The Serotonin Transporter
A Vehicle to Elucidate Pulmonary Hypertension?

E. Kenneth Weir, Zhigang Hong, Anthony Varghese

Primary pulmonary hypertension (PPH) is a life-threatening disease that mostly affects young adults, more commonly women than men. It is characterized by vasoconstriction of the small pulmonary arteries, proliferation in all layers of the vessel wall, thrombosis-in situ, and inflammation. Serotonin (5-hydroxytryptamine, 5-HT) is thought to play a role in the pathogenesis of PPH, in part, because it is a pulmonary vasoconstrictor and smooth muscle mitogen. It is synthesized from L-tryptophan, primarily in the enterochromaffin cells of the small intestine. In the blood, 5-HT is mainly transported in the platelets, some are bound to plasma proteins, and only nanomolar concentrations are free in the plasma. The importance of the lungs in the removal of 5-HT from the blood has been known for more than 50 years. As much as 95% may be taken up or inactivated. 5-HT is taken up by the 5-HT transporter (5-HTT) in several cells including platelets, neurons, and pulmonary artery smooth muscle and endothelial cells. 5-HTT belongs to a large 12 transmembrane–domain gene family including the norepinephrine transporter, glucose transporters, and transporters of other neurotransmitters. The amino acid sequence of cloned human 5-HTT reveals 6 putative phosphorylation sites, targets for PKA and PKC, which may activate 5-HTT. The Figure shows some of the cellular signaling pathways involving 5-HTT and 5-HT receptors.

Herve et al have described elevated plasma levels of 5-HT in PPH patients, even after bilateral lung transplantation. This implies that 5-HT is an etiologic agent or is linked to an etiologic agent, either genetically or by being released through exocytosis, along with an etiologic agent. Other evidence implicating 5-HT is the report of PPH in a patient with platelet storage pool disease who had elevated plasma 5-HT levels. Similarly, the fawn-hooded rat has a genetic defect in platelet storage of 5-HT and develops pulmonary hypertension in mild hypoxia. Finally, the anorectic agents fenfluramine and dexfenfluramine, which block the 5-HTT, have been associated with outbreaks of PPH. Contrary to prior supposition, dexfenfluramine, at concentrations attained clinically, does not cause the release of 5-HT from platelets, but does inhibit platelet uptake of 5-HT and, at least in rats exposed to mild hypoxia, increases the plasma levels of 5-HT. These reports suggest that 5-HT is an etiologic agent in the pathophysiology of PPH. However, it is clear that the majority of patients with carcinoid syndrome, in which high levels of 5-HT are released from this tumor of the enterochromaffin cells, do not develop PPH, nor do most of the patients on dexfenfluramine. Consequently, there have to be other factors that determine susceptibility.

One such susceptibility factor is a mutation in the gene for the bone morphogenetic protein receptor (BMPR II). BMPR II is an inhibitory receptor in the transforming growth factor β (TGF-β) superfamily that is present in most patients with the familial form of PPH and in less than a quarter of those with the sporadic form of PPH. Even in the absence of a BMPR II mutation, BMPR II expression can be reduced in some PPH patients. Loss of inhibitory signaling from this pathway may make the smooth muscle and endothelial cells more susceptible to proliferation induced by 5-HT and other mediators. Another abnormality that may determine susceptibility is a decrease in potassium (K⁺) channel expression in the smooth muscle cells (SMCs) of patients with PPH, specifically Kv1.5. Kv channel genes are known to be downregulated in the lungs of PPH patients. A decrease in the outward K⁺ current causes membrane depolarization and calcium entry through L-type calcium channels that promote both vasoconstriction and proliferation. Anorectic agents, such as dexfenfluramine, also inhibit K⁺ current; thus, a decrease in K⁺ channel function or expression may increase susceptibility to PPH. In addition, dexfenfluramine and K⁺ channel blockers can stimulate 5-HT release from the ileum, which raises the possibility that there might be a connection between K⁺ channel function or expression and increased plasma 5-HT levels.

Susceptibility to PPH is also increased in patients who are homozygous for the L-allele variant of the 5-HTT gene promoter, which is associated with 5-HTT overexpression. The role of 5-HTT is emphasized by the finding that mice deficient for 5-HTT develop less chronic hypoxic pulmonary hypertension (PH) than wild-type mice, whereas mice that overexpress 5-HTT develop more severe PH. In the current issue of Circulation Research, Marcos et al build on their prior body of work implicating 5-HTT in the pathophysiology of PH. Using human lung tissue, they show that immunostaining for 5-HTT is greater in the pulmonary arteries of PPH patients than in secondary PH patients, which is, in turn, greater than in patients without PH. No difference was seen in 5-HT receptors (5-HT₁B, 5-HT₂A, or 5-HT₂C). Likewise, in cultured pulmonary artery SMCs, the mRNA for 5-HTT, the growth in response to 5-HT, and the 5-HT uptake are greater in PPH.
Schematic representation of serotonin (5-HT) signaling pathways in pulmonary artery smooth muscle cells (SMCs). Activation of serotonin receptors 5-HT₁₆, 5-HT₂₃, and 5-HT₂₇ causes vasocostriction, although 5-HT₁₆ is also implicated in the mitogenic action of 5-HT. 5-HT₁₇ activates the G, G-protein, which suppresses the activity of adenylyl cyclase (AC). AC normally increases the levels of cAMP, which activates the protein kinases PKA and PKG. PKA phosphorylates and, thereby, activates L-type voltage-gated calcium channels, as well as voltage-gated potassium channels (Kv) and large-conductance calcium-activated potassium channels (BKCa). PKG, also activated by cAMP, potentiates BKCa as well. These channel effects primarily modulate vasocostriction. The 5-HT₂₃ and 5-HT₂₇ receptors activate phospholipase C-b (PLC) through the Gq complex to increase cytosolic levels of inositol triphosphate (IP₃). IP₃ releases calcium Ca²⁺ from intracellular stores, causing vasocostriction. In addition, 5-HT₁₇ receptors increase c-Src, which blocks the BKCa channel and further promotes vasocostriction. 5-HT₁₆ is also involved in activation of mitogen-activated protein kinase (MAPK), potentially leading to cell proliferation. The serotonin transporter (5-HTT) permits the entry of 5-HT into the cytosol by using the transmembrane sodium (Na⁺) gradient. Intracellular 5-HT activates NAD(P)H oxidase through a combination of Ras and Rac to increase production of superoxide ions (O₂⁻) and hydrogen peroxide (H₂O₂). These reactive oxygen species are thought to activate the extracellular signal regulated (ERK) MAPK, which increases the phosphorylation of the GATA-4 transcription factor, which, in turn, promotes the transcription of cyclin protein genes. Cyclin D2 has been implicated in proliferation of SMCs than controls, with SMCs from secondary PH patients being intermediate. The cell growth and 5-HT uptake could be entirely inhibited by 5-HTT blockers, but not by receptor blockers. These findings, and the confirmation that 5-HTT mRNA levels and 5-HT uptake in pulmonary artery SMCs were higher in patients with the LL genotype, provide strong evidence of the importance of 5-HT and the 5-HTT in the pathogenesis of PH. In the current study, no role could be shown for 5-HT receptors. However, it has been suggested that 5-HT₁₆ and 5-HT₂₇ receptors may be involved in the pulmonary vascular constriction, and in the proliferation seen in chronic hypoxic PH in mice. Similarly 5-HT₁₆ receptors expression is increased in the high-flow model of PH in pigs. The differences between the 5-HTT and 5-HT receptor data may occur because Marcos et al studied human PPH tissue, whereas other studies have used animal models that do not precisely recapitulate PPH. In addition, the mice that lack the gene for the 5-HT₂B receptor have a dilated cardiomyopathy that somewhat complicates the interpretation of the results.

The observation reported in this article that the LL genotype was present in 56% of the lung transplantation patients with PH that was secondary to a wide variety of lung diseases, but only in 27% of controls, is especially intriguing. It is also interesting that patients with chronic obstructive pulmonary disease who carry the LL genotype have more severe secondary PH than those with LS or SS genotype. These findings indicate that 5-HTT plays a significant role in secondary PH, as well as in PPH, and raise the possibility that a better understanding of the role of the 5-HTT may lead to new treatment for many forms of pulmonary hypertension.

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References


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