The Serotonin Pathway in Pulmonary Hypertension


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Introduction: Serotonin Signaling Pathway in Pulmonary Hypertension

The effects of serotonin (5-hydroxytryptamine, 5-HT) on the pulmonary circulation have been investigated because of the reported increased risk of idiopathic pulmonary arterial hypertension (IPH) in patients who used appetite suppressants that interact with 5-HT.[1] An association between the anorexigen aminorex and IPH was first described in the 1960s. In the 1980s, use of the appetite suppressant fenfluramine was shown to be associated with an epidemic of IPH in France and Belgium.[2] The serotonin hypothesis received support from the occurrence of pulmonary hypertension in Fawn-hooded rats, which have an inherited platelet-storage defect.[2]

Early studies focused on circulating 5-HT and its potential effects on the pulmonary vascular bed. In 1990, Herve and colleagues reported elevated plasma 5-HT levels in a patient with a platelet storage disease who developed IPH. Five years later, this group of investigators showed that patients with IPH had increased circulating serotonin levels even after heart-lung transplantation.[3] In addition to its vasoactive effects, 5-HT exerts mitogenic and co-mitogenic effects on pulmonary-artery smooth muscle cells. In contrast to the constricting action of 5-HT on smooth muscle cells, which is mainly mediated by 5-HT receptors (5-HT 1B/D, 2A, and 2B),[2] the mitogenic and co-mitogenic effects of 5-HT require internalization of 5-HT by the 5-HT transporter (5-HTT).[4,5] Accordingly, drugs that competitively inhibit 5-HTT also block the mitogenic effects of 5-HT on smooth muscle cells.[6] The appetite suppressants fenfluramine, d-fenfluramine, and aminorex differ from selective serotonin transporter inhibitors in that they not only inhibit serotonin reuptake but also trigger 5-HT release and interact with 5-HTT and 5-HT receptors in a specific manner.[6,7]

Serotonin Transporter (5-HTT) in Pulmonary Hypertension

We recently tested the hypothesis that the 5-HTT in the lung may be a key determinant of pulmonary vessel remodeling because of its effects on pulmonary artery smooth muscle cell growth. 5-HTT is abundantly expressed in the lung, where it is predominantly located on pulmonary-artery smooth muscle cells.[8,9] It is encoded by a single gene expressed in several cell types such as neurons, platelets, and pulmonary vascular endothelial and smooth muscle cells. The level of 5-HTT expression appears to be considerably higher in human lung than in human brain, suggesting that altered 5-HTT expression may have direct consequences on pulmonary-artery smooth muscle cell function. The requirement for 5-HTT as a mediator for the mitogenic activity of 5-HT appears specific for pulmonary-artery smooth muscle cells, since no such effect has been reported with other smooth muscle cell types. Direct evidence that 5-HTT plays a key role in pulmonary vascular remodeling was recently provided by studies showing that mice with targeted 5-HTT gene disruption developed less severe hypoxic pulmonary hypertension than did wild- type controls and that selective 5-HTT inhibitors attenuated hypoxic pulmonary hypertension.[10] Conversely, increased 5-HTT expression was associated with increased severity of hypoxic pulmonary hypertension.[11] Although a heterogeneous population of 5-HT2A, 5-HT2B, and 5-HT1B receptors exists in pulmonary arteries, 5-HT receptor antagonists are not as efficient as 5-HTT inhibitors in protecting against the development of hypoxic pulmonary hypertension.[12] Moreover, expression of these 5-HT receptors is not altered in IPH.[13] Taken together, these observations suggest a close correlation between 5-HTT expression and/or activity and the extent of pulmonary vascular remodeling during experimental pulmonary hypertension.

Overexpression of 5-HTT Responsible for Pulmonary-Artery Smooth Muscle Cell Hyperplasia in IPH

Evidence that the 5-HTT plays an important role in the pathogenesis of human IPH has recently been demonstrated. 5-HTT expression was shown to be increased in platelets and lungs from patients with IPH, where it predominated in the media of thickened pulmonary arteries and in onion-bulb lesions (**Figure 1**).[9] Interestingly, the higher level of 5-HTT protein and activity persisted in cultured smooth muscle cells isolated from pulmonary arteries of patients with IPH, as compared to cells from controls (**Figure 2**).[9] Moreover, pulmonary-artery smooth muscle cells from patients with IPH grew faster than those from controls when stimulated by serotonin or serum (which contains micromolar concentrations of serotonin), as a consequence of increased expres-

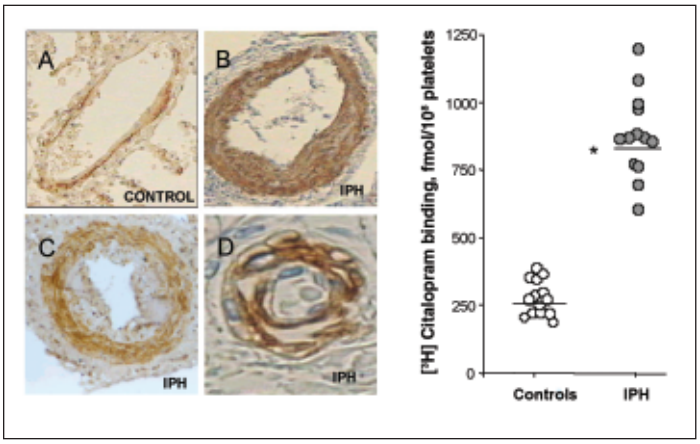

Figure 1. Left panel: 5-HTT-like immunoreactivity in lung sections from control subjects and patients with idiopathic pulmonary arterial hypertension (IPH). Panel A shows a lung section from a control subject, with weak to moderate 5-HTT-like immunoreactivity in pulmonary arterial endothelial cells and strong immunoreactivity in smooth muscle cells. Panels B, C, and D show lung sections from patients with IPH: 5-HTT-like immunoreactivity is much stronger, especially in the medial layer of pulmonary arteries with marked muscular hypertrophy (B); no 5-HTT immunostaining is detected in intimal fibrosis (C); in lesions with onionskin arrangement, 5-HTT-like immunoreactivity is prominent at sites of intense pulmonary-artery smooth muscle cell proliferation (D). Panel E shows no immunoreactivity in a section incubated with secondary antibody but no primary antibody. Scale bar: 100 µm in A, B, C, and E; 200 µm in D. Right panel: Individual platelet [^{3}H]citalopram-binding in normal controls and in patients with IPH.[9]

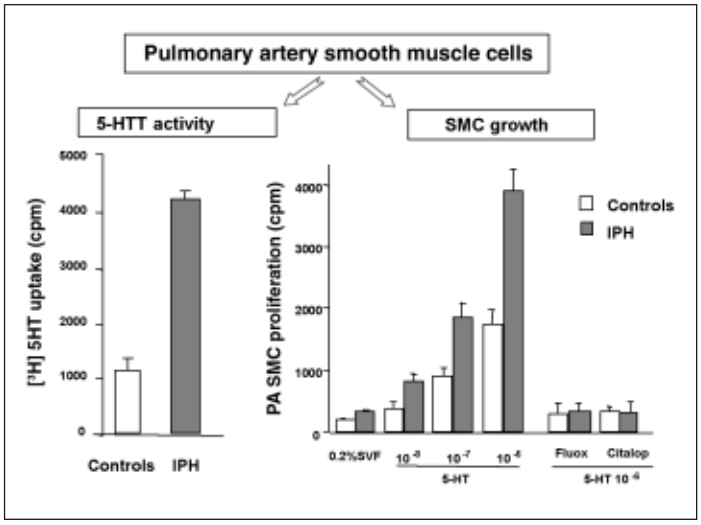

Figure 2. Left panel: 5-HTT activity in pulmonary-artery smooth muscle cells (PA-SMCs) from patients with idiopathic pulmonary arterial hypertension (IPH) and from controls. Right panel: PA-SMC proliferation as assessed by [^{3}H]thymidine incorporation into cells from patients with IPH (solid bars) and from controls (open bars). The cells were incubated with increasing concentrations of 5HT (10^{-8} to 10^{-6} mol/L) in the presence of 0.2% serum. The response was also measured in the presence of ketanserin (10^{-6} mol/L), a 5-HT2A receptor antagonist, GR 127935 (10^{-6} mol/L), a 5-HT1B/1D receptor antagonist, fluoxetine (10^{-5} mol/L), or citalopram (10^{-5} mol/L).[9]

sion of the serotonin transporter (**Figure 2**).[9] In the presence of 5-HTT inhibitors, the growth-stimulating effects of serum and serotonin were markedly reduced, and the difference between growth of pulmonary-artery smooth muscle cells from patients and controls was abolished. The proliferative response of pulmonary-artery smooth muscle cells to various growth factors such as PDGF, EGF, TGFß, FGFa, and IGF did not differ between patients with primary pulmonary hypertension and controls[9]. From these studies in can be concluded that 5-HTT overexpression and/or activity in pulmonary-artery smooth muscle cells from patients with IPH is responsible for the increased mitogenic response to serotonin and to serum.

5-HTT Gene Polymorphism and Increased Expression in Pulmonary Arteries in IPH

That 5-HTT expression is genetically controlled has been convincingly demonstrated: a polymorphism in the promoter region of the human 5-HTT gene alters the level of transcription.[14] This polymorphism consists of two common alleles, a 44-bp insertion or deletion, designated the L and S allele, respectively. The L allele drives a two- to threefold more active transcription of the 5-HTT gene than the S allele. In studies of pulmonary-artery smooth muscle cells from controls, we found that cells from LL subjects expressed twofold more 5-HTT mRNA than did cells from SS subjects, and that LS subjects had an intermediate level of expression (**Figure 3**).[9] Accordingly, the growth-stimulating effects of 5-HT or serum were more marked in cells from subjects with the LL genotype than those with LS or SS genotypes, indicating that the capability of pulmonary-artery smooth muscle cells to proliferate in response to serotonin or serum was directly linked to the functional polymorphism of the 5-HTT gene promoter (**Figure 3**). We have studied a large population of patients with IPH (n = 89) and found that 65% of patients, compared to only 27% of controls,[9] were homozygous for the L allelic variant of the 5-HTT gene promoter, which is associated with 5-HTT overexpression and increased pulmonary-artery smooth muscle cell growth. This finding suggests that the LL allele confers susceptibility to IPH.

Several questions, however, remain unanswered. Although the long allele of the 5-HTT gene promoter is strongly associated with IPH, this does not fully explain the increased 5-HTT expression in patients with IPH. Thus, additional factors are probably needed to produce 5-HTT overexpression. Whether this overexpression results from an alteration in the 5-HTT gene itself or from alterations in other factors involved in regulating 5-HTT gene expression remains to be determined.

No preponderance of the LL genotype has been found in familial IPH. Preliminary results suggest that the bone morphogenetic protein receptor II (BMPR2) agonists BMP4 and BMP6 may inhibit 5-HTT expression. An attractive hypothesis is that decreased BMPR2 function leads to increased 5-HTT expression. Loss of BMPR2 and increased 5-HT transport into pulmonary-artery smooth muscle cells may also interact directly to synergistically produce pulmonary hypertension. There is a pressing need for studies exploring the molecular pathways that connect BMPR2 mutant genotypes, 5-HTT expression, and the IPH phenotype.

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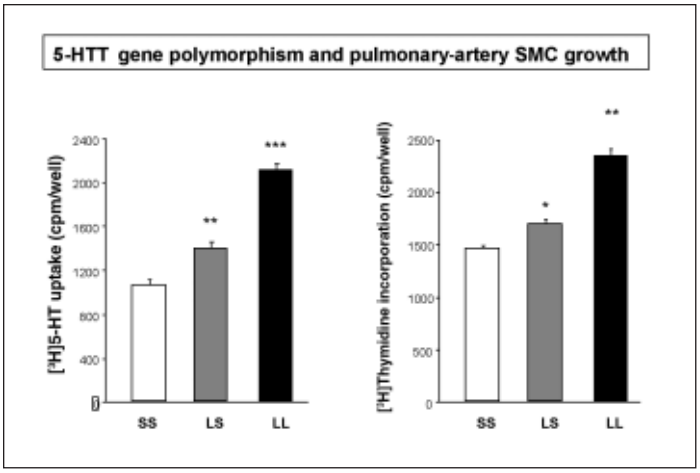

Figure 3. 5-HTT activity as assessed by [^{3}H]5-HT uptake (left panel) and [^{3}H]thymidine incorporation (right panel) in response to 5-HT (10^{-6} mol/L) into smooth muscle cells (SMCs) from controls with the SS, LS, or LL genotype. Each bar is the mean±SEM of data obtained in six individuals in each group. *P <.05, **P <.01, and ***P <.001 compared with respective values for the SS genotype. [^{3}H]5-HT uptake and [^{3}H]thymidine incorporation were also greater in LL cells than in LS cells (P <.01).[9]

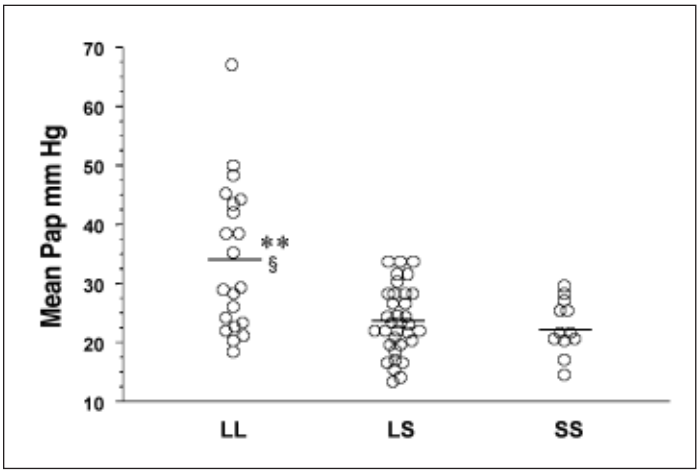

Figure 4. Individual values of pulmonary artery pressure (Pap) measured in patients with COPD classified according to their LL, LS, or SS genotype. Values are mean Pap recorded during right heart catheterization. The mean of each group of values is indicated by a horizontal bar. * P <.05 ** P <.01 compared with respective values for the LS genotype; §P <.01, compared with respective values for the SS genotype. The level of Pap did not differ between patients with the LS or SS genotype.

5-HTT Gene Polymorphism and Mechanisms of Increased Expression in Other Forms of Pulmonary Hypertension

In most forms of pulmonary hypertension in adults, as well as in persistent pulmonary hypertension in neonates, a genetic predisposition has been suggested. Since many factors, such as inflammation or appetite suppressants,[15] interact with 5-HTT expression, we sought to determine whether the associations linking 5-HTT overexpression to pulmonary arterial hypertension, and 5-HTT gene polymorphism to susceptibility to pulmonary arterial hypertension, existed in other types of pulmonary hypertension in humans. In recent studies, we investigated lung transplant recipients with various forms of pulmonary hypertension, including pulmonary venoocclusive disease, scleroderma, sarcoidosis, sickle cell disease, bronchiectasis, and histiocytosis X.[13] We found increased 5-HTT expression in both the lungs and pulmonary-artery smooth muscle cells from these patients, as well as an increased growth response of pulmonary artery smooth muscle cells to 5-HT or serum. The L-allelic variant of the 5-HTT gene promoter polymorphism was present in 14/25 (56%) of lung transplant recipients with these other causes of pulmonary hypertension but in only 27% of controls.[13] Thus, the frequency of the LL genotype was significantly higher in this population of lung transplant recipients with pulmonary hypertension than in a control population, suggesting that the LL genotype may confer genetic susceptibility to pulmonary arterial hypertension as well as to severe pulmonary hypertension associated with other disorders.

Consistent with our finding in IPH, the L/S polymorphism of the 5-HTT gene promoter was only partly responsible for the increased 5-HTT expression in pulmonary hypertension associated with other illness: pulmonary-artery smooth muscle cells from these patients exhibited higher 5-HTT levels than same-genotype cells from controls. No additional promoter sequence alterations were found. This finding indicates that differences in 5-HTT expression between patients and controls cannot be ascribed entirely to 5-HTT gene promoter polymorphism. Additional studies revealed that the sequences of the main regulatory regions of the 5-HTT gene, including the promoter region and intron 2, were not altered in patients with pulmonary hypertension compared to controls. Thus, 5-HTT gene overexpression in pulmonary-artery smooth muscle cells from patients with pulmonary hypertension may not result from alterations in the regulatory sequences of 5-HTT gene. Complex mechanisms are probably involved, such as alterations in related genes or signaling pathways involved in regulating 5-HTT expression, and additional studies will be required to understand this process.

5-HTT Gene Polymorphism in Pulmonary Hypertension Complicating COPD

The pathogenesis of pulmonary hypertension in patients with advanced chronic obstructive pulmonary disease (COPD) is still incompletely understood. Chronic hypoxemia is considered the major contributing factor, in association with inflammation and morphological changes in lung parenchyma. However, pulmonary artery pressure varies greatly among individuals with COPD, with some patients developing severe pulmonary hypertension out of proportion to the severity of their underlying disease.[16] Because we previously showed that hypoxia was a strong inducer of 5-HTT gene expression, we investigated whether 5-HTT gene promoter polymorphism, in combination with hypoxia, determined the extent of pulmonary vascular remodeling and, consequently, the severity of pulmonary hypertension in patients with advanced hypoxemic COPD.[17] Investigating a large series of patients with advanced COPD (n = 103), we found that pulmonary hypertension severity in these patients was closely related to LL genotype of the 5-HTT gene pro-

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moter polymorphism. Mean or systolic pulmonary artery pressure was more than 10 mm Hg higher, and pulmonary vascular resistance twice as high, in patients homozygous for the L allelic variant compared to those with LS or SS genotype.[17] No significant differences in pulmonary artery pressure or pulmonary vascular resistance were found between LS and SS individuals, in keeping with previous evidence that the S variant may exert a dominant influence.

The association between the LL genotype and pulmonary hypertension prompted us to compare the LL, LS, and SS groups regarding variables potentially related to its development. We found no differences regarding blood gas variables or smoking history. Surprisingly, airflow limitation, as a percent reduction in FEV-1, was more severe in SS or LS patients than in LL patients.[17] Conceivably, COPD patients with severe pulmonary hypertension may become symptomatic earlier in the course of their disease, at a stage when they have less airflow limitation than do LS or SS patients, although this requires additional investigation. Since COPD severity is mainly determined by the FEV-1 value, this unexplained difference also indicates that pulmonary hypertension in COPD develops independently from the severity of the underlying lung disease. Given the prognostic impact of pulmonary hypertension, this suggests that early identification of patients with COPD at risk for developing pulmonary hypertension might improve the management of the disease by prompting measures targeting pulmonary hypertension progression.

Conclusion

The present results support a crucial role for enhanced 5-HTT activity in the pathogenesis of pulmonary vascular-remodeling and suggest that functional polymorphism of the 5-HTT gene promoter may confer susceptibility to various forms of pulmonary hypertension. Agents capable of selectively inhibiting 5-HTT-mediated pulmonary artery smooth muscle cell proliferation deserve to be investigated as potential treatments for pulmonary hypertension.

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