

## Commentary

# Anorexigen-induced pulmonary hypertension and the serotonin (5-HT) hypothesis: lessons for the future in pathogenesis

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## Abstract

Epidemiological studies have established that fenfluramine, D-fenfluramine, and aminorex, but not other appetite suppressants, increase the risk of primary pulmonary hypertension (PH). One current hypothesis suggests that fenfluramine-like medications may act through interactions with the serotonin (5-hydroxytryptamine [5-HT]) transporter (5-HTT) located on pulmonary artery smooth muscle cells and responsible for the mitogenic action of 5-HT. Anorexigens may contribute to PH by boosting 5-HT levels in the bloodstream, directly stimulating smooth muscle cell growth, or altering 5-HTT expression. We suggest that individuals with a high basal level of 5-HTT expression related to the presence of the long 5-HTT gene promoter variant may be particularly susceptible to one or more of these potential mechanisms of appetite-suppressant-related PH.

**Keywords:** anorexigens, appetite suppressants, pulmonary hypertension, pulmonary vascular smooth muscle cells, serotonin transporter

## Introduction

Appetite suppressant use is now considered an important risk factor for the development of primary PH. An association between the anorexigen aminorex and PH was first reported in the 1960s, and aminorex was withdrawn from the market in 1972 [1]. In the 1980s, fenfluramine use was linked to primary PH and, subsequently, a 30-fold increase in the risk of PH was found in patients who had received these appetite suppressants for longer than 3 months compared with the general population [2,3].

Understanding the molecular mechanism of appetite-suppressant-induced PH has become a major goal for current and future studies. Because amphetamine-like drugs have potential applications in the treatment of obesity, drug dependence, and other psychiatric disorders, a legitimate concern is that new members of this class introduced in

the future might cause outbreaks of PH similar to those seen with aminorex and fenfluramine. To ward off this danger, we must identify the drug-related effects likely to generate PH and/or the patient characteristics associated with susceptibility to these effects.

Better knowledge of the pathobiology of primary PH can be expected to flow from elucidation of the mechanisms underlying appetite-suppressant-induced PH. These drugs promote the development of vascular lesions confined to the small muscular arteries and arterioles in the lung, suggesting that one of their molecular targets may be selectively present in pulmonary vessels or may have specific functions in pulmonary vessels compared with systemic vessels. Aminorex, fenfluramine, D-fenfluramine, and phentermine belong to a vast class of amphetamine-like drugs that interact with monoamine systems in the brain. Among

5-HT = 5-hydroxytryptamine; 5-HTT = 5-hydroxytryptamine transporter; PH = pulmonary hypertension; SMC = smooth muscle cell.

appetite suppressants, however, only fenfluramine, D-fenfluramine, and aminorex increase the risk of primary PH. The fact that these three drugs not only inhibit neuronal serotonin (5-HT) reuptake, but also trigger indoleamine release has sparked renewed interest for the 'serotonin hypothesis' of PH.

In our laboratory, we recently tested the hypothesis that the 5-HT transporter (5-HTT) in the lung might be a key determinant of pulmonary vessel remodeling because of its action on pulmonary artery smooth muscle cell (SMC) growth [4]. The 5-HTT transporter is abundantly expressed in the lung, where it is predominantly located on SMCs [5]. The recent 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 PH [6].

### **5-HTT as a key determinant of pulmonary vascular remodeling**

A pathological feature shared by secondary and primary PH is increased thickness of the distal pulmonary artery walls, related chiefly to SMC hyperplasia [7]. The 5-HTT in pulmonary vascular SMCs has many attributes suggesting that it may be a key determinant of this process. In addition to contributing to the uptake and subsequent inactivation of 5-HT passing through the lung, 5-HTT mediates the proliferation of pulmonary vascular SMCs through its ability to internalize indoleamine [4,8,9]. The level of 5-HTT expression appears to be much greater in human lung than in human brain, suggesting that altered 5-HTT expression may have direct consequences on pulmonary artery–SMC function [5]. Direct evidence that 5-HTT plays a key role in pulmonary vascular remodeling was recently obtained by showing that mice with targeted 5-HTT gene disruption develop less severe hypoxic PH than wild-type controls [10], and that selective 5-HTT inhibitors attenuate hypoxic PH. Conversely, increased 5-HTT expression is associated with increased severity of hypoxic PH [11]. Although a heterogeneous population of 5-HT<sub>2A</sub> and 5-HT<sub>1B</sub> receptors coexist in pulmonary arteries, 5-HT receptor antagonists do not seem to efficiently protect against development of hypoxic PH (unpublished data). Taken together, these observations suggest a close correlation between 5-HTT expression and/or activity and the extent of pulmonary vascular remodeling during exposure to hypoxia.

5-HTT expression and activity are also increased in platelets and lungs from patients with primary and secondary forms of PH [12] and in pulmonary artery SMCs from patients with primary PH, as compared with SMCs from controls. Furthermore, compared with SMCs from controls, SMCs from PH patients are more susceptible to

the growth promoting effects of 5-HT and serum (which contains high levels of 5-HT) [12], whereas there is no difference for other growth factors such as platelet-derived growth factor, transforming growth factor- $\beta$ , fibroblast growth factor- $\alpha$ , and epidermal growth factor. In the presence of 5-HTT inhibitors, the growth stimulating effects of serum and 5-HT are markedly reduced, and the difference between growth of SMCs from patients and controls is abolished. It follows that 5-HTT overexpression and/or activity in pulmonary artery SMCs from patients with PH is responsible for the increased mitogenic responses to 5-HT and serum [13].

### **Mechanisms by which appetite suppressants may promote primary PH: is 5-HTT a critical target for drugs linked to primary PH?**

#### **5-HT turnover alteration**

The anorexigens aminorex, fenfluramine, and dexfenfluramine are potent 5-HT uptake inhibitors. This inhibition occurs not only in neurons of the serotonergic category, but also in platelets, pulmonary endothelial cells and SMCs, which share the same 5-HTT encoded by a single gene [14]. These facts initially led to the hypothesis that fenfluramine-like medications may elevate circulating 5-HT levels [15,16], which in turn may increase pulmonary artery pressure and pulmonary artery SMC growth, thereby producing primary PH in susceptible individuals. This 'serotonin hypothesis' was consistent with reports of increased plasma 5-HT levels under several conditions leading to PH [17,18]. Recent findings, however, run counter to the serotonin hypothesis: chronic treatment with phentermine and fenfluramine in combination decreases plasma 5-HT levels in humans [19]. Another argument against the serotonin hypothesis is that 5-HTT is also the target of widely used antidepressants, such as selective 5-HT reuptake inhibitors and conventional tricyclic antidepressants, which do not promote the development of primary PH. Conversely, selective 5-HTT inhibitors attenuate the development of experimental PH. Moreover, preliminary data from a case–control study performed in Europe has suggested that the psychoanaleptic drugs may lower the odds ratio for primary PH [20].

The 5-HT concentrations in blood and plasma may not, however, be indicative of 5-HT concentrations in local microenvironments surrounding pulmonary endothelial cells or SMCs. In addition to their 5-HTT inhibiting properties, aminorex and fenfluramine-like drugs are potent triggers of indoleamine release and, consequently, increase the amount of extracellular 5-HT [6]. Selective 5-HTT inhibitors also increased 5-HT levels in extracellular fluid, but to a much smaller degree than fenfluramine-like drugs [21]. This leaves open the possibility that these drugs may promote 5-HT turnover alterations, thereby increasing the availability of free 5-HT near the pulmonary artery wall. Since 5-HT is also released from pulmonary neuro-

endocrine cells and neuroepithelial bodies distributed throughout the airways, and possibly from pulmonary artery SMCs [22], this makes 5-HT more likely to have a role on pulmonary compared with systemic vessels.

### Interaction with 5-HTT

Recent studies investigated the possibility that fenfluramine and other anorexigens might increase the risk of primary PH by interacting directly with 5-HTT. Interestingly, drugs known or suspected to increase the risk of primary PH (namely, aminorex, fenfluramine, and chlorphentermine) were found to be 5-HTT substrates, whereas drugs not associated with an increased risk of primary PH were less potent in this regard [6]. It has been speculated that medications that are 5-HTT substrates may be translocated into pulmonary cells where, depending on the degree of drug retention, intrinsic drug toxicity, and individual patient susceptibility, they may cause effects similar to or greater than those of 5-HT [6]. According to this hypothesis, 5-HTT substrates other than 5-HT may also be mitogenic. Support for this hypothesis has come from a recent study showing that fenfluramine is mitogenic for rat lung SMCs and for lung fibroblasts [9].

Potassium-channel inhibition has also been suggested as a potential mechanism of anorexigen-induced cell toxicity [22]. Although this effect requires drug concentrations 10 times greater than those expected with therapeutic drug dosages, it remains plausible that direct vasoconstriction related to potassium-channel blockade or increased intracellular  $Ca^{2+}$  levels in SMCs may contribute to the toxic effects of appetite suppressants after internalization and accumulation of these drugs by the SMCs. The fact that 5-HT uptake inhibitors and substrates may bind to different domains on 5-HTT suggest that determining the exact mechanism of action of each drug may help to predict the risk of adverse effects [6].

### Stimulation of 5-HTT expression

Another mechanism by which anorexigens may promote pulmonary vascular remodeling is stimulation of 5-HTT expression. Previous studies have shown that hormones and pharmacological agents [23] can modulate 5-HTT levels and activity in serotonergic neurons. Dexfenfluramine given in high doses has been shown to produce long-lasting decreases in both concentration and uptake of 5-HT in forebrain regions, as well as in 5-HTT mRNA levels within the dorsal raphe nucleus [24]. We found that the levels of 5-HTT transcript in lung tissue from rats given chronic dexfenfluramine treatment for 4 weeks remained unchanged compared with those in animals treated with the vehicle alone. However, discontinuation of chronic dexfenfluramine treatment in rats was followed by increased lung 5-HTT expression, which promoted the development of hypoxic PH [25]. 5-HTT overexpression such as that induced by withdrawing dexfenfluramine-like

drugs may therefore represent a complementary mechanism promoting 5-HTT-dependent hyperplasia of pulmonary SMCs.

### Genetic susceptibility to anorexigen-induced PH

A genetic predisposition has been postulated to explain why primary PH develops in only a minority of appetite-suppressant users. In keeping with this possibility, feeding aminorex or dexfenfluramine to experimental animals fails to elicit PH. Interestingly, it has been established that 5-HTT expression is genetically controlled: a polymorphism in the promoter region of the human 5-HTT gene alters transcriptional activity. This polymorphism consists of two common alleles, a 44 base pair insertion (the L allele) or deletion (the S allele) [25]. The L allele drives a twofold to threefold higher level of 5-HTT gene transcription than the S allele. Preliminary results suggest that the L/L genotype is present in 60–70% of the patients with PH as compared with only 20–30% of a control population of Caucasian subjects [13]. The L/L genotype may therefore confer genetic susceptibility to PH in humans, particularly when it is combined with other factors such as hypoxia, HIV infection, portal hypertension, or other conditions. Individuals with a high basal level of 5-HT uptake related to presence of the long 5-HTT gene promoter variant may be particularly susceptible to one or more of the aforementioned potential mechanisms of appetite-suppressant-related primary PH. Studies examining the potential association between appetite-suppressant-related PH and 5-HTT gene polymorphism are warranted.

In recent studies, mutations in the coding sequence of the BMPR2 gene were shown to occur in more than 50% of patients with familial primary PH and in at least 25% of patients with sporadic primary PH [26,27]. The functional impact of BMPR2 mutations into the pathogenesis of pulmonary vascular remodeling is presently under investigation. An attractive hypothesis is that dysfunction of the BMPR2 protein may result in impaired control of cellular proliferation or gene transcription. Whether abnormal signaling through mutated BMPR2 may contribute to appetite-suppressant-related PH also remains to be determined.

### Conclusion

The observation that 5-HTT polymorphism may confer susceptibility to primary PH suggests a mechanism by which appetite suppressants may increase the risk of primary PH in humans. Fenfluramine-like medications may contribute to PH by elevating circulating 5-HT levels, they may act as 5-HTT substrates to produce the same effect as 5-HT or they may alter 5-HTT expression. Individuals with a high basal serotonin uptake that is related to the presence of the long 5-HTT gene promoter variant might be particularly susceptible to one or more of these potential mechanisms of appetite-suppressant-related primary PH.

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