Pulmonary hypertension and the serotonin hypothesis: where are we now?

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Pulmonary arterial hypertension (PAH) has a complex pathobiology involving vascular remodelling, contraction and inflammation. It is characterised by a sustained and progressive elevation in pulmonary arterial pressure, pulmonary vascular remodelling, right heart failure and death. Familial PAH (fPAH) has been shown to be related to heterozygous germ-line mutations in the gene encoding bone morphogenetic protein-receptor 2 (BMPR2) and mutations in activin-receptor kinase-like 1 gene have also been reported (2). However, the majority of individuals with this mutation do not actually develop the disease and it is recognised that other ‘risk factors’ must be involved in the development of PAH. Increased activation of the serotonin system has been proposed as a ‘second hit’ risk factor.

Serotonin promotes pulmonary arterial smooth muscle cell (PASMC) proliferation, pulmonary arterial vasoconstriction and local microthrombosis (3). Proliferation of PASMCs is an important component of pulmonary arterial remodelling in PAH, which accounts for the increased thickness of the medial muscular coat in normally muscularised arteries and extension of muscle into smaller and more peripheral arteries. Elevated circulating peripheral serotonin has been associated with the development of PAH clinically (4). There is also an increasing body of evidence that implicates serotonin in the development of experimental PAH. For example, inhibition of serotonin receptors and the serotonin transporter (SERT) can inhibit the development of PAH in animal models (5–8).

The original ‘serotonin hypothesis of PAH’ was derived from the observation that obese patients using anorexigens such as aminorex developed PAH. Later, studies confirmed that patients taking related drugs such as dexfenfluramine (Dfen) for more than 3 months had a substantial increase in the absolute risk of PAH as compared with non-users (9,10). The appetite-suppressant effect of Dfen is thought to be dependent upon inhibition of neuronal serotonin reuptake via inhibition of the SERT, increased serotonin release and subsequent 5HT-receptor stimulation (11,12). Inhibition of the SERT by Dfen also occurs in platelets, pulmonary endothelial and SMCs as these various cell types share the same SERT encoded by a single gene (13). The increase in serotonin plasma levels observed during treatment with Dfen may therefore be a consequence of impaired serotonin uptake by platelets and pulmonary vascular cells and one school of thought is that it is this action of Dfen that promotes PAH (14). However, Dfen has a very complex pharmacology and the ‘serotonin hypothesis’ for Dfen-induced PAH is now somewhat controversial as Dfen has subsequently been shown to be protective against the development...
of both monocrotaline-and hypoxia-induced PAH (15–17). Events took a further curious twist when a polymorphism of SERT was associated with PAH (18) suggesting that inhibition of SERT might actually be a novel therapeutic approach for PAH. However, recent years have seen the publication of many interesting studies that implicate the serotonin system in the development of PAH, not just via SERT activity but also via serotonin receptors, tryptophan hydroxylase1 (Tph1) and inactivation of potassium channels (Figure 1).

So, where do we stand in our understanding of the serotonin system and its role in the pathobiology of PAH and where are the potential therapeutic targets? This review will address these important issues.

The serotonin transporter

The SERT is encoded by a single gene on chromosome 17q11.2 and a variant in the upstream promoter region of the SERT gene has been described (13). This polymorphism increases SERT expression and function, and has been identified in patients with PAH (18). This suggested that increased activity of SERT may be associated with the development of PAH (18). Other researchers, however, suggest that variation of the SERT gene alone is unlikely to confer significant susceptibility to PAH (19). Others suggest that, in patients with fPAH, the presence of the SERT polymorphism may correlate with an earlier age at diagnosis (20). Over-expression of the gene for human SERT in mice results in more severe hypoxia-induced PAH (21,22). The mechanism behind this increased susceptibility to PAH is thought to be serotonin-induced PASMC proliferation. Serotonin-induced PASMC proliferation is mediated by serotonin entering the PASMC through the SERT and subsequent reactive oxygen species production and activation of down-stream proliferative signalling pathways. Serotonin can activate proliferative pathways via the SERT and also activate serotonin receptors on PASMCs and pulmonary arterial adventitial fibroblasts (23).

5HT receptors in the pulmonary circulation

There are 14 different, structurally distinct 5HT receptors. These are divided up into seven families (5HT1–7). There is evidence that the 5HT1B, 5HT2A and 5HT2B receptors may play a role in the pathobiology of PAH.

5HT2A receptor

In most non-human mammals, the 5HT2A receptor mediated vasoconstriction in both the systemic and pulmonary circulation is mediated by activation of the 5HT2A receptors. This can then act on the underlying smooth muscle cells to induce proliferation and contraction. Proliferation can be mediated either by stimulation of the serotonin receptors or by 5HT entering the cell via the SERT.
pulmonary circulations. For example, 5HT₂A-receptor mediated vasoconstriction is observed in the mouse and rat lung under control conditions (7,24). The 5HT₂A-receptor antagonist ketanserin has proved clinically effective in the treatment of systemic hypertension, especially in the elderly (25). Hence, there is no specificity for the pulmonary circulation and the systemic effects have limited its use in either primary or secondary pulmonary hypertension, where it fails to improve pulmonary haemodynamics significantly (25).

**5HT₂B receptor**

The development of hypoxia-induced PAH in mice is ablated in 5HT₂B-receptor knockout mice (26), and this receptor may control serotonin plasma levels in mice (27). However, there is currently no convincing evidence that this receptor activates either vasoconstriction or proliferation in human PASMCs. In addition, the loss of serotonin 5HT₂B-receptor function may predispose to fenfluramine-associated PPH in man (28).

**The 5HT₁B receptor**

In 1992, MacIntyre et al. (29) published evidence that the 5HT₁B-receptor agonist sumatriptan caused pulmonary vasoconstriction in man and studies subsequent to this provided further evidence that it is the 5HT₁B receptor that mediates constriction in human large and small pulmonary arteries (30,31). Experimentally, whilst serotonin-induced contraction in normoxic rats is mediated by the 5HT₂A receptor, there is an increase in 5HT₁B-receptor mediated vasoconstriction under hypoxic conditions (7,24). Inhibition of 5HT₁B activity, either by genetic knockout or antagonism, ablates hypoxia-induced PAH (7). Others have recently identified a role of this receptor in a pig model of PAH (32) and pulmonary arteries removed from PAH patients can have increased expression of the 5HT₁B receptor (26).

**Tryptophan hydroxylase**

It has recently been shown that expression of the Tph1 gene is increased in lungs and pulmonary endothelial cells from patients with idiopathic PAH (33). Tph catalyses the rate-limiting step in the synthesis of serotonin from tryptophan. By studying mice deficient in Tph1 (Tph1−/− mice), Walther and Bader (34) demonstrated that there are two isoforms of Tph, now classified as Tph1 and Tph2. Tph2 is present exclusively in the brain but not the periphery. The classical Tph gene, now termed Tph1, is mainly expressed in the gut and mediates the generation of serotonin in the periphery (34,35). Interestingly, hypoxia-induced PAH is ablated in Tph1−/− mice which are deficient in peripheral serotonin (36). This is associated with inhibition of pulmonary vascular remodelling. Contractile responses to serotonin are actually increased in pulmonary arteries from Tph1−/− mice, consistent with upregulation of the 5HT receptor in the face of reduced serotonin. Hence, as Tph1 knockout actually increases vascular reactivity, the effect of Tph1 knockout on pulmonary pressures is likely to be due to protection against pulmonary vascular remodelling. This suggests a critical role for serotonin in hypoxia-induced pulmonary vascular remodelling. Both hypoxia and mechanical stretch have been shown to increase Tph1 expression and serotonin release in rabbit lung (37). These observations suggest that chronic hypoxia itself may induce Tph1 synthesis and subsequent serotonin release which acts as a mitogen in pulmonary arteries, contributing to the pulmonary vascular remodelling and subsequent onset of PAH.

**Dfen-induced PAH revisited**

As discussed above, the PAH associated with fenfluramine derivatives was initially thought to be dependent upon inhibition of neuronal serotonin reuptake, increased indoleamine release and subsequent 5HT receptor stimulation (11,12). However, this ‘serotonin hypothesis’ is somewhat controversial as Dfen has actually been shown to be protective against the development of both monocrotaline- and hypoxia-induced PAH (15–17). Other potential, directly-induced mechanisms have been proposed. For example, the metabolite of Dfen, Nordfen, has been shown to have affinity for 5HT₂A-C receptors (38). Nordfen can mediate pulmonary vasoconstriction and this may be via the 5HT₂A receptor (39,40). Other, non-serotonergic effects of Dfen have been proposed: Dfen has been shown to increase [Ca²⁺] in rat pulmonary arteries by both release of Ca²⁺ from the sarcoplasmic reticulum and influx of extracellular Ca²⁺ (41,42).

**Dfen, serotonin and potassium channels**

Another possible mechanism for the effects of Dfen on PASMCs is via inhibition of K⁺ current: human and experimental hypoxic PAH–PASMCs are deficient in K⁺ channels. Consequently, they have depolarised membrane potentials that activate voltage-gated L-type Ca²⁺ channels (43–45). The resulting increase in cytosolic Ca²⁺ boosts cell proliferation and increases pulmonary vascular tone. Aminorex, Nordfen and Dfen have been shown to inhibit K⁺ current in rat PAs (46). Serotonin itself has been shown to inhibit these Kv1.5 currents (47). Therefore, the effects
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