension (PPH), spurred by the interest created by the sudden increase in the disease in patients who had used an anorectic pill (aminorex fumarate) (Humbert 2012). Significant achievements have been made in the field since then, as reflected by the results presented at the second world symposium on PPH (Evian, France, 1998), the third world symposium on pulmonary arterial hypertension (PAH) (Venice, Italy, 2003), and the fourth world symposium on PH (Dana Point, US, 2008) (Humbert 2012).

Table 1 Hemodynamic definitions of pulmonary hypertension. Adapted from Galiè et al. (2009)

Definition	Characteristics	Clinical groups(s)[a]
PH	$\bar{P}_{pa} \geq 25$ mmHg	All
Pre-capillary PH	$\bar{P}_{pa} \geq 25$ mmHg	1. Pulmonary arterial hypertension
	$P_{pcw} \leq 15$ mmHg	3. PH due to lung diseases
	CO normal or reduced[b]	4. Chronic thromboembolic PH
		5. PH with unclear and/or multifactorial mechanisms
Post-capillary PH	$\bar{P}_{pa} \geq 25$ mmHg	2. PH due to left heart disease
	$P_{pcw} > 15$ mmHg	
	CO normal or reduced[b]	
Passive	TPG $\leq$12 mmHg	
Reactive (out of proportion)	TPG >12 mmHg	

$\bar{P}_{pa}$ mean pulmonary arterial pressure; P_{pcw} pulmonary capillary wedge pressure; *CO* cardiac output; *TPG* transpulmonary pressure gradient ($\bar{P}_{pa}$ $\bar{P}_{pcw}$)

[a]According to Table 2

[b]High CO can be present in cases of hyperkinetic conditions such as systemic-to-pulmonary shunts (only in the pulmonary circulation), anemia, hyperthyroidism, etc.

All values measured at rest

2 Definition

Pulmonary hypertension is defined as an increase of mean pulmonary arterial pressure (mPAP) $\geq$ 25 mmHg at rest as assessed by right heart catheterization (RHC) (Galiè et al. 2009). This value has been utilized for selecting patients in all randomized controlled trials on PAH and in PH registries. The normal mPAP at rest is 14 $\pm$ 3.3 mmHg and the upper limit of normal, according to these data, is 20.6 mmHg (Kovacs et al. 2009). It is currently undefined how to consider the values of mPAP between 21 and 24 mmHg (Galiè et al. 2009). In addition, the definition of PH during exercise as a mPAP $>$ 30 mmHg as assessed by RHC is not supported by convincing published data, and healthy individuals can reach higher values (Galiè et al. 2009; Kovacs et al. 2009; Whyte et al. 2012). Therefore, no definition for PH during exercise is currently accepted (Galiè et al. 2009). According to different combinations of values of pulmonary wedge pressure (PWP), pulmonary vascular resistance (PVR), and cardiac output (CO), a hemodynamic classification of PH has been proposed (Table 1) (Galiè et al. 2009). Of major importance is the PWP, which allows to distinguish pre-capillary PH (PWP $\leq$ 15 mmHg) and post-capillary PH (PWP $>$ 15 mmHg). Pre-capillary PH includes the following clinical groups: 1 (PAH), 3 (PH due to lung diseases and/or hypoxia), 4 (chronic thromboembolic PH, CTEPH), and 5 (PH with unclear and/or multifactorial mechanisms) (Simonneau et al. 2009; Galiè et al. 2009). Post-capillary PH includes the clinical group 2 (PH due to left heart diseases) (Simonneau et al. 2009; Galiè et al. 2009). In group 2, the transpulmonary gradient (TPG = mPAP $-$ PWP) allows the distinction of so-called passive "proportionate" post-capillary PH (TPG $\leq$ 12 mmHg) and reactive "out of proportion" post-capillary PH (TPG $>$ 12 mmHg) (Galiè et al. 2009). However, the concept of "proportionate" post-capillary PH is currently debated and the inclusion of other parameters such as the difference between

mPAP and diastolic PAP may allow a more accurate analysis of the pulmonary hemodynamics. In this chapter, the features of each clinical PH group are discussed in specific sections with particular attention to PAH.

3 Classification

The PH classification has gone through a series of changes since the first classification proposed in 1973, which identified only two main categories, namely "primary" or "secondary" PH, depending on the presence or absence of identifiable causes or risk factors (Simonneau et al. 2009; Galiè et al. 2009; Hatano and Strasser 1975). During the second world symposium on PPH (Evian, France, 1998), a new classification was proposed and attempted to create categories of PH that shared similar pathogenesis, clinical features, and therapeutic options (Simonneau et al. 2009). This classification defined homogenous groups of patients to help in conducting clinical trials and obtaining approval for specific therapies. In 2003, the third PH conference (Venice, Italy) proposed only minor changes, with the exception of the introduction of the terms of idiopathic PAH (IPAH), familial PAH, or associated PAH defining three PAH subgroups sharing broadly similar physiopathology and response to therapy (Simonneau et al. 2009). The other prominent change was to move pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) from separate categories into a single subcategory of PH associated with substantial venous or capillary involvement (Simonneau et al. 2009). Indeed PAH and PVOD share similarities with idiopathic PAH, including clinical presentation, hemodynamic characteristics, and risk factors, that justified placing them together in group 1. However, PVOD and PCH differ from PAH in terms of pathological changes and worst responses to vasodilator therapies (Simonneau et al. 2009).

In 2008, the fourth world PH conference held in Dana Point, California, proposed to revise previous classifications in order to accurately reflect information published over the past 5 years, as well as to clarify some areas that were ambiguous (Simonneau et al. 2009). The current Dana Point classification is presented in Table 2.

4 Group 1: Pulmonary Arterial Hypertension

The nomenclature of the PAH subgroups and associated conditions has evolved with time, and additional modifications were included in the 2008 revision (Simonneau et al. 2009; Galiè et al. 2009).

Table 2 Updated clinical classification of pulmonary hypertension. Adapted from Simonneau et al. (2009)

Updated clinical classification of pulmonary hypertension (Dana Point, 2008)
1. Pulmonary arterial hypertension (PAH)
1.1. Idiopathic PAH
1.2. Heritable
1.2.1. BMPR2
1.2.2. ALK1, endoglin (with or without hereditary hemorrhagic telangiectasia)
1.2.3. Unknown
1.3. Drug- and toxin-induced
1.4. Associated with
1.4.1. Connective tissue diseases
1.4.2. HIV infection
1.4.3. Portal hypertension
1.4.4. Congenital heart diseases
1.4.5. Schistosomiasis
1.4.6. Chronic hemolytic anemia
1.5. Persistent pulmonary hypertension of the newborn
1′. Pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)
2. Pulmonary hypertension owing to left heart diseases
2.1. Systolic dysfunction
2.2. Diastolic dysfunction
2.3. Valvular disease
3. Pulmonary hypertension owing to lung diseases and/or hypoxia
3.1. Chronic obstructive pulmonary disease
3.2. Interstitial lung disease
3.3. Other pulmonary diseases with mixed restrictive and obstructive pattern
3.4. Sleep-disordered breathing
3.5. Alveolar hypoventilation disorders
3.6. Chronic exposure to high altitude
3.7. Developmental abnormalities
4. Chronic thromboembolic pulmonary hypertension (CTEPH)
5. Pulmonary hypertension with unclear multifactorial mechanisms
5.1. Hematologic disorders: myeloproliferative disorders, splenectomy
5.2. Systemic disorders: sarcoidosis, pulmonary Langerhans' cell histiocytosis: Lymphangioleiomyomatosis, neurofibromatosis, vasculitis
5.3. Metabolic disorders: glycogen storage disease, Gaucher's disease, thyroid disorders
5.4. Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

ALK1 activin receptor-like kinase type 1; *BMPR2* bone morphogenetic protein receptor type 2; *HIV* human immunodeficiency virus

4.1 Group 1.1/1.2: Idiopathic and Heritable PAH

IPAH corresponds to sporadic disease in which there is neither a familial history of PAH nor identified risk factors (Simonneau et al. 2009; Galiè et al. 2009). When PAH occurs in a familial context, germline mutations in the gene coding for the

bone morphogenetic protein receptor 2 (*BMPR2*), a member of the transforming growth factor beta (TGF-β) signaling family, can be detected in more than 70 % of cases (Simonneau et al. 2009; Sztrymf et al. 2008). *BMPR2* mutations have also been detected in 11–40 % of apparently idiopathic cases with no familial history (Sztrymf et al. 2008; Girerd et al. 2010). Indeed, the distinction between idiopathic and familial PAH with *BMPR2* mutations is artificial, as all patients with a *BMPR2* mutation have heritable disease. Thus, it was decided to abandon the term "familial PAH" in favor of the term "heritable PAH" (Simonneau et al. 2009). Heritable forms of PAH include IPAH with germline mutations (mainly *BMPR2* but also *ACVRL1* or *endoglin*) and familial cases with or without identified mutations (Simonneau et al. 2009; Sztrymf et al. 2008; Girerd et al. 2010). Idiopathic and heritable PAH is more common in women than in men with a gender ratio around 2:1 (Sztrymf et al. 2008; Girerd et al. 2010).

Recent studies have shown that heritable PAH patients carrying a *BMPR2* mutation (irrespective of the familial history of other PAH cases) were younger at diagnosis, had more severe disease, and were less likely to demonstrate vasoreactivity than IPAH patients without a *BMPR2* mutation (Sztrymf et al. 2008; Girerd et al. 2010; Elliott et al. 2006). It is now commonly accepted that genetic testing should be performed as part of a comprehensive program that includes genetic counseling and discussion of the risks, benefits, and limitations of such testing (Girerd et al. 2010).

4.2 Group 1.3: Drug- and Toxin-Induced PAH

In the recent revision of the PAH classification aminorex, fenfluramine, dexfenfluramine, and toxic rapeseed oil represent the only identified "definite" risk factors for PAH (Simonneau et al. 2009; Galiè et al. 2009) (Table 3).

Aminorex fumarate structurally resembles adrenaline and ephedrine and is a potent appetite suppressant and central stimulant. Its use in the 1960s led to an outbreak of rapidly progressive PAH (then termed PPH) in Switzerland, Austria, and Germany, with a median exposure-to-onset time, when known, of 8 months (ranging from 3 weeks to over 1 year), as first described in a Swiss medical clinic (Follath et al. 1971; Gurtner 1979). The incidence of PAH in patients who had used aminorex was shown to be about 0.2 % overall, and proportional to the amount of drug taken. Furthermore, if discontinued early enough, a regression of PPH could be seen. Subsequently, aminorex was withdrawn from the market in 1968.

Both fenfluramine and dexfenfluramine have been marketed as appetite suppressants. An outbreak of drug-induced PAH has been identified with these two agents in the 1980s–1990s (Brenot et al. 1993; Abenhaim et al. 1996; Simonneau et al. 1998; Souza et al. 2008). PAH cases in patients exposed to fenfluramine derivatives share clinical, functional, hemodynamic, and genetic features with IPAH. This suggests that fenfluramine exposure represents a potential trigger for PAH without influencing its clinical course (Souza et al. 2008).

Table 3 Updated risk factors for pulmonary arterial hypertension. Adapted from Simonneau et al. (2009)

Definite	Possible
Aminorex	Cocaine
Fenfluramine	Phenylpropanolamine
Dexfenfluramine	St. John's Wort
Benfluorex	Chemotherapeutic agents
Toxic rapeseed oil	SSRI
Likely	**Unlikely**
Amphetamines	Oral contraceptives
L-tryptophan	Estrogen
Methamphetamines	Cigarette smoking

PAH pulmonary arterial hypertension; *SSRI* selective serotonin reuptake inhibitor

The association of fenfluramine and dexfenfluramine intake with the development of PAH was confirmed by the Surveillance of Pulmonary Hypertension in America (SOPHIA), which enrolled 1,335 subjects at tertiary PH centers in the USA between 1998 and 2001 (Walker et al. 2006). Of note, these agents have also been associated with an increased risk of valvular heart diseases, presumably because of their serotoninergic properties (Connolly et al. 1997). As a result, fenfluramine and dexfenfluramine were withdrawn from the market in the late 1990s (Souza et al. 2008; Humbert et al. 2006).

Benfluorex is a benzoate ester that shares similar structural and pharmacologic characteristics with dexfenfluramine and fenfluramine (Boutet et al. 2009; Savale et al. 2012; Frachon et al. 2010). The active and common metabolite of each of these molecules is norfenfluramine, which itself has a chemical structure similar to that of the amphetamines. Given its pharmacological properties, benfluorex would be expected to have toxic effects similar to the fenfluramine derivatives. An outbreak of valvular heart diseases and/or PAH induced by benfluorex use has been evidenced in France in the 2000s (Boutet et al. 2009; Savale et al. 2012; Frachon et al. 2010). Eighty-five cases of PH associated with benfluorex exposure were identified by the French PH network from June 1999 to March 2011 (Savale et al. 2012). Of these 85 cases, 70 patients had confirmed pre-capillary PH. Interestingly, 33 % of all patients also had prior exposure to fenfluramine or dexfenfluramine, and an additional risk factor for PH was identified in 30 % of the patients with pre-capillary PH (Savale et al. 2012). A quarter of patients in this series showed coexisting PH and mild-to-moderate cardiac valve involvement. These results, together with the accumulated data regarding the known toxic effects of fenfluramine and dexfenfluramine, strongly suggest that benfluorex exposure is a potent trigger for PAH. Benfluorex was withdrawn from the French market in 2009.

St John's Wort and over-the-counter antiobesity agents containing phenylpropanolamine also increase the risk of developing PAH (Walker et al. 2006). The SOPHIA study examined intake of a variety of nonselective monoamine reuptake inhibitors, selective serotonin reuptake inhibitors, antidepressants, and anxiolytics

and found no increased risk for developing PAH (Walker et al. 2006). However, case–control studies of selective serotonin reuptake inhibitors used during pregnancy showed an increased risk of developing persistent PH of the newborn in the offspring (Chambers et al. 2006). Based on this study, selective serotonin reuptake inhibitors have been reclassified to the "possible" category.

Amphetamines use represents a "likely" risk factor, although they are frequently used in combination with fenfluramine. A recent comprehensive, retrospective study suggested a strong correlation between the use of methamphetamine (inhaled, smoked, oral, or intravenous) and the occurrence of PAH (Chin et al. 2006). Based primarily on the results of this study, methamphetamine use is now considered a "very likely" risk factor for the development of PAH.

Cases of pre-capillary PH fulfilling the criteria of drug-induced PAH have been reported in chronic myelogenous leukemia patients treated with the tyrosine kinase inhibitor dasatinib (Montani et al. 2012). At diagnosis, patients had moderate-to-severe pre-capillary PH with functional and hemodynamic impairment. Clinical, functional, and hemodynamic improvements were observed within a few months of dasatinib discontinuation in most patients (Montani et al. 2012). However, after a median follow-up of 9 months (min–max 3–36), the majority of patients failed to demonstrate complete clinical and hemodynamic recovery and no patients reached a normal value of mPAH ($\leq$ 20 mmHg). The lowest estimate of incident PH occurring in patients exposed to dasatinib in France was 0.45 % (Montani et al. 2012). Thus, dasatinib may induce severe pre-capillary PH, fulfilling the criteria of PAH, suggesting a direct and specific effect of dasatinib on pulmonary vessels (Montani et al. 2012).

4.3 Group 1.4.1: PAH Associated with Connective Tissue Diseases

Pulmonary arterial hypertension associated with connective tissue diseases represents an important clinical subgroup (Humbert et al. 2006; Hachulla et al. 2005). The high prevalence of PAH in patients with systemic sclerosis has been well studied. Two prospective studies, using echocardiography as a screening method and RHC for diagnosis and confirmation, found a prevalence of PAH ranging from 8 % to 12 % (Hachulla et al. 2005; Mukerjee et al. 2003). Several long-term studies have showed that the outcomes of patients with PAH associated with systemic sclerosis are worse than those of IPAH patients. However, PAH does not represent the only cause of PH in systemic sclerosis. PH due to chronic lung disease such as pulmonary fibrosis and PH due to heart failure such as diastolic dysfunction are also frequent, emphasizing the importance of a complete evaluation to accurately classify and manage PH (Hachulla et al. 2009).

In systemic lupus erythematosus (Tanaka et al. 2002; Asherson et al. 1990) and mixed connective tissue disease (Burdt et al. 1999), the prevalence of PAH remains unknown, but likely occurs less frequently than in systemic sclerosis. Importantly,

corticosteroids and immunosuppressants may improve outcomes in a subset of patients with PAH complicating the course of systemic lupus erythematosus or mixed connective tissue disease, while this is not the case in IPAH or PAH associated with systemic sclerosis (Jais et al. 2008; Sanchez et al. 2006). In the absence of chronic pulmonary parenchymal involvement, PAH has been reported infrequently in other connective tissue diseases such as Sjögren syndrome (Launay et al. 2007), polymyositis (Bunch et al. 1981), or rheumatoid arthritis (Dawson et al. 2000).

4.4 Group 1.4.2: Human Immunodeficiency Virus Infection

Pulmonary arterial hypertension is a rare complication of human immunodeficiency virus (HIV) infection (Speich et al. 1991; Opravil et al. 1997; Mehta et al. 2000; Nunes et al. 2003; Sitbon et al. 2008; Zuber et al. 2004; Opravil and Sereni 2008; Degano et al. 2010). This PAH subcategory is the strongest evidence of virus-induced PAH. The HIV-associated PAH has clinical, hemodynamic, and histologic characteristics similar to those of IPAH. Epidemiologic data in the early 1990s, a time when highly active antiretroviral therapy was not yet available, showed a PAH prevalence of 0.5 % and a dramatic early mortality (Speich et al. 1991; Opravil et al. 1997). Since the wide use of highly antiretroviral therapies, a stable prevalence of 0.46 % was confirmed, while the incidence of PAH has declined (due to the longer survival of the HIV-associated PAH patients) (Sitbon et al. 2008; Opravil and Sereni 2008). Uncontrolled studies show that patients with severe HIV-associated PAH benefit from specific PAH therapies (Degano et al. 2010). Interestingly, normalization of hemodynamics has been regularly reported in a substantial number of the HIV-associated PAH patients treated with specific PAH drugs and highly active antiretroviral therapy (Degano et al. 2010).

4.5 Group 1.4.3: Portopulmonary Hypertension

Portopulmonary hypertension (PoPH) is defined by the development of PAH associated with increased pressure in the portal circulation (Hadengue et al. 1991; Rodriguez-Roisin et al. 2004; Kawut et al. 2008; Le Pavec et al. 2008). Prospective hemodynamic studies have shown that 2–6 % of patients with portal hypertension have PH (Hadengue et al. 1991; Rodriguez-Roisin et al. 2004). As always, RHC is mandatory for the accurate diagnosis of PoPH, because several mechanisms may increase mPAP in the setting of advanced liver disease: hyperdynamic circulatory state with high CO, fluid overload, and diastolic dysfunction; in such circumstances, PVR is within the normal range. Pathologic changes in the small arteries appear identical to those seen in IPAH. A recent multicenter case–control study conducted in the USA identified that female gender and autoimmune hepatitis were independent risk factors for the development of PoPH and that hepatitis C infection was associated

with a decreased risk (Kawut et al. 2008). Long-term prognosis of PoPH was related to the presence and severity of cirrhosis as well as to right heart function in a recently reported large French cohort study (Le Pavec et al. 2008).

4.6 Group 1.4.4: Congenital Heart Diseases

A significant proportion of patients with congenital heart disease, in particular those with systemic-to-pulmonary shunts, will develop PAH if left untreated (Simonneau et al. 2009; Galiè et al. 2009; Wood 1958; Hoffman and Rudolph 1965). Eisenmenger's syndrome is defined as congenital heart disease with an initial large systemic-to-pulmonary shunt that induces progressive pulmonary vascular disease and PAH, with resultant reversal of the shunt, hypoxemia, polycythemia, and cyanosis (Wood 1958; Hoffman and Rudolph 1965). It represents the most advanced form of PAH associated with congenital heart disease. The prevalence of PAH associated with congenital systemic-to-pulmonary shunts in Europe and North America has been estimated to be between 1.6 and 12.5 cases per million adults, with 25–50 % of this population affected by Eisenmenger's syndrome. These numbers are much higher in developing countries. The histological and pathophysiological changes seen in patients with PAH associated with congenital systemic-to-pulmonary shunts, in particular endothelial dysfunction, are similar to those observed in idiopathic or other associated forms of PAH. Following the fourth world symposium on PH, it was decided to update the pathologic and pathophysiological classification of congenital heart disease with systemic-to-pulmonary shunts (Table 4). In order to provide a more detailed description of each condition, four distinct phenotypes have been individualized (Table 5) (Simonneau et al. 2009; Galiè et al. 2009).

4.7 Group 1.4.5: Schistosomiasis

Embolic obstruction of pulmonary arteries by *Schistosoma* eggs was initially thought to be the main mechanism responsible for the development of PH in schistosomiasis (Simonneau et al. 2009; Chaves 1966; Lapa et al. 2009). However, it has been demonstrated that PH associated with schistosomiasis may have a similar clinical presentation and histologic findings than IPAH (Lapa et al. 2009). The mechanisms of PH in patients with schistosomiasis are probably multifactorial including PoPH and local vascular inflammation, whereas mechanical obstruction by *Schistosoma* eggs seems to play a minor role. Therefore, PH associated with schistosomiasis has been included in Group 1 (PAH) in the Dana Point updated classification (Simonneau et al. 2009; Galiè et al. 2009). More than 200 million people are infected by *Schistosoma* worldwide and 4–8 % of the infected individuals subsequently develop hepatosplenic disease. Therefore, PAH associated with schistosomiasis represents a frequent form of the disease,

Table 4 Anatomic-pathophysiological classification of congenital systemic-to-pulmonary shunts associated with PAH. Adapted from Simonneau et al. (2009)

1. Type
1.1. Single pre-tricuspid shunts
1.1.1. Atrial septal defect (ASD)
1.1.1.1. Ostium secundum
1.1.1.2. Sinus venosus
1.1.1.3. Ostium primum
1.1.2. Total or partial unobstructed anomalous pulmonary venous return
1.2. Simple post-tricuspid shunts
1.2.1. Ventricular septal defect (VSD)
1.2.2. Patent ductus arteriosus
1.3. Combined shunts (describe combination and define predominant defect)
1.4. Complex congenital heart disease
1.4.1. Complete atrioventricular septal defect
1.4.2. Truncus arteriosus
1.4.3. Single ventricle physiology with unobstructed pulmonary blood flow
1.4.4. Transposition of the great arteries with VSD (without pulmonary stenosis) and/or patent ductus arteriosus
1.4.5. Other
2. Dimension (specify for each defect if >1 congenital heart defect)
2.1. Hemodynamic (specify Qp/Qs)[a]
2.1.1. Restrictive (pressure gradient across the defect)
2.1.2. Nonrestrictive
2.2. Anatomic
2.2.1. Small to moderate (ASD ≤2.0 cm and VSD ≤1.0 cm)
2.2.2. Large (ASD >2.0 cm and VSD >1.0 cm)
3. Direction of shunt
3.1 Predominantly systemic-to-pulmonary
3.2 Predominantly pulmonary-to-systemic
3.3 Bidirectional
4. Associated cardiac and extracardiac abnormalities
5. Repair status
5.1. Unoperated
5.2. Palliated (specify type of operation(s), age at surgery)
5.3. Repaired (specify type of operation(s), age at surgery)

[a]Ratio of pulmonary (Op)-to-systemic (Qs) blood flow

especially in countries where the infection is endemic. Data from Brazil based on invasive hemodynamic measurements indicated the prevalence of PAH in patients with hepatosplenic disease of 4.6 % (Lapa et al. 2009) Of note, the prevalence of post-capillary PH was also important (3.0 %) reinforcing the need of RHC to appropriately characterize PH (Lapa et al. 2009).

Table 5 Clinical classification of congenital systemic-to-pulmonary shunts associated with PAH. Adapted from Simonneau et al. (2009)

A. Eisenmenger's syndrome	Includes all systemic-to-pulmonary shunts resulting from large defects and leading to a severe increase in PVR and a reversed (pulmonary-to-systemic) or bidirectional shunt; cyanosis, erythrocytosis, and multiple organ involvement are present
B. PAH associated with systemic-to-pulmonary shunts	Includes moderate to large defects; PVR is mildly to moderately increased, systemic-to-pulmonary shunt is still prevalent, and no cyanosis is present at rest
C. PAH with small defects	Small defects (usually ventricular septal defects <1 cm and atrial septal defects <2 cm of effective diameter assessed by echocardiography); clinical picture is very similar to idiopathic PAH
D. PAH after corrective cardiac surgery	Congenital heart disease has been corrected, but PAH is still present immediately after surgery or recurs several months or years after surgery in the absence of significant postoperative residual lesions

PAH pulmonary arterial hypertension; *PVR* pulmonary vascular resistance

4.8 Group 1.4.6: Chronic Hemolytic Anemia

There has been increasing evidence that PH is a complication of chronic hereditary and acquired hemolytic anemias, including sickle cell disease (Castro et al. 2003; Gladwin et al. 2004; Parent et al. 2011; Fonseca et al. 2012) and thalassemia (Aessopos et al. 1995). Pulmonary hypertension has been reported most frequently in patients with sickle cell disease; however, the prevalence of PAH is not clearly established. A large US study of patients with sickle cell disease, which screened PH by means of Doppler echocardiography by the presence of tricuspid regurgitation jet velocity (TRV) $\geq$2.5 m/s, found that 32 % of patients met this criteria (Gladwin et al. 2004). However, TRV $\geq$ 2.5 m/s is low threshold and leads to a substantial number of false-positive cases of PH if not confirmed by RHC (Parent et al. 2011; Fonseca et al. 2012). In this study, RHC was carried out in only 18 of 63 patients with TRV $\geq$ 2.5 m/s and PH defined by a mPAP $\geq$ 25 mmHg was confirmed in 17 patients (Gladwin et al. 2004). Nevertheless, PWP was elevated in a number of patients, emphasizing the complex mechanisms of PH in sickle cell patients. Indeed, a substantial proportion of sickle cell disease patients develop post-capillary PH (Parent et al. 2011). In addition, some patients present with a hyperkinetic state with moderate elevation in mPAP and normal PVR. Thus, the prevalence of pre-capillary PH in sickle cell disease is undoubtedly much lower than 32 %.

Two recent studies (Parent et al. 2011; Fonseca et al. 2012) have addressed the precise prevalence of pre- and post-capillary PH in sickle cell disease. In these two studies, patients with sickle cell disease underwent Doppler echocardiography with measurement of TRV. RHC had to be performed in all patients in whom PH was suspected on the basis of TRV $\geq$ 2.5 m/s. Pulmonary hypertension was defined as a

mPAP $\geq$ 25 mmHg. The results of these two studies performed in France (Gladwin et al. 2004) and in Brazil (Parent et al. 2011) are remarkably similar showing a prevalence of PH of 6.2 % and 10 %, respectively, which was lower than previously reported. Interestingly, post-capillary PH was the most frequent cause with a prevalence of 3.3 % and 6.2 %, respectively, whereas the prevalence of pre-capillary PH was only 2.9 % and 3.8 %, respectively (Parent et al. 2011; Fonseca et al. 2012). In addition, these two studies clearly demonstrated that when a threshold TRV of $\geq$2.5 m/s was used to define PH, the positive predictive value of echocardiography for the detection of pulmonary hypertension was only 25 % and 32 %, respectively (Parent et al. 2011; Fonseca et al. 2012). Lastly, pre-capillary PH associated with sickle cell disease appears substantially different from other forms of PAH, in terms of both hemodynamic profile and response to specific PAH therapies. These observations call into question the rationale of keeping sickle cell disease in group 1 (PAH) of the clinical PH classification (Parent et al. 2011).

4.9 Lessons from the French PAH Registry in the Modern Management era

Pulmonary arterial hypertension is a rare and severe vascular disease for which the trend is to manage the patients in designated centers with multidisciplinary teams working in a shared-care approach (Humbert et al. 2006; Humbert et al. 2010a; Humbert et al. 2010b). The French registry was initiated in 17 university hospitals following at least 5 newly diagnosed patients per year. All consecutive adult ($\geq$ 18 years) patients seen between October 2002 and October 2003 were to be included and followed up for at least 3 years: 674 patients (age of 50 $\pm$ 15 years (mean $\pm$ SD); range 18–85) were entered in the registry. Idiopathic, familial, anorexigen, connective tissue diseases, congenital heart diseases, portal hypertension, and HIV-associated PAH accounted for 39.2 %, 3.9 %, 9.5 %, 15.3 %, 11.3 %, 10.4 %, and 6.2 % of the population, respectively (Humbert et al. 2006). At the time of diagnosis, 75 % of patients were in the New York Heart Association (NYHA) functional class III or IV (Humbert et al. 2006). Six-minute walk distance was 329 $\pm$ 109 m. mPAP, cardiac index, and PVR index were 55 $\pm$ 15 mmHg, 2.5 $\pm$ 0.8 L/min/m^2, and 20.5 $\pm$ 10.2 mmHg/L/min/m^2, respectively (Humbert et al. 2006). Delay between symptom onset (mainly dyspnea on exercise) and PAH diagnosis was still $\geq$ 2 years (27 months), similar to that observed in the National Institutes of Health Registry in the 1980s, emphasizing the need for better PAH awareness and diagnostic strategy (Humbert et al. 2006). The low estimates of prevalence and incidence of PAH in France were 15.0 cases/million of adult population and 2.4 cases/million of adults per year, respectively (Humbert et al. 2006). Among the 354 consecutive adult patients with idiopathic, familial, or anorexigen-induced PAH who were prospectively enrolled, 56 had incident disease (PAH diagnosis was made at the time of recruitment in the registry) and

298 were prevalent cases (PAH diagnosis was made months or years before recruitment in the registry) (Humbert et al. 2010a; Humbert et al. 2010b). Patients were followed for 3 years and survival rates were analyzed. For incident idiopathic, familial, and anorexigen-induced PAH, estimated survival (95 % confidence intervals) at 1, 2, and 3 years was 85.7 % (76.5–94.9 %), 69.6 % (57.6–81.6 %), and 54.9 % (41.8–68.0 %), respectively (Humbert et al. 2010a). In a combined analysis population (incident patients and prevalent patients diagnosed within 3 years prior to study entry; $n = 190$), 1-, 2-, and 3-year survival estimates were 82.9 % (72.4 %–95.0 %), 67.1 % (57 · 1 %–78.8 %), and 58.2 % (49.0 %–69.3 %), respectively (Humbert et al. 2010a). Individual survival analysis identified the following factors as significantly and positively associated with survival: being female, NYHA functional class I/II, a greater six-minute walk distance, a lower right atrial pressure, and a higher cardiac output (Humbert et al. 2010a). Multivariable analysis showed that being female and having a greater six-minute walk distance and a higher cardiac output were jointly significantly associated with improved survival (Humbert et al. 2010a). This contemporary registry highlights current practice and shows that PAH is still detected late in the course of the disease with a majority of patients displaying severe functional and hemodynamic compromise. In the modern management era, idiopathic, familial, and anorexigen-induced PAH remains a progressive, fatal disease. Mortality is most closely associated with male gender, right ventricular hemodynamic function, and exercise limitation (Humbert et al. 2010a; Humbert et al. 2010b).

5 Group 1′: Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are uncommon conditions, but they are increasingly recognized as causes of PH (Montani et al. 2008). Similarities in pathological features and clinical presentation suggest that PVOD and PCH may in fact overlap, and it has been shown that PCH may be an angioproliferative process frequently associated with PVOD (Lantuejoul et al. 2006). Both conditions are difficult to categorize, as they share characteristics with PAH but also have a number of distinct differences. Given the current evidence, it has been decided at the Dana Point world symposium that PVOD/PCH should be a distinct category but not completely separated from PAH, and they are currently designated as a category 1′ in the updated classification (Simonneau et al. 2009; Galiè et al. 2009).

Pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis share a number of characteristics with PAH. First, some histologic changes in the small pulmonary arteries (intimal fibrosis and medial hypertrophy) observed in PAH are also found in PVOD/PCH. Second, clinical presentation and hemodynamic characteristics of PVOD/PCH and PAH are often indistinguishable (Montani et al. 2008; Montani et al. 2009). Third, PVOD/PCH and PAH share similar risk

factors, including connective tissue diseases such as systemic sclerosis (Dorfmuller et al. 2007; Günther et al. 2012), HIV infection (Escamilla et al. 1995), and anorexigen intake (Montani et al. 2009). Last, familial occurrence has been reported in both PVOD/PCH, and mutations in the *BMPR2* gene have been documented in patients with PVOD (Montani et al. 2008; Montani et al. 2009). These findings suggest that PVOD, PCH, and PAH may represent different components of a single spectrum of disease.

Although PVOD/PCH may present similarly to PAH, there are also a number of important differences. These include the presence of crackles on clinical examination, radiologic abnormalities best seen on high-resolution computed tomography of the chest (nodular ground glass opacities, septal thickening, mediastinal lymph node enlargement) (Montani et al. 2008; Montani et al. 2009), hemosiderin-laden macrophages on bronchoalveolar lavage (Rabiller et al. 2006), a more pronounced hypoxemia, and a lower diffusing capacity for carbon monoxide (DLCO) in patients with PVOD/PCH (Montani et al. 2008; Montani et al. 2009). In addition, the response to PAH therapy and prognosis of PVOD/PCH are worse than in PAH, with an increased risk of pulmonary edema with continuous intravenous epoprostenol, calcium-channel blockers, endothelin receptor antagonists, and phosphodiesterase type 5 inhibitors (Montani et al. 2008; Montani et al. 2009; Humbert et al. 1998).

6 Group 2: Pulmonary Hypertension due to Left Heart Disease

Left-sided ventricular or valvular diseases may produce an increase in left atrial pressure, leading to a backward transmission of the pressure and a passive increase of pulmonary pressures. Left heart disease probably represents the most frequent cause of PH (Simonneau et al. 2009; Galiè et al. 2009; Oudiz 2007). In this situation, PVR is normal or near normal (< 3 mmHg/L/min) and there is no gradient between mPAP and PWP (TPG ≤ 12 mmHg) (Galiè et al. 2009). The increasing recognition of the left-sided heart dysfunction with a preserved ejection fraction has led to changes in the subcategories of Group 2, which now includes left heart systolic dysfunction, left heart diastolic dysfunction, and valvular diseases (Simonneau et al. 2009; Galiè et al. 2009). In some patients with left heart disease, the elevation of mPAP is out of proportion to that expected from the elevation of left arterial pressure (TPG > 12 mmHg) and PVR is higher than 3 mmHg/L/min (Galiè et al. 2009). Some patients with valvular disease or left heart dysfunction can develop severe PH of the same magnitude as that seen in PAH (Zener et al. 1972). The elevation of mPAP and PVR may be due to either the increase of pulmonary artery vasomotor tone and/or pulmonary vascular remodeling (Delgado et al. 2005; Moraes et al. 2000). No large, randomized controlled trial using medications approved for PAH have been performed in this patient population, and the efficacy and safety of PAH medications are currently unknown.

7 Group 3: Pulmonary Hypertension due to Lung Diseases and/or Hypoxia

In this group, the predominant cause of PH is alveolar hypoxia as a result of either chronic lung disease, impaired control of breathing, or living at high altitudes (Simonneau et al. 2009; Galiè et al. 2009; Weitzenblum et al. 1981; Thabut et al. 2005; Chaouat et al. 2005; Chaouat et al. 2008; Cottin et al. 2005). However, the precise prevalence of PH in all these conditions remains largely unknown. In the updated PH classification, a category of lung disease characterized by a mixed obstructive and restrictive pattern was added, including combined pulmonary fibrosis and emphysema (Simonneau et al. 2009; Cottin et al. 2005). In PH associated with parenchymal lung disease, the increase of mPAP is usually modest (mPAP $<$ 35 mmHg) (Simonneau et al. 2009; Weitzenblum et al. 1981; Chaouat et al. 2008). In a retrospective study of 998 patients with chronic obstructive pulmonary disease who underwent RHC, only 1 % had severe PH defined as mPAP above 40 mmHg (Chaouat et al. 2005). These patients with severe PH had a mild-to-moderate airway obstruction, severe hypoxemia, hypocapnia, and a very low DLCO (Thabut et al. 2005; Chaouat et al. 2005). Large, randomized controlled trials of PAH therapies in patients with parenchymal lung disease and severe PH have not been performed, and there is no current recommendation supporting the use of PAH drugs in this group of patients (Humbert and Simonneau 2010).

8 Group 4: Chronic Thromboembolic Pulmonary Hypertension

Even if the exact incidence and prevalence of chronic thromboembolic pulmonary hypertension (CTEPH) are uncertain, it represents a frequent cause of PH and occurs in up to 4 % of patients after an acute pulmonary embolism (Tapson and Humbert 2006; Pengo et al. 2004). In the previous classification, CTEPH was divided into two subgroups: proximal CTEPH and distal CTEPH, depending on the technical possibility to manage PH by means of surgical pulmonary endarterectomy (Simonneau et al. 2009). However, there is no clear consensus about the exact definitions of proximal and distal CTEPH, and the decision regarding surgical treatment may vary depending on individual centers (Simonneau et al. 2009). Thus, in the new classification, it was decided to keep in Group 4 only a single category of CTEPH without distinction between proximal and distal forms (Simonneau et al. 2009; Galiè et al. 2009). Patients with suspected or confirmed CTEPH need to be referred to a center with expertise in the medical and surgical management of this condition, in order to consider the feasibility of performing surgery. The latter depends on the location of the obstruction, the correlation between hemodynamic findings, and the degree of mechanical obstruction assessed by pulmonary angiography, comorbidities, the willingness of the patient, and the experience of the medical and surgical teams

(Simonneau et al. 2009; Galiè et al. 2009; Kim 2006; Dartevelle et al. 2004; Jamieson et al. 2003). Patients who are not candidates for surgery may benefit from off-label use of PAH medical therapy (Suntharalingam et al. 2008; Rubin et al. 2006). However, further evaluation of these therapies in randomized control trials is needed (Rubin et al. 2006).

The clinical characteristics and management of patients enrolled in an international CTEPH registry have been recently reported (Pepke-Zaba et al. 2011). The international registry included 679 newly diagnosed ($\leq$ 6 months) consecutive patients with CTEPH (February 2007 to January 2009). Diagnosis was confirmed by RHC, ventilation–perfusion lung scintigraphy, computerized tomography, and/or pulmonary angiography. At diagnosis, a median of 14.1 months had passed since first symptoms; 427 patients (62.9 %) were considered operable, 247 (36.4 %) non-operable, and 5 (0.7 %) had no operability data; 386 patients (56.8 %, ranging 12.0–60.9 % across countries) underwent surgery (Pepke-Zaba et al. 2011). Operable patients did not differ from non-operable patients relative to symptoms, NYHA functional class, and hemodynamics. A history of acute pulmonary embolism was reported for 74.8 % of patients (77.5 % operable, 70 % non-operable) (Pepke-Zaba et al. 2011). Associated conditions included thrombophilic disorder in 31.9 % (37.1 % operable, 23.5 % non-operable) and splenectomy in 3.4 % of patients (1.9 % operable, 5.7 % non-operable) (Pepke-Zaba et al. 2011). At the time of CTEPH diagnosis, 37.7 % of patients initiated at least one PAH therapy (28.3 % operable, 53.8 % non-operable) (Pepke-Zaba et al. 2011). Pulmonary endarterectomy was performed with a 4.7 % documented mortality rate (Pepke-Zaba et al. 2011). Long-term follow-up of this CTEPH cohort is currently being studied with a goal to analyze outcomes according to clinical presentation and treatment strategy.

9 Group 5: Pulmonary Hypertension with Unclear or Multifactorial Etiologies

9.1 Group 5.1: Hematologic Disorders

Pulmonary hypertension has been reported in chronic myeloproliferative disorders including polycythemia vera, essential thrombocythemia, and chronic myelogenous leukemia (Guilpain et al. 2008; Adir and Humbert 2010). Several mechanisms may be implicated in PH associated with chronic myeloproliferative disorders including high cardiac output, spleen involvement (asplenia or spleen enlargement), direct obstruction of pulmonary arteries by circulating megakaryocytes, CTEPH, portopulmonary PH, drug-induced PAH (dasatinib), and congestive heart failure (Montani et al. 2012; Adir and Humbert 2010). Splenectomy, as a result of trauma or as a treatment for hematologic disorders, may increase the risk of developing PH (Adir and Humbert 2010). CTEPH and several cases of PAH with medial hypertrophy, intimal fibrosis, and plexiform lesions in the pulmonary vasculature have been reported in association with splenectomy (Hoeper et al. 1999; Jais et al. 2005).

9.2 Group 5.2: Systemic Disorders

The second subgroup includes systemic disorders, including sarcoidosis, pulmonary Langerhans' cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, or vasculitis (Simonneau et al. 2009; Galiè et al. 2009).

Sarcoidosis is a common systemic granulomatous disease of an unknown etiology. PH is an increasingly recognized complication of sarcoidosis, with a reported prevalence of 1–28 % (Shorr et al. 2005; Nunes et al. 2006). PH is often attributed to the destruction of the capillary bed by the pulmonary fibrotic process and/or results from chronic hypoxia (Shorr et al. 2005; Nunes et al. 2006). However, the severity of PH does not occasionally correlate to the severity of parenchymal lung disease and blood gas abnormalities, suggesting that other mechanisms may contribute to the development of PH (Nunes et al. 2006). Among these mechanisms, one can consider extrinsic compression of large pulmonary vessels by lymph node enlargement or mediastinal fibrosis, granulomatous infiltration of the pulmonary vasculature, affecting especially the pulmonary veins (which sometimes mimics PVOD), cardiac sarcoidosis which may cause heart failure and post-capillary PH, and hepatic sarcoidosis which may cause portopulmonary PH (Shorr et al. 2005; Nunes et al. 2006).

Pulmonary Langerhans' cell histiocytosis is an uncommon lung disease that predominantly affects young adults and develops almost exclusively in those with a history of current or prior cigarette smoking. In pulmonary Langerhans' cell histiocytosis, pre-capillary PH is frequently observed in patients with advanced lung destruction, though no clear relationship exists between PH and the extent of parenchymal lung disease and/or hypoxia (Fartoukh et al. 2000). This observation suggests that alternative or additional mechanisms contribute to an intrinsic pulmonary vasculopathy that involves both the pre-capillary arterioles and post-capillary venous compartment, in addition to possible PVOD-like lesions (Fartoukh et al. 2000). Patients with pulmonary Langerhans' cell histiocytosis that develop PH have a particularly poor prognosis and an early referral for lung transplantation assessment is recommended (Fartoukh et al. 2000; Le Pavec et al. 2012). Recent encouraging data suggest that agents licensed for use in PAH confer improvements in pulmonary hemodynamics and are generally well tolerated. Further investigations into the use of PAH medical therapy in this population are warranted (Le Pavec et al. 2012).

Lymphangioleiomyomatosis is a rare multisystem disorder predominantly affecting women, characterized by cystic lung destruction, lymphatic abnormalities, and abdominal tumors. PH is relatively uncommon in patients with lymphangioleiomyomatosis (Cottin et al. 2012). A retrospective, multicenter study evaluated 20 patients with lymphangioleiomyomatosis and pre-capillary PH (Cottin et al. 2012). This study confirmed that PH of mild hemodynamic severity may occur in patients with lymphangioleiomyomatosis, even with mild pulmonary function impairment (Cottin et al. 2012). Off-label use of PAH therapies might improve hemodynamic parameters but requires further investigations (Cottin et al. 2012).

Neurofibromatosis type 1, also known as von Recklinghausen's disease, is an autosomal dominant disease that can be recognized by characteristic "café au lait" skin lesions and by cutaneous fibromas (Montani et al. 2011; Stewart et al. 2007). Neurofibromatosis type 1 is occasionally complicated by systemic vasculopathy. Several cases of PH have recently been reported in patients with von Recklinghausen's disease (Montani et al. 2011; Stewart et al. 2007). The mechanism of PH is unclear, and lung fibrosis and CTEPH may play a role in the development of PH. In rare cases, histological examination found both arteries and veins narrowed by medial and/or intimal hypertrophy and fibrosis (Montani et al. 2011; Stewart et al. 2007).

Last, some rare cases of PH have been observed in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis with clinical presentation similar to PAH; however, histological data are not available (Launay et al. 2006).

9.3 Group 5.3: Metabolic Disorders

PH has been reported in a few cases of type Ia glycogen storage disease, a rare autosomal recessive disorder caused by a deficiency of glucose-6-phosphatase (Hamaoka et al. 1990; Humbert et al. 2002; Pizzo 1980). The mechanisms of PH are uncertain, but portocaval shunts, atrial septal defects, severe restrictive pulmonary disease, or thromboembolic disease are thought to play a role. In one case, postmortem examination revealed the presence of plexiform lesions (Pizzo 1980).

Gaucher's disease is a rare disorder characterized by a deficiency of lysosomal B glucosidase, which results in an accumulation of glucocerebroside in reticuloendothelial cells. PH has been reported in Gaucher's disease with several potential mechanisms, including interstitial lung disease, chronic hypoxia, capillary plugging by Gaucher's cells, and splenectomy (Elstein et al. 1998; Theise and Ursell 1990).

The association between thyroid diseases and PH has been reported in a number of studies (Badesch et al. 1993; Chu et al. 2002; Li et al. 2007). High prevalence of autoimmune hypo- and hyperthyroidism suggests that these conditions may share a common (auto)immune susceptibility (Badesch et al. 1993; Chu et al. 2002; Li et al. 2007).

9.4 Group 5.4: Miscellaneous Conditions

The last subgroup includes a number of miscellaneous conditions, including tumoral extrinsic or intrinsic obstruction, fibrosing mediastinitis, and chronic renal failure with dialysis.

A progressive obstruction of proximal pulmonary arteries leading to PH may be observed when a tumor grows into the central pulmonary arteries with additional thrombosis. Such cases are mainly associated with pulmonary artery sarcomas (Anderson et al. 1995; Mayer et al. 2001). The differential diagnosis with CTEPH can be difficult and findings on angiography by computed tomography or

magnetic resonance imaging, as well as 18 F-fluorodeoxyglucose positron emission tomography, may be useful to differentiate an obstruction by tumor or thrombotic material. Occlusion of the microvasculature by metastatic tumor emboli represents another rare cause of rapidly progressive PH (Roberts et al. 2003; Dot et al. 2007). The initial laboratory evaluation often shows severe hypoxemia. CT scanning does not reveal proximal thrombi but often shows thickening of septa. In contrast, the ventilation/perfusion lung scan is generally abnormal with multiple subsegmental perfusion defects. Pulmonary microvascular cytology sampling through a pulmonary artery catheter in the wedge position is an important diagnostic tool (Dot et al. 2007). The majority of reported cases occur in association with breast, lung, or gastric carcinoma (Roberts et al. 2003; Dot et al. 2007).

Fibrosing mediastinitis may be associated with severe PH due to compression of both pulmonary arteries and veins (Davis et al. 2001; Loyd et al. 1988; Goodwin et al. 1972). Ventilation/perfusion lung scan, computerized tomography of the chest, and pulmonary angiography are useful for accurate diagnosis; however, findings can mimic proximal thrombotic obstruction (Seferian et al. 2012). The predominant etiologies are histoplasmosis (Loyd et al. 1988; Goodwin et al. 1972), tuberculosis (Goodwin et al. 1972; Seferian et al. 2012), and sarcoidosis (Nunes et al. 2006).

Lastly, PH has been reported in patients with end-stage renal disease maintained on long-term hemodialysis (Yigla et al. 2003). Based on echocardiographic studies, elevated estimated PAP may be high in this patient population (Yigla et al. 2003). There are several potential explanations for the development of PH in these patients: mPAP may be increased by high CO (resulting from the arteriovenous access and anemia), as well as fluid overload. In addition, diastolic and systolic left heart dysfunctions are also frequent in this setting, leading to a significant proportion of post-capillary PH (Yigla et al. 2003; Nakhoul et al. 2005). Furthermore, hormonal and metabolic derangement associated with end-stage renal disease might promote dysfunction of pulmonary vascular tone.

10 Conclusion

Since the mid-twentieth century, a significant progress has been made in the PH field starting from the development of RHC techniques and the first description of PPH. The National Institutes of Health Registry and the world symposiums on PH have played an important role in this process. The most common causes of PH remain post-capillary PH due to left heart diseases (group 2) and pre-capillary PH due to chronic lung diseases and/or hypoxia (group 3). Besides these two common causes, CTEPH is of a major importance because it can be surgically managed. Lastly, PAH patients should be identified and classified early, as several targeted therapies have been approved in this group of diseases. The proceedings of the fifth world PH symposium will highlight current knowledge of PH that should help the international community to better manage PH patients (Humbert 2012).

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Pulmonary Hypertension: Pathophysiology and Signaling Pathways

Bradley A. Maron and Joseph Loscalzo

Abstract Pulmonary hypertension (PH) is characterized by pathological changes to cell signaling pathways within the alveolar-pulmonary arteriole–right ventricular axis that results in increases in pulmonary vascular resistance and, ultimately, the development of right ventricular (RV) dysfunction. Cornerstone histopathological features of the PH vasculopathy include intimal thickening, concentric hypertrophy, and perivascular fibrosis of distal pulmonary arterioles. The presence of plexogenic lesions is pathognomonic of pulmonary arterial hypertension (PAH); when present, this severe form of remodeling is associated with subtotal obliteration of the blood vessel lumen. The extent of RV remodeling in PH correlates with clinical symptom severity and portends a poor outcome. Currently available PH-specific pharmacotherapies that aim to improve symptom burden by targeting pulmonary vasodilatory/vasoconstrictor cell signaling pathways do not fully reverse pulmonary vascular remodeling and, thus, are largely unsuccessful at maintaining normal cardiopulmonary hemodynamics long term. Thus, determining the molecular mechanisms that are responsible for pulmonary vascular remodeling in PH is of great potential therapeutic value, particularly pathways that promote apoptosis-resistant cellular proliferation, disrupt normal cellular bioenergetics to alter cell function, and/or modulate severely abnormal responses to pulmonary vascular injury. This chapter reviews current insights into PH pathophysiology and disease mechanisms, and discusses novel cell signaling pathways that implicate microRNAs and mitochondrial dysfunction in the development of the PH phenotype.

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M. Humbert et al. (eds.), *Pharmacotherapy of Pulmonary Hypertension*,
Handbook of Experimental Pharmacology 218, DOI 10.1007/978-3-642-38664-0_2,

Keywords Network medicine • Systems pharmacology • Complex diseases • Pharmacogenetics

Contents

Abbreviations

BH_4	Tetrahydrobiopterin
BMP-RII	Bone morphogenetic protein receptor II
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
COX	Cyclooxygenase
EGFR	Epidermal growth factor receptor
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
ET_A	Endothelin-type A receptor
ET_B	Endothelin-type B receptor
FeNO	Iron-nitrosyl
GTP	Guanosine triphosphate
HAPE	High altitude pulmonary edema syndrome
HHT	Hereditary hemorrhagic telangiectasia
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus
IL	Interleukin
ISCU1/2	Iron–sulfur cluster assembly proteins
KL	Kruppel-like factor
LO	Lipooxygenases
LV	Left ventricle
MAPK	Mitogen-activated protein kinase
miR	MicroRNA
$NO^{\bullet}$	Nitric oxide
NOX	NADPH oxidase

$^{\bullet}O_2^-$	Superoxide
O_2NOO^-	Peroxynitrate
$ONOO^-$	Peroxynitrite
PAEC	Pulmonary artery endothelial cells
PAH	Pulmonary arterial hypertension
PDE	Phosphodiesterase inhibitor
PDGF	Platelet-derived growth factor
PDK	Pyruvate dehydrogenase kinase
PG	Prostaglandin
PH	Pulmonary hypertension
PKG	Protein kinase G
PPAR-γ	Peroxisome proliferator-activated receptor
PSMC	Pulmonary artery smooth muscle cells
PTPC	Permeability transition pore complex
ROS	Reactive oxygen species
RV	Right ventricle
sGC	Soluble guanylyl cyclase
SOD	Superoxide dismutase
TAPSE	Tricuspid annular plane systolic excursion
TGF	Transforming growth factor
TXA_2	Thromboxane
VEGF	Vascular endothelial growth factor

1 Introduction

Maladaptive changes to the phenotype of pulmonary arterioles resulting in pulmonary vascular dysfunction, right ventricular (RV) pressure loading, and, ultimately, right heart failure are a central pathophysiological mechanism leading to the development of clinically evident pulmonary hypertension (PH). The "two-hit" hypothesis of PH proposes that in the presence of a predisposing genetic and/or molecular substrate, exposure to certain environmental or biological mediators of vascular injury initiates a cascade of adverse cell signaling events culminating in gross structural malformation and functional deterioration to pulmonary arterioles. Although no single inciting event is known to trigger universally the development of PH, pulmonary endothelial dysfunction and decreased levels of bioavailable nitric oxide ($NO^{\bullet}$) are observed in early stages of many PH disease forms. Importantly, the pulmonary vascular bed hosts the greatest density of vascular tissue within the human circulatory system (Barst and Rubin 2011); thus, even subtle perturbations to signaling pathways that regulate structure and function of cells within the alveolar-pulmonary circulation interface may translate into meaningful changes to cardiopulmonary performance.

The cornerstone histopathological feature of PH is adverse remodeling of distal pulmonary arterioles that is characterized by intimal thickening (Farber and

Loscalzo 2004), dysregulated proliferation of apoptosis-resistant pulmonary artery endothelial cells (PAECs) and pulmonary vascular smooth muscle cells (PSMCs) (Abe et al. 2010), increased perivascular fibrosis, and, in certain forms of PH, the genesis of plexogenic lesions (Archer et al. 2010). Subtotal luminal obliteration of small- and medium-sized pulmonary arterioles, abnormal pulmonary vascular reactivity, and increased pulmonary blood vessel tone contribute to elevations in pulmonary vascular resistance and uncoupling of RV-pulmonary circulatory function (Rondelet et al. 2010). Enhanced understanding of cross talk between signaling pathways in PAECs, PSMCs, lung fibroblasts, and RV myocytes that occurs in response to injury has led to the development of PH-specific pharmacotherapies. These treatments aim to improve pulmonary vascular tone by restoring nitric oxide ($NO^{\bullet}$)- or prostacyclin-mediated signaling pathways, or through inhibition of endothelin-1 (ET-1)-dependent and -independent activation of vascular calcium channels that promotes vascular mitogenesis and vasoconstriction (Schneider et al. 2007; McLaughlin et al. 2009). Despite this progress, however, clinical outcome in PH remains poor, particularly among patients afflicted with pulmonary arterial hypertension (PAH), in which mortality rates approach 10 % within 1 year of diagnosis (Benza et al. 2010). This observation has stimulated novel dimensions of investigation that emphasize abnormalities in mitochondrial function, cellular metabolism, and microRNA (miR)-dependent responses to hypoxia as potentially under-recognized mechanisms involved in the pathogenesis of PH.

2 PH Pathophysiology

In PH, pulmonary circulatory performance is impaired as a consequence of adverse changes to the compliance of medium- and small-sized pulmonary arterioles that occur in response to chronic pulmonary vascular injury. In the majority of patients, these changes occur owing to hypoxic pulmonary vasoconstriction; vascular congestion in the setting of left atrial hypertension (i.e., impaired left ventricular [LV] function, mitral valve disease); or impedance to pulmonary blood flow as a consequence of primary lung, cardiac, pulmonary, or vascular thromboembolic disease (Maron and Loscalzo 2013). In PAH, the interplay between specific molecular and genetic factors induces the effacement of pulmonary arterioles and disrupts homeostatic mechanisms that control normal blood vessel tone and platelet function. This results in the classic PAH phenotypic triad of microvascular thrombosis, increased pulmonary vascular reactivity, and plexiform lesions (Fig. 1).

The contemporary definition of PH stipulates that the following hemodynamic criteria be met: a sustained elevation in mean pulmonary artery pressure (>25 mmHg) and pulmonary vascular resistance (>3 Wood units) in the setting of a normal pulmonary capillary wedge pressure. These measures emphasize *pulmonary vascular dysfunction* as the central determinate mitigating the diagnosis of PH. This distinction departs from previous iterations of this definition by identifying the pulmonary circulatory system as a specific entity within the larger

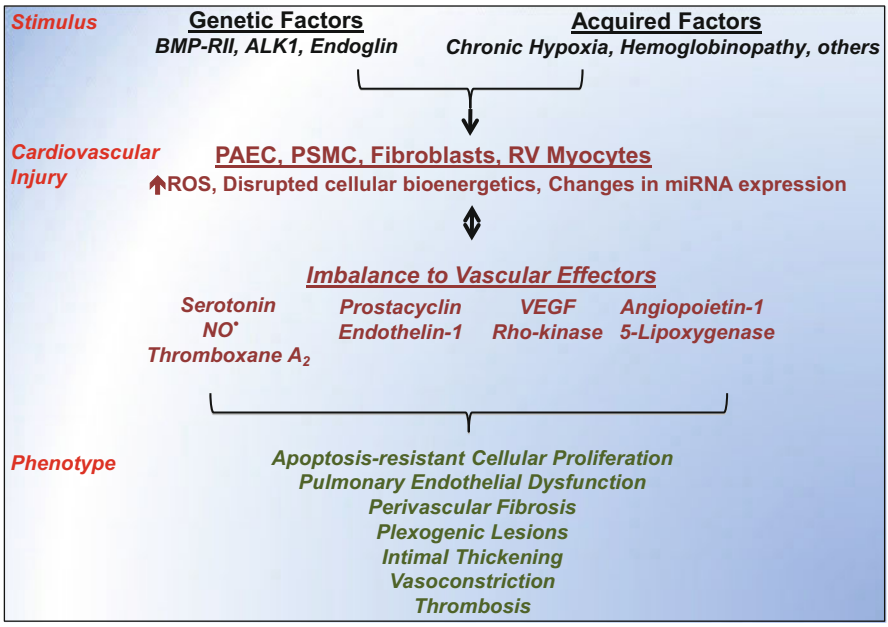

Fig. 1 Pathobiology of pulmonary arterial hypertension. The "two-hit" hypothesis of PAH contends that in the presence of certain genetic and/or molecular risk factors, exposure to environmental and/or biological stimulators of pulmonary/cardiovascular injury increases oxidant stress levels, promotes mitochondrial dysfunction, and upregulates specific microRNAs, which, in turn, dysregulate cell signaling pathways responsible for maintaining normal pulmonary vascular structure and function. Ultimately, these changes are associated with the classical PAH vasculopathy that is characterized by pulmonary endothelial dysfunction, plexogenic lesions, vasoconstriction, and microvascular thrombosis. *BMP-RII* bone morphogenetic protein receptor II, *PAEC* pulmonary artery endothelial cell, *PSMC* pulmonary smooth muscle cell, *RV* right ventricle, *ROS* reactive oxygen species, *NO•* nitric oxide, *VEGF* vascular endothelial growth factor, *miRNA* microRNA

cardiopulmonary apparatus. This approach furthermore reflects the fact that traditional PH treatment strategies, which emphasize PH-associated comorbidities (i.e., hypoxic lung disease, impaired left ventricular diastolic function) to alleviate symptoms, are often unsuccessful at providing patients with sufficient and sustained improvements to cardiopulmonary hemodynamics. Analyzing PH pathophysiology and, thus, the pursuit of novel therapies in the modern era must be predicated upon an understanding of biological/molecular factors that drive disease progression.

Increases in pulmonary vascular resistance are tolerated poorly by the RV, which, compared to the LV, is a thin-walled and non-compacted structure. Chronic changes to RV volume- and/or pressure-loading conditions result in adverse remodeling of the RV that is characterized by increased end-diastolic volume, geometric conformational changes from a normal tetrahedron to a crescentic trapezoid, and RV free wall hypertrophy (Voelkel et al. 2006) (Fig. 2). Eventual RV systolic dysfunction may be accelerated or compounded in severity by

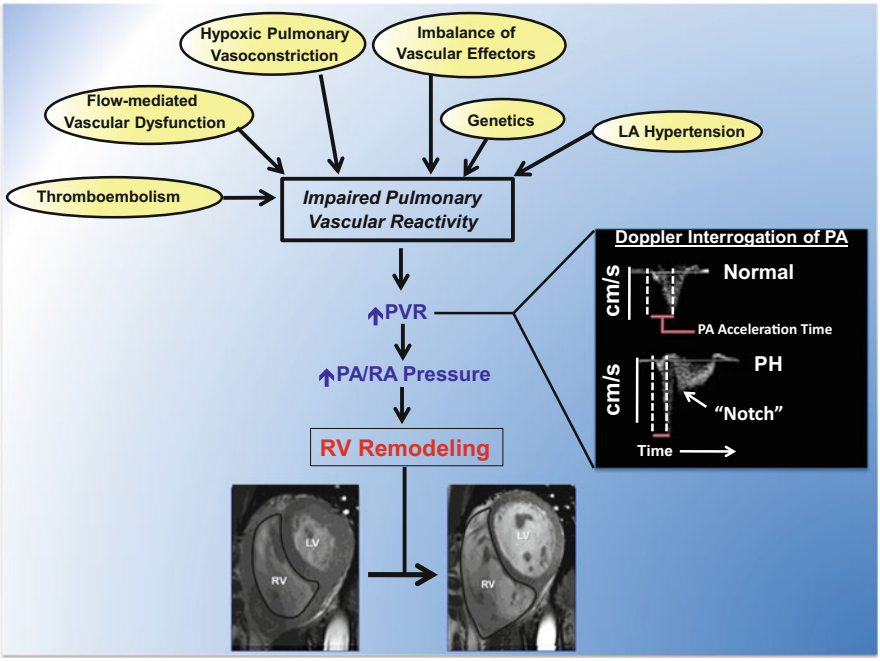

Fig. 2 Pathophysiology of pulmonary hypertension. Pulmonary hypertension (PH) is characterized by impaired pulmonary vascular reactivity due to a heterogeneous range of pathological cellular/molecular processes that mediate pulmonary vascular injury. Pulmonary vascular dysfunction in PH is detected clinically by elevations in pulmonary vascular reactivity, which may be assessed invasively by cardiac catheterization or noninvasively by transthoracic echocardiography (*inset*). Under normal conditions, Doppler interrogation of the pulmonary artery outflow tract results in a broad-based triangular signal envelope. In the presence of decreased vascular compliance, as is the case in moderate to severe forms of PH, the time to peak blood flow acceleration is decreased (PA acceleration time) and a reflected (i.e., retrograde) Doppler signal is detected as a mid- or late-systolic 'notch.' Increased right ventricular (RV) pressure loading results in geometric conformational changes to the RV cavity (outlined in black) as well as RV hypertrophy and systolic dysfunction. *LA* left atrial, *PA* pulmonary artery, *RA* right atrial, *cm/s* centimeters/second. Cardiac magnetic resonance images are reproduced with permission from Fernandez-Friera L, Alvarez-Garcia A, Guzman G et al. (2011) Apical right ventricular dysfunction in patients with pulmonary hypertension demonstrated with magnetic resonance. Heart 97:1250–1256

progressive tricuspid valve regurgitation that increases RV end-diastolic volume and enhances cavitary dilation. The pathobiological mechanisms involved in the development of frank, irreversible RV failure (i.e., cor pulmonale) are unresolved, but likely involve RV (subendocardial) ischemia (Gautier et al. 2007), strain/stress-induced intramural replacement fibrosis (Umar et al. 2012), and torsional effects on RV myocytes that are mediated by global changes to RV shape (Puwanant et al. 2010).

Pulmonary artery pressure is dependent partly upon RV systolic function; thus, in the setting of diminished RV contractility, pulmonary artery pressure may be normal despite severe pulmonary vascular disease. Along these lines, decremental changes in RV systolic function in patients with PH are associated with worsening symptomatology (e.g., dyspnea, fatigue, abdominal/peripheral edema), decreased functional capacity, and increased mortality. This is the case in patients with even *mild* heart failure (New York Heart Association Class II) and left atrial hypertension-associated PH due to LV systolic dysfunction, in which an RV ejection fraction $\leq$39 % is an independent predictor of early mortality (de Groote et al. 1998). Similarly, in patients with PAH, decreases in tricuspid annular plane systolic excursion (TAPSE), an echocardiographic measurement of RV systolic function, correlate inversely with 1-year mortality rates (Forfia et al. 2006). In turn, clinical benefits afforded to PAH patients by endothelin receptor antagonists and prostacyclin replacement therapy (see Part II, Olschewski 2013; Clozel et al. 2013) occur by virtue of their favorable effect on RV loading conditions, which promotes reverse RV remodeling and restores RV-pulmonary vascular coupling (Oikawa et al. 2005; Chin et al. 2008).

3 Cell Signaling Mechanisms in the Pathobiology of PH

3.1 Endothelial Nitric Oxide Synthase in PH

Nitric oxide ($NO^{\bullet}$) is a 30 Da lipophilic gaseous molecule, which may diffuse through PAEC/PSMC membranes to participate in intercellular signaling. Nitric oxide is synthesized in mammalian tissues via activation of three nitric oxide synthase (NOS) isoforms, each of which are homodimeric enzymes containing a calmodulin-binding domain that separates an N-terminal heme-binding domain and a C-terminal reductase domain (Porter et al. 1990). Nitric oxide synthases catalyze the formation of $NO^{\bullet}$ from L-arginine in a reaction that consists of two distinct monooxygenation steps. In the first monooxygenation step, two moles of electrons are donated by one mole of NADPH to a heme-bound oxygen via flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). This allows for the two-electron oxidation of a guanidine nitrogen of L-arginine to form one mole each of omega-*N*-hydroxy-L-arginine and water (Delker et al. 2010). In the second monooxygenation step, one-half mole of NADPH transfers one electron to a second heme-bound oxygen, and omega-*N*-hydroxy-L-arginine undergoes a three-electron oxidation to form one mole each of $NO^{\bullet}$ and L-citrulline (Griffith and Stuehr 1995).

Activation of endothelial NOS (eNOS), such as in response to vascular endothelial shear stress, is modulated by various intracellular posttranslational modifications, including *S*-nitrosylation (e.g., Cys94, Cys99), phosphorylation (e.g., Ser1177, Ser65, Thr495), and palmitoylation, among others (Dudzinski et al. 2006). The classical extracellular signaling pathways involved in eNOS

activation include G-protein-coupled receptor signal transduction, which increases intracellular Ca^{2+} levels and, subsequently, levels of Ca^{2+}-calmodulin; Akt signaling via sphingosine 1-phosphate; vascular endothelial growth factor (VEGF) via phosphatase calcineurin; and hormonal stimuli (e.g., estrogen and insulin) (Murata et al. 2002; Egom et al. 2011; Cerqueira et al. 2012). Decreased pulmonary vascular eNOS activity is observed in numerous animal models of PH in vivo and in humans with this disease (Steudel et al. 1998; Gangopahyay et al. 2011). Specifically, loss of $NO^{\bullet}$ bioavailability is linked to impaired endothelium-dependent and -independent vasodilation, increased PSMC mitogenesis, and platelet aggregation. Proposed mechanisms to account for diminished levels of functional eNOS in PH are provided below.

3.1.1 Hypoxia and eNOS in PH

The mechanism(s) by which hypoxia influences eNOS gene expression is (are) controversial, as PAEC exposure to $PaO_2 < 70$ mmHg has been associated with both increased *and* decreased eNOS protein expression levels. Fish and colleagues demonstrated that hypoxia induces a decrease in acetylation and lysine 4 methylation of eNOS proximal promoter histones to decrease eNOS gene transcription (Fish et al. 2010). In contrast, others have suggested that hypoxia-inducible factor-1α (HIF-1α), a master transcription factor that modulates a wide range of cellular processes in response to hypoxia, binds to a HIF response element near the promoter region of eNOS to increase eNOS gene expression (Coulet et al. 2003). However, in this scenario, hypoxia-mediated upregulation of eNOS expression does not necessarily imply increased eNOS *activity*. To the contrary, tonic stimulation of eNOS is associated with a paradoxical *decrease* in eNOS activity, likely owing to the consumption and subsequent depletion of key cofactors (i.e., 5,6,7,8-tetrahydrobiopterin [BH_4]) necessary for normal eNOS function. Under these conditions, eNOS is 'uncoupled,' resulting in the preferential generation of superoxide ($^{\bullet}O_2^-$) over $NO^{\bullet}$ (see Sect. 3.3). Data from PH experiments in vivo support this claim: eNOS deficiency and/or impaired eNOS function is a key factor in disease pathogenesis. For example, eNOS knockout mice ($eNOS^{-/-}$) exposed to *mild* hypoxia demonstrate significantly increased RV systolic pressure and diminished markers of eNOS bioactivity as compared to wild-type controls (Fagan et al. 1999). Diminished eNOS activity is also implicated in inflammatory (monocrotaline), genetic (bone morphogenetic protein receptor II [BMP-RII] deficient), and angioproliferative (VEGF inhibition with SU-5416) experimental models of PAH in vivo (Tang et al. 2004).

Hypoxia may also decrease eNOS activity by inducing posttranslational modification(s) of eNOS and/or caveolin-1, which decreases Ca^{2+} sensing by eNOS and results in dissociation of eNOS from its regulatory proteins, heat shock protein 90 and calmodulin (Murata et al. 2002). Alternatively, hypoxia may decrease levels of bioavailable $NO^{\bullet}$ through eNOS-independent mechanisms. In red blood cells, for example, hypoxia promotes increased levels of heme iron-nitrosyl (FeNO) that

limits hemoglobin *S*-nitrosylation, which, in turn, is a key PaO_2-sensitive mechanism implicated in the regulation of pulmonary vascular tone (McMahon et al. 2005).

3.1.2 Oxidant Stress and eNOS

Perturbations to the redox status of PAECs, PSMCs, RV myocytes, and lung fibroblasts due to activation of reactive oxygen species-generating (ROS) enzymes, such as NADPH oxidase (NOX), xanthine oxidase, and uncoupled eNOS, or via disrupted electron transport chain function in mitochondria promote pulmonary vasculopathy characterized by impaired $NO^{\bullet}$-dependent vasodilation, intimal thickening, and perivascular fibrosis (Mittal et al. 2012). In humans, increases in pulmonary vascular ROS generation may occur as a pathological response to chronic hypoxia, or increased pulmonary vascular blood flow (e.g., secondary to intracardiac shunt); or due to impaired antioxidant enzyme function, as is the case in sickle cell anemia-associated PH in which glutathione peroxidase deficiency is observed (Gizi et al. 2011). ROS may impair eNOS activity through the oxidation of enzyme cofactors (i.e., BH_4), or inactivate $NO^{\bullet}$ such as in the case of $^{\bullet}O_2^-$ which reacts with $NO^{\bullet}$ to generate peroxynitrite ($ONOO^-$). Additionally, the interaction of $^{\bullet}O_2^-$ with the stable $NO^{\bullet}$ by-product nitrite (NO_2^-) forms peroxynitrate (O_2NOO^-) and, thus, decreases levels of NO_2^-, which is a key substrate for NOS-independent synthesis of $NO^{\bullet}$ ($2HNO_2 \rightarrow N_2O_3 + H_2O$; $N_2O_3 \rightarrow NO^{\bullet} + NO_2^{\bullet}$) (Lundberg et al. 2011); (Spiegelhalder et al. 1976).

3.1.3 Genetic Mediators of eNOS in PH

BMP-RII is a serine–threonine kinase and member of the transforming growth factor-β (TGF-β) superfamily of receptors (Rosenzweig et al. 1995). Approximately 70 % of familial PAH cases involve mutations in BMP-RII, and receptor dysfunction is increasingly recognized as a contributor to non-PAH forms of PH (Machado et al. 2006). Although BMP-RII is believed to contribute to remodeling of pulmonary blood vessels through a wide range of signaling pathways, including SMAD-dependent PSMC migration (Long et al. 2009), it was recently demonstrated that two BMP-RII ligands, BMP2 and BMP4, are involved in BMP-RII-dependent phosphorylation of eNOS at Ser-1177 to upregulate eNOS activity (Gangopahyay et al. 2011). Similarly, abnormalities in the function of endoglin, a key BMP receptor accessory protein in human PAECs, are linked to the development of PAH when present in the setting of the clinical syndromes hereditary hemorrhagic telangiectasia (HHT) type 1 and type 2. Mice heterozygous for vascular endothelial endoglin expression ($Eng^{+/-}$) develop PH spontaneously in vivo due, in part, to increased pulmonary vascular ROS generation, eNOS uncoupling, and decreased NOS-inhibitable $NO^{\bullet}$ production (Toporsian et al. 2010).

Several eNOS polymorphisms are implicated in the development of PH and other vascular diseases. For example, a single nucleotide polymorphism leading to a substitution of aspartic acid for glutamic acid at position 298 (Glu298Asp) of eNOS and an increased NOS4a allelic frequency of 27-bp variable number of repeats increase susceptibility to developing the high altitude pulmonary edema (HAPE) syndrome, including elevations in pulmonary artery pressure and pulmonary vascular resistance. These changes may occur owing to function-limiting changes in the conformation of eNOS, although the precise mechanism by which to account for the phenomenon is unknown (Miyamoto et al. 1998; Droma et al. 2002; McDonald et al. 2004).

3.2 Endothelin-1 System

Endothelin-1 (ET-1) is a 21-amino acid vasoactive peptide that contains two disulfide bridges between Cys1-Cys15 and Cys3-Cys11 (Yeager et al. 2012), which are necessary for endothelin converting enzyme-mediated proteolytic cleavage of ET-1 from its precursor, 'Big ET-1.' Endothelin-1 is constitutively expressed in a wide range of mammalian cell types, including hepatic sinusoidal cells, renal epithelial cells, and PAECs (Huggins et al. 1993). Endothelin-1 gene expression levels are upregulated significantly in RV myocytes, PAECs, PSMCs, and lung fibroblasts in the presence of stimuli associated with pulmonary vascular injury in PH, including cytokines that mediate vascular inflammation (i.e., TGF-β, IL-6) (Olave et al. 2012), increased levels of pulmonary vascular ROS (An et al. 2007), hypoxia (Yamashita et al. 2001), and decreased levels of bioavailable $NO^{\bullet}$ (Kourembanas et al. 1993). In fact, plasma ET-1 levels may be increased fourfold in patients with PAH or PH due to left atrial hypertension, and anti-ET-1 immunohistochemical analysis demonstrates significantly increased immunoreactivity in PAECs and PSMCs of plexiform lesions compared to blood vessels harvested from normal controls (Giaid et al. 1993). Endothelin-1 is also released from sickled red blood cells and interacts with the blood vessel wall to promote vasoconstriction in a process that contributes to the systemic and pulmonary vasculopathy of sickle cell anemia (Gladwin and Vichinsky 2008).

Endothelin-1 regulates pulmonary vascular tone through its interaction with the vasoconstrictor endothelin-type A (ET_A) and -type B (ET_B) receptors in PSMCs and vasodilatory ET_B receptors in PAECs, which do not constitutively express ET_A. Endothelin-type A and ET_B receptors are members of the superfamily of G-protein-coupled receptors and are overall highly homologous (55 %), with the exception of the cysteine-rich 35-amino acid sequence distal to the seventh transmembrane domain, in which homology between receptors is only 75 % (Doi et al. 1999). Since cysteine(s) in this region are believed to regulate G-protein coupling to both ET_A and ET_B receptors, and, thus, are integral to receptor signal transduction, it has been postulated that differences in this region between receptor subtypes may account, in part, for their differential functions (Okamoto et al. 1997).

In PSMCs, stimulation of $ET_{A/B}$ receptors by ET-1 induces G_i and G_q coupling to modulate phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP_3). In turn, opening of IP_3-sensitive calcium (Ca^{2+}) channels as well as ET-1-mediated opening of the store-operated and nonselective Ca^{2+} channels induces an increase in intracellular Ca^{2+} flux ($[Ca^{2+}]_i$), Ca^{2+} waves, and Ca^{2+} oscillations that promotes vasoconstriction (Liu et al. 2012). Importantly, ET-1-induced vasoconstriction persists following ET-1 dissociation from the ET_A receptor, indicating that the $[Ca^{2+}]_i$ flux response mediated by ET-1 is robust, Ca^{2+}-dependent hyperpolarization is delayed during ET-1 signaling, or both (Zhang et al. 2003; Liu et al. 2012). The functional consequence of ET_A receptor signaling on vascular tone is noteworthy: relative to norepinephrine, the concentration of ET-1 required to induce 50 % blood vessel contraction (i.e., EC_{50}) in pig coronary arteries, rat aorta, and rat pulmonary artery is 0.52, 1.4, and 0.68, respectively (Huggins et al. 1993). ET-1 binding to ET_A receptors ($K_i = 0.6$ nmol l^{-1}) also promotes vascular smooth muscle cell mitogenesis by activating various signaling intermediaries that regulate protein synthesis, including protein kinase C; mitogen-activated protein kinase (MAPK); p70S6K, which targets the ribosomal protein S6K to increase cellular protein synthesis; and epidermal growth factor receptor (EGFR) via tyrosine phosphorylation (Iwasaki et al. 1999; Kapakos et al. 2010). Interestingly, upregulation of the proto-oncogene transcription factor *c-fos* by ET-1 (or hypoxia) is linked to cellular proliferation and fibrosis of PSMCs, lung fibroblasts, and myocytes in experimental animal models of PH (Rothman et al. 1994; Nishimura et al. 2003; Recchia et al. 2009), providing molecular evidence to account for the proliferative phenotypic overlap between plexogenic lesions of PAH and various solid tumors.

In contrast to PSMCs, ET-1 binding to the ET_B receptor (K_i of 0.12 nmol l^{-1}) in PAECs results in the activation of eNOS and synthesis of $NO^{\bullet}$, which is required to maintain normal pulmonary vascular tone and prevent vascular remodeling. Endothelin-type B receptor-dependent activation of eNOS is believed to occur via G-protein coupling to the ET_B receptor that stimulates $[Ca^{2+}]_i$ flux and subsequent elevations in Ca^{2+} binding to calmodulin, which is a key allosteric modulator of eNOS activity. Recent work from our laboratory has demonstrated that pathophysiological levels of the mineralocorticoid hormone aldosterone akin to those observed in humans with PH increase NOX4-dependent ROS generation in PAECs in vitro, which is associated with ET_B receptor dysfunction, impaired ET_B receptor-dependent activation of eNOS, and oxidation of $NO^{\bullet}$ to $ONOO^-$ (Maron et al. 2012).

Endothelin-type B receptor signal transduction also results in the synthesis of vasodilatory prostaglandins (PG). In isolated guinea pig lungs exposed to ET-1, a ~50-fold increase in ET_B receptor-dependent PGI_2 synthesis is observed (D'Orleans-Juste et al. 1991), although the mechanism by which ET_B receptor activation stimulates PGI_2 synthesis is not well characterized. Internalization of the ET_B receptor/ET-1 complex and subsequent proteasomal degradation is the chief mechanism by which ET-1 elimination occurs. This conclusion is supported

in vivo by experiments involving the transgenic spotting lethal rat (*sl*/*sl*), which lacks constitutively expressed vascular ET_B receptors. Compared to wild-type rats, these rats demonstrate significantly higher circulating levels of ET-1 and more severe PH following monocrotaline injection to induce pulmonary vascular injury (Nishida et al. 2004).

3.3 Soluble Guanylyl Cyclase and Phosphodiesterase Inhibition in PH

Nitric oxide is the primary biological activator of the heterodimeric (α_1/β_1 or α_1/β_2) enzyme soluble guanylyl cyclase (sGC) that catalyzes the conversion of cytosolic GTP to cGMP, which is a critical secondary signaling molecule necessary for activation of cGMP-dependent protein kinase (i.e., protein kinase G [PKG]) to promote PSMC relaxation and inhibit platelet aggregation and thrombosis. Nitric oxide binding to the heme (Fe^{2+}) prosthetic group of sGC results in the formation of a hexa-coordinated histidine–heme–$NO^{\bullet}$ intermediate. Subsequent cleavage of the heme–histidine bond leads to a dramatic upregulation of enzyme activity: nanomolar concentrations of $NO^{\bullet}$ may induce an appropriate 100-fold increase in sGC activation (Evgenov et al. 2006). In PH, elevated levels of ROS accumulation may impair sGC activity through the oxidation of heme from the ferrous (Fe^{2+}) to ferric (Fe^{3+}) state that converts sGC to an $NO^{\bullet}$-insensitive state, presumably owing to decreased affinity of $NO^{\bullet}$ for oxidized heme. Alternatively, others have demonstrated that sGC activity is influenced by the redox status of functional sGC cysteinyl thiol(s) in a manner that is independent of the heme redox state (Fernhoff et al. 2009; Yoo et al. 2012). Work from our laboratory has demonstrated that pathophysiological concentrations of H_2O_2 induce the formation of higher cysteinyl thiol oxidative states of Cys-122 on the β_1 subunit of sGC in vascular smooth muscle cells, including sulfenic acid, sulfinic acid, and the disulfide form. Posttranslational oxidative modification of Cys122, in turn, functions as a redox 'switch' that regulates enzyme function, resulting in decreased $NO^{\bullet}$-sensing by sGC and impaired enzyme activity (Maron et al. 2009). The importance of abnormal sGC function in the pathogenesis of PH is well established. Transgenic mice deficient in the sGC α_1-subunit develop exaggerated elevations in RV systolic pressure and muscularization of intraacinar pulmonary arterioles following exposure to chronic hypoxia compared to wild-type mice (Vermeersch et al. 2007). Moreover, hypoxia alone is associated with decreased mRNA and protein levels of sGC as well as sGC-dependent cGMP formation (Hassoun et al. 2004).

Collectively, these observations implicate the pharmacotherapeutic potential of heme-independent sGC activators in PH. Work from Ko and colleagues and drug discovery experiments performed at Bayer HealthCare in the early 1990s identified YC-1 [3-5′-hydroxymethyl-2′furyl)-1-benzyl indazole] and 5-substituted-2-furaldehyde-hydrazone derivative compounds (i.e., BAY compounds), respectively,

as synthetic heme-(in)dependent activators of sGC (Ko et al. 1994; Stasch et al. 2006). BAY 58-2667, perhaps the best studied among these compounds, activates sGC with a K_m of 74 μM and a V_{max} of 0.134 μmol min^{-1} mg^{-1} (Schmidt et al. 2003), and although the precise mechanism by which this (and other) BAY compounds activates heme-oxidized sGC is not fully resolved, one leading hypothesis contends that BAY 58-2667 competes with the oxidized heme moiety for binding to the sGC-activating motif to induce enzyme activation (Pellicena et al. 2004) (see Part III, Sect. 1). The effect of these compounds on sGC-$NO^{\bullet}$ vasodilatory signaling and $NO^{\bullet}$-dependent vascular remodeling has been assessed in PH in vivo. In one study, the administration of YC-1 to hypoxic mice decreased PSMC proliferation and pulmonary artery pressure (Huh et al. 2011). The effect of BAY 63-2521 (Riociguat™) on cardiopulmonary hemodynamics was also assessed in a small cohort of patients with PAH, chronic thromboembolic PH, or PH from interstitial lung disease. Drug therapy (3.0–7.5 mg day^{-1}) over 12 weeks decreased pulmonary vascular resistance by 215 dyne s cm^{-5}, which was associated with an increase in the median 6-min walk distance by 55.0 m from baseline (Ghofrani et al. 2010).

3.4 Phosphodiesterase Inhibition in PH

In 1962, Butcher and Sutherland implicated phosphodiesterase enzymatic activity in endogenous degradation of adenosine 3′, 5′ phosphate (cAMP) (Butcher and Sutherland 1962). Eleven PDE isoforms have since been detected in mammalian tissue (Table 1). The fields of PDE biochemistry and PH intersected following the identification of cGMP-specific PDE type-5, at elevated concentrations in PSMCs, platelets, and myocytes. Phosphodiesterase type-5 regulates cGMP bioactivity via (1) the hydrolysis of cGMP to 5′-GMP, and (2) allosteric binding of cGMP to PDE-5 GAF[1] domains, which induces a conformational change to the structure of PDE-5 and positively feeds back to promote cGMP metabolism (Fig. 3). PDE-5-cGMP binding ranges from K_d of 2.4 μM (pH = 5.2) to 0.15 μM (pH = 9.5) (Turko et al. 1999). The pH-dependent manner by which this occurs is in concert with reports demonstrating a regulatory role for the cyclic nucleotide ionizing residues (i.e., pH-sensitive) Asp-289 and Asp-478 in modulating PDE-5 function (McAllister-Lucas et al. 1995). The intracellular concentration of cGMP may also be influenced by flux through membrane-bound cGMP-gated channels and the multidrug transporter, although the contribution of these to total levels of bioactive cGMP is negligible in pulmonary vascular tissue (Serre et al. 1995).

[1] GAF is an acronym of the various tissues in which these domains were originally described: cGMP-dependent phosphodiesterases (PDEs), *nabaena* adenylyl cyclases, and *E. coli* FhlA (Francis et al. 2010).

Table 1 cGMP-specific kinetic properties of phosphodiesterases and their tissue distribution

Isoenzyme	K_m cGMP (μM)	V_{max} cGMP (μM)	Tissue distribution
PDE1A	3–4	50–300	VSMC, cardiomyocyte, brain
PDE1B	1–6	30	VSMC, cardiomyocyte, brain
PDE1C	1–2	Not determined	VSMC, cardiomyocyte, brain
PDE2A	10	123	VSMC, cardiomyocyte, brain, corpus cavernosum
PDE3A	0.02–0.2	0.3	VSMC, cardiomyocyte, brain, corpus cavernosum, platelets
PDE3B	0.3	2	VSMC, cardiomyocytes
PDE5A	1–6	1–3	Corpus cavernosum, PSMC, skeletal muscle, platelets, cardiomyocytes
PDE6A/B	15	2,300	Retina
PDE 6C	17	1,400	Retina
PDE9A	0.2–0.7	Not determined	Various
PDE 10A	13	Not determined	Various
PDE 11A	0.4–0.2	Not determined	Skeletal muscle, heart, VSMC

PDE phosphodiesterase, *VSMC* vascular smooth muscle cell, *PSMC* pulmonary smooth muscle cell. Adapted from Francis SH et al. (2010) cGMP-dependent protein kinase and cGMP phosphodiesterases in nitric oxide and cGMP action. Pharm Rev 62:525–563 and Reffelman T, Kloner RA (2003) Therapeutic role of phosphodiesterase 5 inhibition for cardiovascular disease. Circulation 108:239–244

In PAH, expression of PDE-5 is increased in PSMCs and in RV myocytes (Wharton et al. 2005; Nagendran et al. 2007), which is associated with decreased levels of bioactive $NO^{\bullet}$, pulmonary vascular dysfunction, and impaired RV lusitropy (Waxman 2011). In cultured PSMCs, PDE-5 inhibition attenuates key indices of adverse remodeling, including DNA synthesis/cell growth, cellular proliferation, and suppression of apoptosis (Wharton et al. 2005). Phosphodiesterase type-5 is also linked to decreased thrombotic burden in chronic thromboembolic PH, presumably by increasing bioactive cGMP levels in platelets to inhibit platelet aggregation (Suntharalingam et al. 2007).

3.5 *Prostacyclin Signaling in PH*

Arachidonic acid, or 5,8,11,14-eicosatetraenoic acid, is released from membrane phospholipids in response to mechanical or chemical stimuli, resulting in the synthesis of two major classes of eicosanoids: prostanoids, via cyclooxygenase (COX) pathway, and leukotrienes, via the lipooxygenase (LO) pathway. Cyclooxygenase (COX) exists in at least two isoforms. The constitutively expressed form, COX-1, is present in various cell types including the vascular endothelium, gastric mucosa, and platelets. In contrast, COX-2, which is the inducible form of COX, is present in cells involved in inflammation, particularly macrophages. COX-2 is also present in normal vascular endothelial cells and appears to be upregulated in

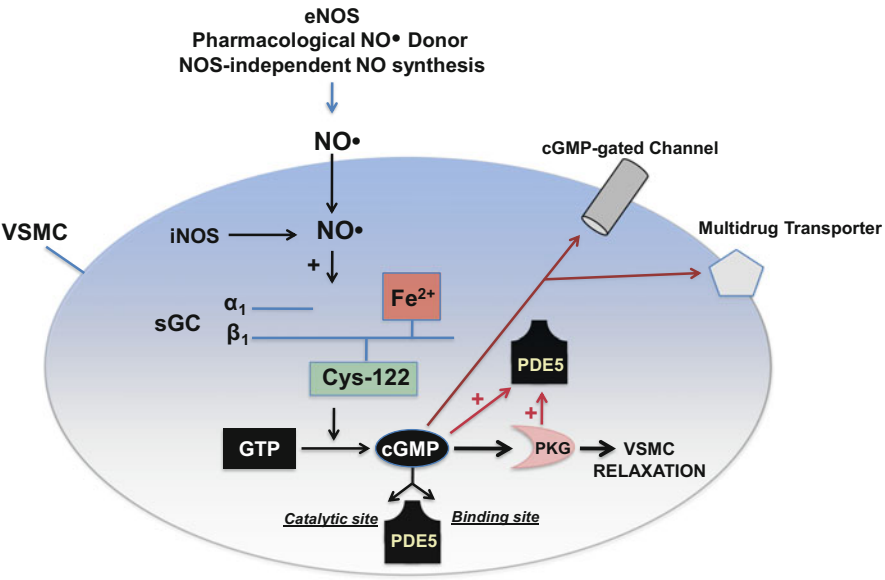

Fig. 3 Influence of phosphodiesterase type-5 on the nitric oxide signaling axis. Nitric oxide ($NO^{\bullet}$) derived from endothelial nitric oxide synthase (eNOS), pharmacological $NO^{\bullet}$ donors, or via induction of inducible NOS in vascular smooth muscle cells (VSMCs) activates soluble guanylyl cyclase (sGC) to generate cGMP. Normal $NO^{\bullet}$–sGC signaling is regulated by the redox status of the prosthetic heme ligand and functional cysteinyl thiol(s), such as Cys122, which are present near the catalytically active region of the β_1-subunit of sGC. The interaction of cGMP with its most relevant biological target, protein kinase G (PKG), results in VSMC relaxation. This vasodilatory effect of this pathway is offset through the interaction of cGMP with phosphodiesterases (PDEs), which decreased bioactive cGMP levels through hydrolysis of cGMP to form 5′GMP, or by allosteric binding of cGMP to PDE. In the case of PDE type-5 (PDE5), increased levels of cGMP and/or PKG upregulates PDE5 activity. The contribution of gated cGMP channels and the multidrug transported to the regulation of bioactive cGMP levels is negligible in pulmonary vascular tissue. Adapted from Francis SH et al. (2010) cGMP-dependent protein kinase and cGMP phosphodiesterases in nitric oxide and cGMP action. Pharm Rev 62:525–563, with permission

response to stimuli associated with vascular injury, such as shear stress (Topper et al. 1996). Key products of the COX pathway that are pertinent to the pathophysiology of PH include the potent vasoconstrictor and stimulus for platelet aggregation, thromboxane A_2 (TXA_2), and prostaglandin I_2 (prostacyclin), which exerts opposing effects to TXA_2, including vasodilation and inhibition of platelet activation. Prostacyclin is synthesized from PGH_2 by prostacyclin synthase in a reaction that occurs primarily in vascular endothelial cells. Early investigators speculated that the pulmonary vasoconstriction phenotype in PAH was a consequence of imbalanced TXA_2/PGI_2 synthesis. This hypothesis was supported by the observation that, compared to normal pulmonary blood vessels, pre-capillary pulmonary arterioles harvested from patients with PAH, hepato-pulmonary PH, and HIV-associated PH demonstrate significantly decreased PGI_2 synthase mRNA and protein expression levels (Tuder et al. 1999). In contrast, elevated levels of

TXA_2 and increased pulmonary vascular *sensitivity* to TXA_2 have been reported in PH, such as in the lamb model of hypoxia-induced neonatal persistent pulmonary hypertension (Hinton et al. 2007). These observations are consistent with other reports in adults demonstrating that, compared to healthy controls, patients with PAH or PH due to hypoxic lung disease demonstrate elevated levels of urinary excretion of 11-dehydro-thromboxane B_2, a stable metabolite of TXA_2, and decreased levels of the stable prostacyclin metabolite, 2,3-dinor-6-keto-prostaglandin F1α (Christman et al. 1992).

Shear stress, ET-1, hypoxia, and BMP-RII dysfunction are each associated with overactivation of the TXA_2 synthesis pathway relative to PGI_2 in vascular tissue (Zaugg et al. 1996; Song et al. 2005; Racz et al. 2010). Although the precise mechanism by which to account for this imbalance is unresolved, abnormal COX-2 function in the setting of vascular injury may play a role. It was recently demonstrated that compared to control mice, COX-2 knockdown mice administered monocrotaline to induce vascular inflammation exhibit a robust increase in NOX4 gene expression, dihydroethidium fluorescence (indicative of ROS accumulation), and ET_A receptor expression in pulmonary arterioles, whereas prostacyclin levels were decreased significantly (Seta et al. 2011). These findings are consistent with reports in cultured COX-2-deficient PSMCs, in which hypoxia results in a hypertrophic remodeling response and a vasoconstrictor phenotype (Fredenburgh et al. 2008).

5-lipooxygenase (5-LO) catalyzes the conversion of arachidonic acid ultimately into various leukotrienes that mediate cellular processes involved in vascular remodeling and cellular responses to injury. Leukotriene B_4, for example, exerts both chemotactic and chemokinetic activity on polymorphonuclear leukocytes and eosinophils (Ford-Hutchinson et al. 1980), and leukotrienes C_4, D_4, and E_4 (each of which contain a cysteine) are implicated in pulmonary vasoconstriction and increased pulmonary vascular permeability. Although rats that overexpress 5-LO do not develop PAH spontaneously, pulmonary vascular dysfunction and abnormal cardiopulmonary hemodynamics are accelerated in the presence of pulmonary vascular inflammation (Jones et al. 2004). This supports other observations indicating that an inflammatory milieu is conducive to 5-LO-dependent synthesis of the vasoactive cysteinyl leukotrienes (Listi et al. 2008). Molecular inhibition of the 5-LO-activating protein (FLAP) has, in turn, been shown to prevent pulmonary hypertension in rats exposed to chronic hypoxia (Voelkel et al. 1996).

3.6 Mitochondrial Dysfunction

Mitochondria regulate bioenergetics, cellular respiration, and the intracellular redox status and, thus, have the potential to regulate PAEC/PSMC signaling pathways linked to cell survival, proliferation, and ROS production. Hydrogen (hydride) derived from dietary carbohydrate and fats is oxidized by molecular oxygen (O_2) via the tricarboxylic acid (TCA) cycle and β-oxidation pathways, respectively, to

generate adenosine triphosphate (ATP). These biochemical events occur via the electron transport chain, in which two electrons donated by $NADH + H^+$ flow sequentially from complex I to ubiquinone (coenzyme Q) to complex III (ubiquinol: cytochrome c oxidoreductase) and then to cytochrome c. Electrons are then transferred to complex IV to reduce $½O_2$ and generate H_2O (Wallace 2005). Protons are pumped across the inner mitochondrial membrane to establish the significantly negative electrochemical gradient ($\Delta\psi m$: ~ −200 mV) across that membrane, which provides the electromotive force necessary for ATP synthesis.

Changes to mitochondrial membrane permeability, and, hence, the normal $\Delta\psi m$, are antecedent to reversible structural and functional changes in mitochondria and, if unchecked, commit the cell to apoptosis (Kroemer and Reed 2000; Michelakis et al. 2008). Numerous mechanisms to account for the relationship between mitochondrial membrane permeability and changes to cell survival have been proposed and include increased permeability of the voltage-sensitive permeability transition pore complex (PTPC), alkalinization of the local pH, and perturbations to the intramitochondrial redox status that results in oxidation of a key thiol involved in regulating PTPC opening and/or oxidation of pyridine nucleotides (i.e., $NADH/NAD^+$) to favor PTPC opening (Woodfield et al. 1998; Zamzami and Kroemer 2001). Collectively, these changes afford egress of apoptosis-associated proteins (e.g., Bax, Bcl-2, others) from the intramitochondrial to extramitochondrial space, thereby activating programmed cell death signaling pathways (Mossalam et al. 2012).

Pathological disruptions to mitochondria-dependent regulation of cell survival are a central mechanism in the pathobiology of various angioproliferative diseases, including solid tumor cancers. Apoptosis-resistant proliferation of PAECs/PSMCs is likewise a prominent pathophenotypic feature of PH, particularly with respect to plexigenic lesions in PAH. This observation has raised attention to the possibility that mitochondrial dysfunction is an under-recognized pathobiological factor by which to account for the phenotypic overlap between these two broad categories of disease. Evidence in support of this concept is derived partly from observations made in the fawn-hooded rat, a unique animal strain that develops PAH spontaneously. Pulmonary vascular smooth muscle cells harvested from these animals demonstrate mitochondria that are decreased in size and fragmented prior to the development of pulmonary vascular remodeling (Bonnet et al. 2006). The functional effects of these changes are linked to a shift in mitochondrial metabolism from oxidative phosphorylation toward glycolysis, impairment to electron flux, and subsequent activation of hypoxia-inducible factor (HIF)-1α (Archer et al. 2008). In turn, HIF-1α has been shown in endothelial cells cultured from patients with idiopathic PAH to target carbonic anhydrase IX, which decreases levels of the antioxidant enzyme manganese superoxide dismutase (SOD2) to increase vascular ROS generation and decrease levels of $NO^{\bullet}$ (Fijalkowska et al. 2010). Interestingly, in these experiments, increased HIF-1α expression correlated inversely with low numbers of mitochondria, indicating that negative control of mitochondrial biogenesis by HIF-1α may be one mechanism by which to account for abnormal cellular respiration patterns observed in in vivo models of PAH. Conventional

factors associated with PAH may also influence mitochondrial dysfunction directly. For example, compared to healthy controls, stimulation of PSMCs with ET-1, platelet-derived growth factor (PDGF), or IL-6 harvested from PAH patients results in Kruppel-like factor 5 (KL-5)-mediated activation of cyclin B1 that hyperpolarizes the mitochondrial inner membrane to inhibit apoptosis (Courboulin et al. 2011b).

Under normoxic conditions, electron transport chain complexes I or II generate $^{\bullet}O_2^-$ that is dismutated to form H_2O_2, which is a key signaling molecule required for activation of Kv channels necessary to maintain the negative electrochemical gradient of the mitochondria (Bonnet et al. 2006). At $PaO_2 < 70$ mmHg, there is decreased intramitochondrial H_2O_2 generation, opening of O_2-sensitive Kv1.5 channels, and subsequent activation of L-type Ca^{2+} channels that promotes pulmonary vasoconstriction (Archer et al. 2004). Human PSMCs in PAH, however, are deficient in Kv1.5 channels, and data from experimental animal models of PAH suggest that the effect of this deficiency is mitochondrial hyperpolarization, and, consequently, tonic activation of L-type Ca^{2+} channels associated with vasoconstriction and proliferation of PSMCs (Reeve et al. 2001).

Less well established is the role of abnormal mitochondrial bioenergetics in the development of pulmonary vascular dysfunction and/or RV hypertrophy in PH. There is increasing evidence suggesting that in cardiomyocytes, an abnormal shift in cellular fuel utilization vis-à-vis the glucose–fatty acid cycle (i.e., *Randle's* cycle) (Randle et al. 1963) accounts for changes to myocardial structure (i.e., hypertrophy) and function (i.e., impaired contractility) (Fig. 4). In the monocrotaline and pulmonary artery banding rat models of PAH, for example, decreased RV O_2 consumption is observed and modulates a shift from oxidative phosphorylation to glycolysis by a mechanism involving increased Glut-1 expression and upregulation of pyruvate dehydrogenase kinase (PDK) expression with consequent increased phosphorylation of pyruvate dehydrogenase leading to its inhibition (Piao et al. 2010). The functional effects of this process include impaired RV systolic function and prolongation of the QT interval, which can be reversed by PDK inhibition or through inhibition of fatty acid oxidation to induce an indirect reciprocal shift in the mitochondrial fuel source back to glucose (oxidation) (Fang et al. 2012).

3.7 Peroxisome Proliferator-Activated Receptor-γ

Peroxisome proliferator-activated receptor (PPAR-γ) is a transcription factor most commonly associated with its regulatory effect on genes involved in fatty acid storage and glucose metabolism in adipocytes (Kilroy et al. 2012); PPAR-γ, and its transcription target apoE, are also key downstream targets of BMP-RII signal transduction. In turn, loss of function to BMP-RII via somatic mutation or dissociation of BMP-RII-interacting proteins is associated with PSMC proliferation in vitro and the development of PAH in vivo (Merklinger et al. 2005; Chan et al. 2007; Song et al. 2008). In PSMCs, the antiproliferative effect of BMP-RII

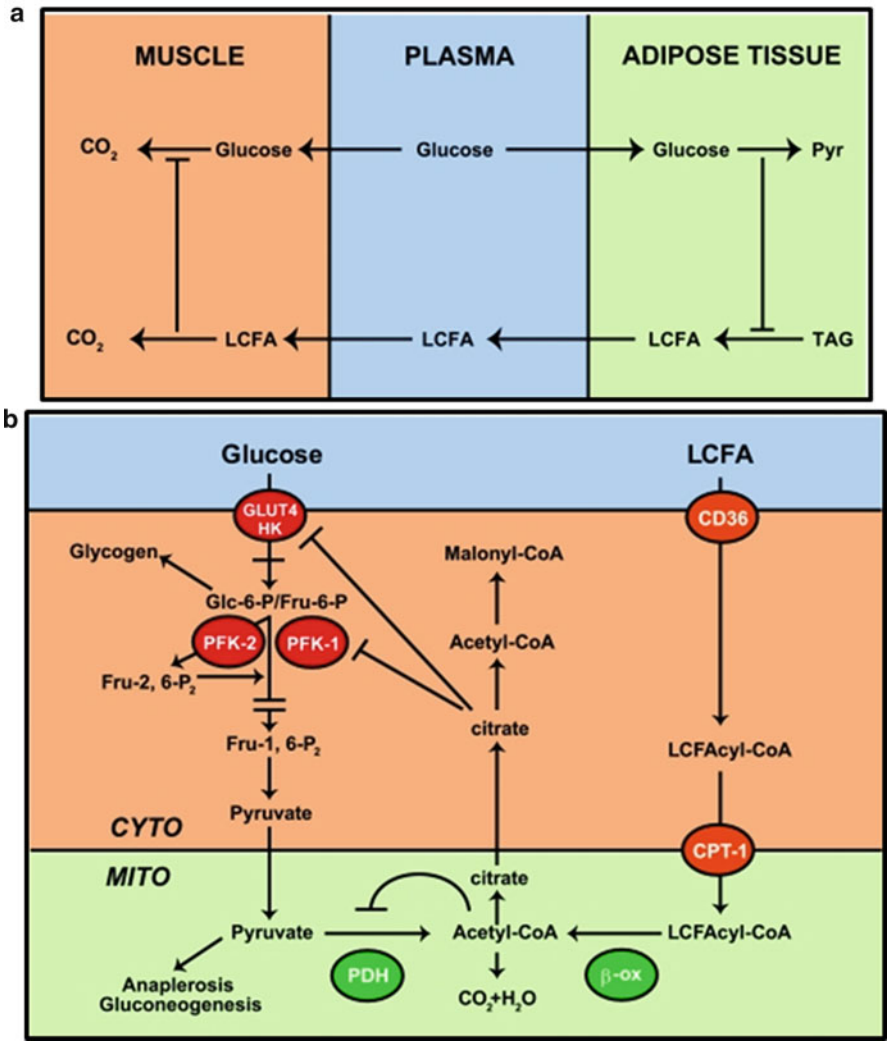

Fig. 4 Mitochondrial bioenergetics. (**a**) The "glucose–fatty acid cycle" or *Randle Cycle* describes a mechanism to maintain homeostatic control of circulating levels of glucose and fatty acids. (**b**) Inhibition of glucose utilization by fatty acid oxidation is regulated most strongly by pyruvate dehydrogenase (PDH) and, to a lesser extent, by 6-phosphofructo-1-kinase (PFK) and glucose uptake. Phosphofructokinase inhibition owing to citrate accumulation or via pharmacological/molecular strategies shifts glucose toward glycogen synthesis and pyruvate to gluconeogenesis or the synthesis of TCA intermediates (i.e., anaplerosis). Overactivation of PDK in right ventricular myocytes in an in vivo model of pulmonary hypertension has been associated with a shift from glucose oxidation to glycolysis and subsequent myocardial dysfunction (Piao et al. 2010). *LCFA* long-chain fatty acids, *TAG* triacylglycerol, *Pyr* pyruvate, *Cyto* cytosol, *MITO* mitochondria, *GLUT4* glucose transporter 4, *HK* hexokinase, *Glc-6-P* glucose-6-phosphate, *Fru-6-P* fructose 6-phosphate, *CPT I* carnitine palmitoyltransferase I, *β-ox* β-oxidation. Reproduced with permission from Hue et al. (2009) Am J Physiol Endocrinol Metab 297:E578–E591