Mediators and modulators of pulmonary arterial hypertension

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Said, Sami I. Mediators and modulators of pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol 291: L547–L558, 2006. First published May 12, 2006; doi:10.1152/ajplung.00546.2005.—Pulmonary hypertension (PH), defined as a mean pulmonary arterial (PA) pressure of >25 mmHg at rest or >30 mmHg during exercise, is characterized by a progressive and sustained increase in pulmonary vascular resistance that eventually leads to right ventricular failure. Clinically, PH may result from a variety of underlying diseases (Table 1 and Refs. 50, 113, 124). Pulmonary arterial hypertension (PAH) may be familial (FPAH) or sporadic (idiopathic, IPAH), formerly known as primary pulmonary hypertension, i.e., for which there is no demonstrable cause. More often, PAH is due to a variety of identifiable diseases including scleroderma and other collagen disorders, liver disease, human immunodeficiency virus, and the intake of appetite-suppressant drugs such as phentermine and fenfluramine (72). Other, more common, causes of PAH include left ventricular failure (perhaps the most common cause), valvular lesions, chronic pulmonary diseases, sleep-disordered breathing, and prolonged residence at high altitude. This classification, now widely accepted, was first proposed at a meeting in Evian, France, in 1998, and modified in Venice, Italy, in 2003 (124).

transforming growth factor-β/bone morphogenetic protein receptor; vasoactive compounds; serotonin transporter; growth factors; knockout mice

PATHOGENESIS OF PAH

SEVERAL PATHOLOGICAL PROCESSES contribute to the development and progression of pulmonary arterial hypertension (PAH). These begin with pulmonary vasoconstriction, which may become evident only during exercise or hypoxic breathing. This is followed by remodeling of the pulmonary vessels, notably through muscleization of smaller arteries and arterioles (32, 130). Additional contributing responses include inflammation, apoptosis of endothelial cells followed by proliferation of an apoptosis-resistant endothelial phenotype (135), fibrosis, and in situ thrombosis (52, 66, 93, 113, 142, 154).

A special morphological feature of the pulmonary vasculature in advanced PAH is the plexiform lesion, a result of proliferation of monoclonal, phenotypically distinct endothelial cells, migration and proliferation of smooth muscle cells/myofibroblasts, and accumulation of circulating cells, including macrophages and endothelial progenitor cells. Although plexiform lesions are typically seen in idiopathic PAH (IPAH) and familial PAH (FPAH), they may also be present in other forms of severe pulmonary hypertension (PH) (93, 113, 142).

Major strides have recently been made toward a better understanding of the pathogenesis and pathophysiology of PAH. These advances include: 1) the discovery of specific gene mutations that predispose to the FPAH and IPAH forms of the disease (22, 60); and 2) the increasing recognition that a variety of vasoactive compounds and growth factors are capable of enhancing or reducing the risk of disease occurrence, and promoting or ameliorating the pulmonary vascular changes associated with experimental or clinical PAH.

Recent reviews are available of the clinical, pathological, pathophysiological, epidemiologic, pathogenetic, and genetic aspects of PAH, as well as its pharmacological treatment (2–4, 22, 28, 30, 50, 51, 60, 62, 82, 83, 92–97, 110, 112, 113, 154).

The objectives of this review are to: 1) summarize current views of the special role of compounds that can influence vascular tone, vascular remodeling, the response to hypoxia, and other processes involved in the pathogenesis of PAH; 2) examine the interrelationships among these compounds and their possible interactions with the genetic mutations already shown to predispose to PAH, to either increase or decrease the likelihood of clinical expression of the disease; and 3) show how this recently acquired knowledge leads to the generation of novel experimental models of PAH that should clarify its molecular mechanisms and the introduction of new, rationally designed drugs that promise greater therapeutic potential.

THE GENETIC BASIS OF PAH

Foremost among the gene mutations in PAH are heterozygous mutations of the bone morphogenetic protein (BMP) receptor type II gene (BMPRII) on chromosome 2q33 (Fig. 1), coding for a type II receptor member of the transforming growth factor-β (TGF-β) family (22, 67, 70). TGF-β signaling controls a diverse set of cellular processes, including cell proliferation, recognition, differentiation, apoptosis, and determination of developmental fate during embryogenesis as well as in mature tissues in species ranging from worms to mammals (10, 74–76, 123). A TGF-β ligand initiates signaling by binding to and bringing together type I and type II receptor
serine/threonine kinases on the cell surface. This allows receptor II to phosphorylate the receptor I kinase domain, which then propagates the signal through phosphorylation of the Smad proteins (Fig. 1). There are eight distinct Smad proteins, constituting three functional classes: the receptor-regulated Smad (R-Smad), the comediator Smad (Co-Smad), and the inhibitory Smad (I-Smad). R-Smads (Smad1, 2, 3, 5, and 8) are directly phosphorylated and activated by the type I receptor kinases, forming heteromeric complexes with the Co-Smad, Smad4. The activated Smad complexes are translocated into the nucleus where, in conjunction with other nuclear cofactors, they regulate the transcription of target genes (60).

Mutations in two receptors of this group have been identified in the majority (>70%) of cases of inherited FPAH: BMPRII and activin-like kinase type-1 (ALK1). Exonic mutations in BMPRII are present in ~50% of patients with FPAH and 10–20% of IPAH patients (28). Additional, intronic mutations have recently been found in a significant proportion of these patients (20). ALK1 mutations are seen in a minority of patients with hereditary hemorrhagic telangiectasia, associated with PAH.

Numerous different mutations of the TGF-β type II receptor BMPRII in PAH were recently identified (67, 98). The mutations lead to a reduction of the signaling activity routed through the receptor, creating the most common inherited predisposition to the disease (33, 67). In pulmonary arterial (PA) smooth muscle cells from FPAH and IPAH patients, the antiproliferative effect of BMP is converted to a proliferative one, and the proapoptotic action is significantly attenuated. In PA endothelial cells, the antiapoptotic, pro-survival effect of BMP becomes proapoptotic. Thus, reduced BMPRII expression and the reversal of BMP effects on PA smooth muscle cells and endothelial cells in PAH patients may play an important pathogenetic role in pulmonary vascular remodeling, vascular obliteration, and pulmonary hypertension (3, 53).

While the mutations in BMPRII confer an increased risk of developing PAH over a carrier’s lifetime, the mutations have reduced penetrance that is both age and sex dependent. Be-

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<th>Pulmonary arterial hypertension</th>
<th>Familial PAH, idiopathic (Primary) PAH</th>
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<tr>
<td>PH with left heart disease</td>
<td>Left-sided atrial, ventricular, or valvular heart disease</td>
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<td>PH associated with chronic lung disease or hypoxemia</td>
<td>Chronic obstructive pulmonary disease, interstitial lung disease, sleep-disordered breathing, alveolar hypoventilation syndromes, prolonged residence at high altitude</td>
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<td>PH due to chronic thrombo-embolic disease</td>
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PH, pulmonary hypertension; PAH, pulmonary arterial hypertension. [Adapted from Humbert (50).]
cause only a small (10–20) percentage of persons with such mutations develop PAH, it is likely that the development of clinical PAH depends on the presence of genetic or environmental “modifiers” that accentuate the risk for full expression of the disease (96). This means that mutations of BMPRII predispose the mutant carrier to develop PAH in the presence of abnormalities of other genes and gene products or in the presence of exogenous stimuli (Fig. 2). Such additional genes include those encoding for various vasoactive compounds that can influence the pulmonary circulation, such as serotonin and its transporter, thromboxane A₂ (TXA₂), endothelins (ET) and their receptors, endothelial nitric oxide (NO) synthase, prostacyclin, atrial natriuretic peptide (ANP), adrenomedullin (AM), and vasoactive intestinal peptide (VIP). Exogenous stimuli of potential influence include certain viral infections, anorexigen intake, and chronic exposure to hypoxia (93). Thus the loss-of-function mutations in BMPRII or dysfunction of BMP receptors and their downstream signal transduction by Smad proteins appear to be insufficient alone to “trigger” PAH (9). Rather, in the opinion of many investigators, “multiple hits,” in the form of genetic or acquired factors, are required for the progression to full expression of PAH (67, 70, 133, 153).

VASOACTIVE COMPOUNDS THAT ENHANCE PULMONARY VASCULAR TONE AND SMOOTH MUSCLE CELL PROLIFERATION

ETs, Serotonin (5-HT) and Serotonin Transporter, TXA₂, and Angiotensin II

ETs. Vascular endothelium normally generates a number of vasodilator substances, notably prostacyclin and NO, which also inhibit platelet aggregation and vascular smooth muscle proliferation (Table 2). Under pathological conditions, however, endothelium can produce potent vasoconstrictor compounds, notably the three peptides making up the ET system: ET-1, -2, and -3 (70). The predominant isoform of these, ET-1, also promotes smooth muscle cell proliferation as well as inflammation and fibrosis. Two receptor subtypes, ETA and ETB, mediate the actions of ET (21). Convincing evidence suggests that local ET-1 synthesis in vascular endothelial cells probably plays a special role in the pathophysiology of PAH (39, 83b, 132):

1) ET-1-like immunoreactivity and messenger RNA, rarely present in vascular endothelial cells from control subjects, were abundant in IPAH patients with pulmonary hypertension associated with medial thickening and intimal fibrosis.

2) There was a strong correlation between the intensity of ET-1-like immunoreactivity and pulmonary vascular resistance in the patients with plexogenic pulmonary arteriopathy, but not in those with secondary PH.

3) ET-1 receptor density was considerably greater in smaller pulmonary arteries and lung parenchyma from pulmonary hypertensive patients than in control subjects.

4) ET-1 stimulated DNA synthesis in human PA smooth muscle cells.

5) Inhibition of endogenous ET-1 release or its action attenuated serum-stimulated proliferation of pulmonary vascular smooth muscle cells (21, 39).

Serotonin (5-HT) and serotonin transporter (5-HTT). Considerable research has been carried out to validate the hypothesis that 5-HTT in the lung might be a key determinant of pulmonary vascular remodeling in PAH. The 5-HTT is encoded by a single gene on chromosome 17q11.2 and is expressed in various cell types, including neurons, blood platelets, and pulmonary artery endothelial and smooth muscle cells. The level of 5-HTT expression appears to be much greater in human lung than in human brain (26, 27).

Several features about the 5-HTT in pulmonary vascular smooth muscle cells suggest that it may have a major influence on pulmonary vascular smooth muscle function. In addition to contributing to the uptake of 5-HT passing through the lung,
5-HTT mediates the proliferation of pulmonary vascular smooth muscle cells through its ability to internalize indoleamine. Additional observations, reported by others (26, 27, 113), support a key role for 5-HTT in pulmonary vascular remodeling, both under experimental conditions and in PAH.

1) Chronic exposure to hypoxia is a well-recognized stimulus for pulmonary vascular remodeling and the development of PAH. Chronic hypoxia elicited less severe PAH, vascular modeling, and right ventricular hypertrophy, in mice with targeted 5-HTT gene disruption than in paired wild-type controls. Conversely, increased expression of 5-HTT was associated with increased severity of hypoxic PAH (26).

2) Selective 5-HTT inhibitors attenuated hypoxic PAH.

3) Intraperitoneal infusion of serotonin over a 2-wk period in heterozygous BMPRII-deficient mice caused these mice to develop PH with pulmonary vascular remodeling (64).

4) 5-HTT expression and activity are increased in platelets and lungs from patients with PAH and in pulmonary artery smooth muscle cells from patients with IPAH compared with corresponding cells from control subjects.

5) Compared with pulmonary vascular smooth muscle cells from controls, those from PAH patients are more susceptible to the growth-promoting effects of 5-HT and serum (which contains high levels of 5-HT). In the presence of 5-HTT inhibitors, the growth-stimulating effects of serum and 5-HT are markedly reduced.

6) In one study, a higher percentage (65%) of IPAH patients than of normal controls (27%) were found to be homozygous for a 5-HTT promoter polymorphism linked with PA smooth muscle cell proliferation in response to serotonin exposure (27, 113).

7) The observation that aminorex and fenfluramine derivatives interact with 5-HTT in a specific manner has provided further support to the hypothesis that this transporter may be a critical target for appetite suppressants and perhaps for other insults initiating the process of PAH. In rats receiving dexfenfluramine treatment for 4 wk, 5-HTT expression in lung tissue remained unchanged, but upon discontinuation of the dexfenfluramine treatment, the expression level increased together with the development of hypoxic PH. This may be an example of overexpression of 5-HTT providing a complementary mechanism for promoting 5-HTT-dependent hyperplasia of pulmonary vascular smooth muscle cells (27).

Despite this impressive body of supportive evidence, two recent reports have cast some doubt on the significance of serotonin transporter polymorphism in the pathogenesis of PAH. In the first study, no significant correlation was detected between alleles of the 5-HTT gene and PH (69). In the second report, the long (LL) transporter genotype correlated with an earlier age at diagnosis of FPAH than the SL or short (SS) allele (148).

TXA₂. Like prostacyclin, TXA₂ is a product of arachidonate metabolism, but, in contrast to prostacyclin, it constricts pulmonary vessels and stimulates platelet aggregation. Urinary levels of the stable metabolite of TXA₂ were elevated in patients with primary or secondary PH, whereas those of the stable metabolite of prostacyclin were decreased (19). Such findings have led to the concept that an imbalance of the prostacyclin:TXA₂ ratio may play a role in the development of sustained pulmonary vasoconstriction and vascular remodeling in IPAH patients (Fig. 2; Refs. 2, 19, 31, 73). In a related study, terbogrel, a combined thromboxane synrhase inhibitor-receptor antagonist, attenuated pulmonary vasoconstriction in hypoxic piglets and increased the pulmonary vasodilator response to acetylcholine (31). Similarly, ozagrel, another thromboxane synthesis inhibitor, reduced PH and improved symptoms in a patient with portopulmonary hypertension (73).

Angiotensin II and angiotensin-converting enzyme. The expression of angiotensin-converting enzyme (ACE), the enzyme that catalyzes the activation of angiotensin I to angiotensin II, is increased in advanced intimal and plexiform lesions of IPAH. Because angiotensin II exerts a mitogenic effect on human PA smooth muscle cells, the higher expression of the enzyme may contribute to pulmonary vascular remodeling in this disease. In support of this view, ACE inhibition in pneumonectomized, monocrotaline-treated rats, an established model of severe PH, delayed pulmonary vascular neointimal formation. These observations notwithstanding, the role of ACE in the pathogenesis of PAH remains uncertain (113). A recent 12-mo pilot study found no significant clinical benefit from losartan, an angiotensin II antagonist, in 40 patients with chronic obstructive pulmonary disease and PH (83a).

Vasoactive Compounds That Modulate Pulmonary Vascular Tone and Smooth Muscle Cell Proliferation

Prostacyclin, NO, ANP, AM, VIP, and Pituitary Adenylate Cyclase Activating Peptide

Prostacyclin. Among the first endogenous vasodilator compounds to be linked to PAH, prostacyclin acts by stimulating cAMP formation (Table 3, Fig. 2). Prostacyclin has two additional beneficial effects: inhibition of platelet aggregation and suppression of smooth muscle proliferation (137). A product of arachidonate metabolism, prostacyclin is a key mediator of pulmonary vasodilation in the perinatal period, its synthesis in pulmonary vascular endothelium increasing during late gestation due to enhanced expression of cyclooxygenase-1, the rate-limiting enzyme (29, 54).

Prostacyclin production is decreased in patients with PH, particularly IPAH, relative to normal controls, leading to an imbalance of the prostacyclin:thromboxane ratio (141). This impaired production is attributable to suppression of prostacyclin synthase expression in small- and medium-sized pulmonary arteries. Prostacyclin receptor expression is also reduced in remodeled PA smooth muscle in severe PH (49, 127). As noted below, prostacyclin was the first truly effective drug for the therapy of PAH.

NO. Synthesized in endothelial cells by endothelial NO synthase (eNOS or NOS3), NO is an important endogenous modulator of pulmonary vasodilator tone and inhibitor of smooth muscle cell proliferation. Several lines of evidence

Table 3. Vascular compounds that modulate pulmonary vasoconstriction, vascular remodeling, or both

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<td>Prostacyclin</td>
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<td>Nitric oxide</td>
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<td>Atrial natriuretic peptide</td>
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<td>Adrenomedullin</td>
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<tr>
<td>Vasoactive intestinal peptide</td>
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<td>Pituitary adenylate cyclase-activating peptide</td>
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support a major role for NO, and in particular NOS3, in the pulmonary circulation.

1) NOS3-deficient mice had mild PH (pulmonary artery pressure 19.0 ± 0.8 compared with 16.4 ± 0.6 mmHg in wild-type mice). In the same study, isolated pulmonary arteries of NOS3-deficient mice failed to show the normal vasodilator response to acetylcholine but were morphologically unaltered (129).

2) In a complementary study by the same group of investigators, mice with congenital deficiency of NOS3 (which exhibit major systemic and mild PH under normoxic conditions) developed more severe degrees of PH after a 3- to 6-wk period of hypoxic (11% oxygen) breathing. The NOS-deficient mice also showed greater increases in vascular remodeling and right ventricular hypertrophy than wild-type mice, and right ventricular hypertrophy was prevented by breathing 20 parts per million NO (129).

3) Deletion of the NOS3 gene was associated with histological evidence of PH in both male and female mice during fetal life and at birth, but PH and right ventricular hypertrophy persisted only in the adult males. In neither sex did inducible or neuronal NOS compensate for the deletion of eNOS (80).

4) Lung tissue from patients with chronic PH showed decreased NOS3 expression in vascular endothelium, especially in patients with severe histological abnormalities (i.e., with plexiform lesions); the intensity of the enzyme immunoreactivity correlated inversely with the severity of histological changes. The findings support the conclusion that the reduction of this vasodilator enzyme may contribute to the development of PH (38).

5) Consistent with the above conclusion, mice deficient in the de novo production of tetrahydrobiopterin (BH4), a cofactor for NO synthase, selectively express a pulmonary hypertensive, but not systemic hypertensive, phenotype (90).

6) Inhaled NO attenuated PH and improved lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor (134).

ANP. A member of a group of natriuretic peptides, including brain natriuretic peptide (B-type, BNP) and urodilatin, ANP is a cardiac hormone with potent diuretic, vascular relaxant, and antiproliferative properties. In the systemic circulation, it modulates intravascular volume and the hypertensive response to salt loading (58). Like other natriuretic peptides, ANP acts on a membrane-bound receptor that activates particulate guanylate cyclase and promotes cGMP accumulation (63).

In the pulmonary circulation, too, ANP appears to be a physiological modulator of vascular resistance and vascular remodeling. Mice with targeted disruption of the ANP gene had moderately elevated right ventricular systolic pressures (22 ± 2 vs. 15 ± 1 mmHg) and thickened right ventricular walls compared with heterozygous or wild-type mice. After 3 wk of hypobaric hypoxia (0.5 atmosphere), homozygous mutants had higher mean right ventricular systolic pressures (29 ± 3) than heterozygous or wild-type mice (23 ± 1 and 22 ± 2 mmHg, respectively). There was a greater degree of remodeling of peripheral pulmonary vessels in homozygous mutants than in heterozygous or wild-type mice under both normoxic and hypoxic conditions (58). Relevant to these findings, ANP inhibits ET-1 synthesis and ET receptor expression (21). In a recent open-label study, a 3-h infusion of BNP did not significantly improve pulmonary hemodynamics in most patients with PAH, but it was well tolerated and augmented the acute pulmonary vasodilator effect of sildenafil (57a).

Adrenomedullin. Adrenomedullin (AM), a 52-amino acid residue peptide that exhibits structural homology to calcitonin gene-related peptide, is a systemic vasodilator peptide, was originally isolated from human pheochromocytoma. Acting by stimulation of cAMP production, it produces systemic vasodilation and hypotension (57). In addition to its presence in normal adrenal medulla, AM is now known to be expressed in a wide variety of tissues, including lungs, myocardium, blood and blood vessels, and other tissues (147). Of special relevance to this review, AM is produced by pulmonary as well as systemic endothelial and smooth muscle cells. In the pulmonary circulation as well, AM induces vasodilation, and it also inhibits the migration and proliferation of vascular smooth muscle cells. These properties suggest the peptide may help regulate the pulmonary circulation and modulate PH (85, 86). Additional beneficial effects of AM: it downregulates lipopolysaccharide-induced secretion of TNF-α by rat macrophages (149) and inhibits ovalbumin-induced bronchoconstriction and airway microvascular leakage in guinea pigs (101).

Because AM is a nonselective vasodilator of both the pulmonary and systemic vascular beds, if administered intravenously it will cause both systemic and pulmonary vasodilation. For this reason, AM has recently been given by inhalation in tests of its potential benefit in PH. With this method of administration, AM led to sustained and selective reduction of pulmonary artery pressure in a surfactant-depleted piglet model (145) and in rats with monocrotaline-induced PH (88) and improved pulmonary hemodynamics and exercise tolerance in patients with IPAH (87).

VIP. Extensive research over many years has documented a key physiological role for VIP as a potent pulmonary vasodilator and inhibitor of vascular smooth muscle proliferation as well as an anti-inflammatory and antiapoptotic agent (103, 117, 118). These properties have promoted VIP as having a promising potential as an effective pharmacological agent for the treatment of PAH. Pertinent evidence along these lines includes the following observations.

1) VIP relaxes isolated PA segments from several mammalian species in vitro (44, 114, 115, 121).

2) In the same preparations, VIP neutralizes the pulmonary vasoconstrictor effect of such diverse agents as ET (11), leukotriene D4 (45), and amyloid β-peptide (120).

3) VIP reduces pulmonary vascular resistance in the cat in vivo (91) and in rabbits with monocrotaline-induced PH (43) and attenuates hypoxic pulmonary vasoconstriction in newborn lambs (139) and in Fawn-Hooded rats, known to exhibit an exaggerated pulmonarypressor response to alveolar hypoxia (46).

4) Together with NO, VIP is a likely mediator of the nonadrenergic, noncholinergic relaxant system of pulmonary vasculature (59). The combined activation of the guanylate cyclase and adenylate cyclase systems, respectively, by NO and VIP, maximizes smooth muscle relaxation (116, 119).

5) VIP inhibits the proliferation in vitro of pulmonary vascular smooth muscle cells from patients with IPAH (107).

6) Given for a period of 3 mo by daily inhalations, it markedly improved exercise tolerance and pulmonary hemodynamics in patients with the same disease (107).
7) VIP-containing nerves, normally plentiful in the walls of pulmonary arteries (23), are totally absent from pulmonary arteries of patients with IPAH (107). Whether the VIP deficiency was causally related to the pathogenesis of the disease or was merely an associated finding could not be ascertained from this study.

**Pituitary adenylate cyclase-activating peptide.** Showing strong sequence homology to VIP, pituitary adenylate cyclase-activating peptide (PACAP) possesses similar biological activities. The two peptides act via three different G protein-coupled receptors: VPAC1 and VPAC2, which bind with equal affinity to both peptides, and PAC1, which binds to PACAP with greater affinity than to VIP. Like VIP, PACAP and its receptors are widely expressed in various structures within the lung (13, 143).

Like VIP, and as its name implies, PACAP acts mainly by stimulating cAMP production, but some of its actions are mediated via other pathways, including calcium ion mobilization and NO and ANP production (7, 41, 84) and via ATP-mediated signaling in the maintenance of normal pulmonary vascular structure and function during early postnatal life (105).

**GROWTH FACTORS AND PULMONARY CIRCULATION: PLATELET-DERIVED GROWTH FACTOR, VEGF, AND TGF-β**

**PDGF.** Compelling evidence suggests that platelet-derived growth factor (PDGF) is closely involved in the pulmonary vascular pathology of PH. 1) PDGF acts as a potent mitogen and chemoattractant for pulmonary vascular smooth muscle cells (152). 2) Both PDGF receptors (PDGFR)-α and -β are upregulated in lambs with chronic intrauterine PH (5). 3) The PDGFR antagonist STI571 (imatinib or Gleevec, currently approved for the treatment of certain malignancies) could reverse advanced pulmonary vascular remodeling and cor pulmonale in two experimental models of severe, established PH (122): a) rats with monocrotaline-induced PH and b) mice exposed to chronic hypoxia (ventilation with 10% O2 for 35 days), which activates a PDGF-related pathway. In both models, the drug treatment resulted in markedly improved survival, together with reduced right ventricular pressure and right ventricular hypertrophy (122). These studies led to a successful clinical trial of the drug, as cited below.

**VEGF.** VEGF (or VEGF-A), expressed at high levels in the lungs, is the principal growth factor for endothelial cells. It is required both for lung development and for the structural maintenance of the adult lung, including pulmonary endothelial cell survival (144). The complex regulation of VEGF production, its multiple actions and physiological roles, and its interactions with other biologically active compounds were recently reviewed (144). VEGF ligand binding to its main receptor, VEGFR-2 tyrosine kinase, results in increased expression of eNOS and increased prostacyclin production (144).

Blockade of VEGFR-2 in newborn rats by the tyrosine kinase inhibitor SU-5416 resulted in PH with right ventricular hypertrophy (61). The same inhibitor, in combination with chronic hypobaric hypoxia, caused severe PH in rats. The hypertension was associated with precapillary occlusion by proliferating endothelial cells. Before and during the development of PH, the lungs showed significant pulmonary endothelial cell death, as evidenced by activation of caspase-3, and by prevention of the PH and the obliterator pulmonary endothelial cell proliferation by a broad-spectrum caspase inhibitor (135, 144). These findings suggested that endothelial cell apoptosis, induced by VEGFR-2 blockade, was followed by the emergence of excessive proliferation of an apoptosis-resistant endothelial cell phenotype (144).

VEGF is strongly expressed in the pleiform lesions associated with severe PH, both primary (IPAH) and secondary. In experimental models of PAH, however, the expression of VEGF may be either increased or decreased, depending on the particular model. Despite lack of agreement on whether chronic hypoxia increases lung VEGF expression, its overexpression obtained by adenovirus-mediated gene transfer attenuates the development of PAH and right ventricular hypertrophy in rats exposed to chronic hypoxia (106). In support of this interpretation, treatment with VEGF reduced the severity of PH in fetal sheep with partial ligation of the ductus arteriosus (42), and VEGF gene transfer attenuated PH induced by monocrotaline in rats (14) or by bleomycin in immature rabbits (40).

Several other isoforms of VEGF besides VEGF-A exist in the lung. One of these, VEGF-B, resembles VEGF-A in that its overexpression in the lung attenuates experimental PH. But VEGF-B differs from VEGF-A in several respects: its expression is not regulated by hypoxia or cytokines, it acts mainly on VEGFR-1, and it does not stimulate eNOS expression or increase vascular permeability (65).

**TGF-β/BMPRII.** As outlined above, TGF-β regulates cell fate by signaling through two receptor serine kinases that act in sequence. Signaling by these receptors is mediated by the Smad protein family, which upon phosphorylation by the activated receptors, carries the TGF-β signals from the cell membrane receptors to target genes (74–76, 89, 123). Dysfunction of the BMPRII protein is thought to result in impaired control of cellular proliferation, a cardinal feature of PAH (69, 70, 131, 150).

Pulmonary hemodynamics and vascular morphometry in BMPRII-deficient (BMPR+/−) mice were similar to those in wild-type littermate controls under normoxic and hypoxic conditions. After chronic infusion of serotonin, however, the animals developed PA and right ventricular hypertension and pulmonary vascular remodeling (64). Heterozygous BMPR2 mutant mice, subjected to inflammatory stress resulting from adenovirus-mediated pulmonary overexpression of 5-lipoxygenase, developed PH within 2 wk and pulmonary vascular remodeling 2 wk later (127a).

**Other peptides and growth factors possibly related to PH.** It is likely that additional peptides to those mentioned here have a role in the regulation of the pulmonary circulation and PH (55). One example is Relaxin, a hormonal peptide thought to be
involved in loosening collagen bundles in ligaments during parturition (140). In rats exposed to alveolar hypoxia (10% O_2) for 10 days, subcutaneous administration of recombinant human Relaxin dose dependently reduced PH and ameliorated collagen accumulation in the pulmonary arteries (140).

Another growth factor with potential influence on PAH is basic fibroblast growth factor (bFGF). One study revealed elevated urinary and plasma levels of bFGF in patients with PH compared with normal control subjects, and the highest levels were found in IPAH patients (8).

EXPERIMENTAL MODELS OF PAH

Experimental models of classical disorders, particularly such relatively rare ones as FPAH or IPAH, are invaluable in clarifying the pathogenesis and pathophysiology of the disease and in suggesting potentially useful new therapies. Numerous models of PAH have been used. The focus here is on those models based on altered gene expression of one or more of the vasoactive compounds and growth factors discussed earlier.

1) Targeted deletion of the eNOS (NOS3) gene leads to a mild form of PAH, without demonstrable anatomic changes in the pulmonary vessels (129).
2) Mice with targeted disruption of the ANP gene show moderate elevations of right ventricular pressure, with thickened right ventricular walls. The addition of hypobaric hypoxia accentuates the pulmonary and right ventricular hypertension (58).
3) Mice deficient in the PAC1 receptor, one of three receptors shared between VIP and PACAP, develop a severe form of PAH with right ventricular failure and die early in neonatal life.
4) Mice lacking the VIP gene live for at least two years and express a phenotype of PH with thickened pulmonary arteries and arterioles and varying degrees of right ventricular hypertrophy and dilatation (unpublished observations).
5) It is noteworthy that although VIP and PACAP are structurally related and share many biological features, including tissue distribution, actions, receptors and receptor distribution, selective gene deletion of either peptide is not compensated for by the remaining peptide.
6) The combination of VEGFR-2 blockade and hypoxia in rats results in PH (135, 136).
7) Similarly, activation of the PDGF pathway in mice, as by chronic hypoxia, leads to PH with pulmonary vascular remodeling (122).

IMPLICATIONS FOR THERAPEUTIC INTERVENTIONS

Goals of Therapy

Early approaches to therapy for PAH centered on the use of available vasodilators, which often reduced systemic more than PA blood pressure. Later, vasodilators such as prostacyclin, which afforded more selective pulmonary vasodilatation, were introduced. Over the past decade, with improved understanding of the pathophysiology, pathology, and pathogenesis of PAH, treatment has progressed to include drugs that target other pathogenetic mechanisms. These drugs have the additional objectives of inhibiting vascular smooth muscle proliferation, neutralizing mediators of vascular injury, inflammation, and cell death, antagonizing agents, such as ET, that promote vascular remodeling, and supplementing endogenous compounds, such as VIP, that counteract the pathological changes in the pulmonary vasculature. To help accomplish these goals, two or more drugs are often given in combination, especially if they work by different but complementary mechanisms (110).

Newer Therapeutic Agents

The therapeutic use of prostacyclin and its analogs, NO, ET antagonists, and phosphodiesterase inhibitors have recently been discussed (4, 6, 17, 34–36, 48, 62, 78, 79, 102, 110, 111, 125, 126, 128). Only two drugs already in use, although mostly for other indications (simvastatin and imatinib), and another still being investigated (VIP), are briefly discussed here with emphasis on the rationale for their clinical use.

Simvastatin. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, known as statins, were initially developed as lipid-lowering drugs that reduce morbidity and mortality from coronary artery disease. Statins may also attenuate, prevent, or reverse the development of experimental pulmonary hypertensive vascular disease (24). Pneumonectomized rats injected with monocrotaline developed severe pulmonary hypertensive vascular disease with neointimal formation (99). If treated with simvastatin from day 5 to day 35, such rats had markedly attenuated PH (99). Lung eNOS (NOS3) gene expression was relatively well preserved in the simvastatin-treated rats compared with vehicle-treated animals (99). In a complementary study, 2- to 6-wk treatment with simvastatin rescued rats from severe, established, and fatal PAH in the same model (100). The favorable effects of statins on pulmonary (and systemic) vessels are attributable, in part, to the induction of endothelial progenitor cell differentiation (24). In a recent open-label observational study on 16 patients with PAH, simvastatin (20–80 mg/day) appeared safe, and individual patients demonstrated improvement in a 6-min walk test, cardiac output, or right ventricular systolic pressure (54a).

VIP. As noted above, VIP has several favorable pharmacological actions that make it potentially useful in PAH. Such actions include pulmonary vasodilation, inhibition of human PA smooth muscle proliferation, inhibition of platelet aggregation, and anti-inflammatory, antiapoptotic, antioxidant, and cytoprotective effects (25, 103, 117, 118). The absence of VIP-containing nerves from pulmonary arteries of patients with PAH (107) and the finding that VIP knockout mice present a PAH-like phenotype suggest that therapy with VIP may not only be pharmacologically appropriate but may actually represent a form of “replacement therapy.”

Clinical trials of VIP in PAH are currently under way in Europe, and a trial in the United States is being planned. Preliminary data show that several weeks of treatment of VIP as an aerosol resulted in considerable improvement in pulmonary hemodynamics and in exercise tolerance, as measured by the 6-min walk test (107). Because VIP acts mainly by stimulating cAMP production, its combination with a phosphodiesterase inhibitor, such as sildenafil, which acts by promoting cGMP levels, may enhance the benefit from either drug alone.

Imatinib. The demonstrated ability of imatinib, a PDGFR antagonist, to reverse PH and pulmonary vascular remodeling (see above and Ref. 122), prompted a recent clinical trial of the drug (also known as Gleevec) in a patient with a rapidly progressive form of FPAH. The drug was added to the thera-
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Invited Review

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Great strides have been taken in the past few years toward a better understanding of the genetic basis and pathogenesis of PAH and a more effective treatment. There is every reason to believe that progress will continue at the same, if not an accelerated, pace. The report of a workshop held in March 2003 under the sponsorship of the National Heart, Lung, and Blood Institute and the Office of Rare Diseases, National Institutes of Health, summarized the status of research at the time and presented a set of recommendations for future studies (94). Those recommendations hold true today. There is no doubt research in the coming years will address, among others, the following unanswered questions.

What are the full genetic determinants of PAH? One of the important challenges will be to build on the breakthrough advances made within the past decade concerning the genetic basis of the pathogenesis of PAH. We already know that BMPRII mutations are the key genetic defect (3, 9, 10, 47, 70, 74–76, 83, 93, 96–98, 112, 138, 146, 150). But additional questions arise.

How does downregulation or other malfunction of BMPR signaling cause PAH pathology?

Since only a small proportion of persons with a BMPRII mutation express the clinical picture of PAH, what are the other factors, genetic or environmental, required to induce the pathological and clinical features of the disease?

What are the “modifier genes”? Understanding how other modifying genetic alterations may interact with the BMPR/SMAD pathway in PAH would no doubt contribute to further demystification of this disease. Many investigators have focused on the possible influence of serotonin transporter overexpression, and the intake of appetite-suppressant drugs, which appear to work via a common mechanism (26, 27, 71). But even there, the question has not been fully settled (69, 148).

How are these genes interact with BMPRII/Smad signaling?

Identifying the interactions between modifier gene signaling pathways and BMPRII/Smad signaling should help clarify the molecular mechanisms leading to the initiation of PAH pathology and thus promote the design of more rational therapy.

Are the findings in mice with gene disruption or overexpression of vasoactive compounds applicable to humans? A case in point is the finding that mice lacking the VIP gene present with the phenotype of PAH.

Does that imply that genetic mutations of the VIP gene could be one of the modifier genes referred to above? Similarly, do PAH patients exhibit mutations in the genes of other vasoactive compounds or their receptors?

How do these genes cause PAH pathology? The answer might uncover helpful information relating to the pathogenesis of the disorder, particularly the role of VIP as a modifier gene. Several possible interactions are known to exist between VIP and BMPR/Smad signaling: 1) R-Smads bind the transcriptional coactivator CBP (cAMP-regulated enhancer-binding protein) (75), and VIP is known to have a similar effect on CBP (21a); 2) the ERK-activated Ras pathway can modify TGF-β signaling pathways at different levels (75), and VIP also acts via the ERK pathway (61a); and 3) TGF-β induces transcription of the VIP gene through a 180-bp cytokine response element (CyRE) in the VIP promoter; to exert this action, Smad3 and Smad4 proteins bind to two distinct sites within the VIP CyRE (108).

Can the study of experimental models of the disease lead to more effective drugs? Many of the drugs currently in use or still on the horizon were developed on the basis of investigations in experimental animal models. Examples are ET receptor antagonists, phosphodiesterase inhibitors, the statins, and VIP.

How beneficial is combined therapy likely to be? Recent experience suggests that selected drug combinations are likely to be more effective than treatment with any single drug. Such an outcome is predictable on pharmacological grounds provided the drugs act by complementary or synergistic mechanisms. Examples of such combinations are: two drugs, one acting by increasing cAMP production and the other by promoting its preservation by inhibiting the selective phosphodiesterase enzyme; and drugs with combined vasodilator and anti-inflammatory properties that also suppress vascular smooth muscle proliferation and neointimal growth.

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