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Targeting the serotonin pathway for the treatment of pulmonary arterial hypertension

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ABSTRACT

As we uncover the complex pathophysiology underlying idiopathic and familial pulmonary arterial hypertension, multiple disease associated pathways, cell types and processes reveal links to elements of the serotonin system. Beyond the original 'serotonin hypothesis' observed with anorexigens, and the latterly demonstrated association with vascular tone and pulmonary artery smooth muscle cell proliferation, recent studies suggest links to BMPR2, PDGF and RhoK pathways, as well as an impact upon more complex lesion formation and pathologic bone marrow progenitor mobilization.

Clinical experience with antagonists targeting the various elements of the serotonin pathway has been unsatisfactory, yet perhaps this is less than surprising given our expanding knowledge around serotonin production and signaling biology, which indicate opportunities for novel therapeutic options.

Contents

1. Evolution of the 'serotonin hypothesis'

Serotonin (5-HT) has 15 receptors (5-HTR) divided into 7 families, whose wide distribution and function both peripherally and within the central nervous system indicate the complexity of this signaling system (Berger et al., 2009). Said complexity has hampered attempts to fully elucidate the interactions of serotonin with other signaling cascades and its consequent role/s in pathophysiological processes — illustrated by the emergence of the 'serotonin hypothesis' for pulmonary arterial hypertension (PAH). In the 1960s &80s a profound rise in PAH cases was linked to the anorexigens aminorex, fenfluramine (Dfen) and phentermine (MacLean, 1999). As these are substrates for the serotonin transporter (SERT), it was hypothesized that PAH was caused by both reversal of 5-HT flux and disruption of monoamine-dependent 5-HT metabolism (Rothman et al., 2000; Ulus et al., 2000). These data were in keeping with the association of elevated 5-HT levels in plasma and PAH (Herve et al., 1995). Further studies suggested alternative mechanisms of action: the Dfen metabolite nor-Dfen was demonstrated to be an agonist of 5HTR2A and 2B (Rothman et al., 2000). Dfen was also shown to directly inhibit potassium channels associated with PAH (Weir et al., 1996). Dfen may also directly increase intracellular Ca^{2+} release from sarcoplasmic reticulum as well as extracellularly in pulmonary artery tissue (Reeve et al., 1999). An additional 'gateway hypothesis' to explain Dfen-induced PAH suggests fenfluramine accumulation inside cells which promote mitogenesis via mechanisms including post-translational transamidation of small proteins (Rothman et al., 1999). However, the hypothesis that Dfen acts directly to induce PAH was questioned by its protective effects in hypoxia and monocrotaline preclinical rodent models of the disease (Rochefort et al.,

Abbreviations: 5-HT (5-hydroxytryptamine), serotonin; TPH1, tryptophan hydroxylase 1; 5HTR, 5-HT receptor; 5HTT, 5-HT transporter; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; SMA, smooth muscle actinECs pulmonary arterial endothelial cells; Im, Imatinib; MCT, monocrotaline; PAH, pulmonary arterial hypertension; RVP, right ventricular pressure; RVH, right ventricle hypertrophy; PASMCs, pulmonary artery smooth muscle cells; PAECs, pulmonary artery endothelial cells; BMPRII (BMPR2), bone morphogenetic protein receptor II.

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2006) and more recent studies in genetically engineered murine strains. Mice lacking tryptophan hydroxylase 1 (TPH1) – the enzyme mediating the rate-limiting step in serotonin synthesis – display a 96% reduction in peripheral 5-HT (Walther et al., 2003), and protection from Dfen-induced PAH (Dempsie et al., 2008). Such protection may point toward a pathologic role for 5-HT in the Dfen-induced transamidation of small proteins such as Rho A., or may reflect adaptive changes in non-serotonergic PAH-related genes (Rothman et al., 2011). Furthermore, the augmented PAH-like pathology observed in hypoxic SERT over-expressing mice (Dempsie et al., 2008) was inhibited by Dfen treatment. A potential mechanism was suggested from ex vivo studies of pulmonary artery fibroblast proliferation in which Dfen inhibited hypoxia-induced p38 mitogen-activated protein kinase phosphorylation (Dempsie & MacLean, 2008). Therefore, the mechanisms by which anorexigen-associated PAH and perturbation of the serotonin signaling system remain obscure (MacLean & Dempsie, 2010). Yet research into the 'original' serotonin hypothesis has sparked more broad interest in the links between 5-HT and the pathophysiology of PAH.

2. Serotonin and pulmonary arterial hypertension pathophysiology

PAH is characterized by increased pulmonary vascular tone, adventitial and medial hypertrophy, neointimal hyperplasia and fibrosis (Humbert et al., 2004). Pro-proliferative, apoptosis resistant smooth muscle cells (SMCs), endothelium and fibroblasts observed in pulmonary arterial lesions have been accredited to perturbation of a number of mechanisms including inflammatory mediators, progenitor cell influx, metabolic signaling, vasoactive factors, elastases, proteases and growth factors (Humbert et al., 2004). The resultant increases in pulmonary vascular resistance and pulmonary artery (PA) stiffness cause pressure and volume overloading of the right ventricle, leading to right heart failure and death (Morrell et al., 2009; Drake et al., 2011; Schermuly et al., 2011) — processes illustrated in Fig. 1.

2.1. Pulmonary vascular tone

An imbalance of vasoactive factors leading to increased pulmonary vasoconstriction is an important aspect of PAH, and is the principal target of currently available therapies. Serotonin is a potent vasoconstrictor, with activity on the systemic vascular bed mediated through 5-HTR2A (MacLean & Dempsie, 2010). In rat PASMCs, signaling through the 5-HT2A receptor inhibits native K–V and hKv1.5 currents, and this may contribute to vasoconstriction observed in disease (Cogolludo et al., 2006). Ex vivo studies in isolated human small and large pulmonary arteries demonstrated 5-HTR1B to mediate serotonin-induced vasoconstriction, thus providing a potential mechanism of more relevance to PAH (MacLean, 1999). Indeed, preclinical efficacy of 5-HTR1B inhibition has been tested via both genetic deletion and pharmacologic antagonism, demonstrating the inhibition of enhanced pulmonary vasoactivity and of the development of PAH-like pathologies within the chronic hypoxia model (Keegan et al., 2001). Contractile responses mediated by 5-HTR1B receptor signaling may be increased in blood vessels with damaged endothelium, but may also be augmented in the presence of low concentrations of other vasoconstrictors such as thromboxane A (2), endothelin-1, prostaglandin F(2alpha), angiotensin II, histamine, noradrenaline, phenylephrine or KCl (MacLean & Morecroft, 2001; MacLean & Dempsie, 2009).

2.2. Pulmonary artery smooth muscle cells

Addressing the vasoconstrictive elements of PAH provides only partial and transitory relief from the disease — indeed evidence from the French PAH registry would suggest that the advent of the modern treatment era has only improved mean survival times by 10% of that predicted by the NIH in 1985 (Humbert et al., 2004). Therefore, it is the vascular remodeling aspects of PAH which underlie the disease and offer further potential to impact the course of disease progression. Pulmonary artery smooth muscle cells (PASMCs) from PAH patients and preclinical models display a pro-proliferative and invasive phenotype. Transgenic mice overexpressing the SERT gene globally, display an exaggerated pulmonary arterial remodeling in response to chronic hypoxia (MacLean et al., 2004), which was latterly associated with female gender. Restriction of SERT overexpression to smooth muscle results in spontaneous development of pulmonary hypertension (Guignabert et al., 2005). Indeed mice genetically lacking SERT develop less PAH and vascular remodeling in response to chronic hypoxia (Eddahibi & Adnot, 2006). Further mechanistic links between the 5-HT signaling cascade and proliferation were demonstrated in vitro using human PASMCs in which SERT-initiated Rho kinase signaling allowed 5-HTR1B mediated MEK phosphorylation of ERK. Translocation of pERK to the nucleus activated transcription factors such as GATA-4, cyclin D1, Egr-1 and Elk-1 — subsequent expression of S100A4/Mts1 then promoted calcium dependent proliferation of the PASMCs (Liu et al., 2004; Lawrie et al., 2005). There is also evidence for co-operation between SERT and 5HT1B in vivo (Morecroft et al., 2010). In addition to aberrant proliferation, PASMCs become more invasive as demonstrated by neointima formation in concentric vascular lesion characteristic of PAH (Guignabert et al., 2005; Eddahibi & Adnot, 2006). Pharmacologic inhibition of JNK (via SP-600125) in bovine PASMC migration assays identified 5-HT as an important point for cross talk between MAPK and PI3K pathways. Interestingly, 5-HTR1B and 5-HTR2A receptors were proposed to mediate pro-migratory effects rather than SERT, and inhibition of ERK and p38 MAPKs had little effect on PI3K-mediated Akt phosphorylation and subsequent cytoskeletal reorganization necessary for cell movement (Wei et al., 2010). PASMCs from PAH patients have also shown elevation in RhoA and Rho kinase activities and a strong increase in 5-HT binding to RhoA indicating RhoA serotonylation. SERT inhibition prevented 5-HT-induced RhoA serotonylation and RhoA/Rho kinase activation, as well as 5-HT-induced proliferation of PASMCs, demonstrating interplay between ROCK signaling, 5-HT and vascular remodeling (Guilluy et al., 2009).

An association between SERT and platelet-derived growth factor receptor (PDGFR) – a growth factor central to PAH pathogenesis – has been proposed. SERT was shown to transactivate PDGFRβ in serotonin-stimulated PASMC proliferation from PAH patients (Liu et al., 2007). Further studies used PDGFR inhibitors, genetic ablation, and construct overexpression of SERT to demonstrate a role for SERT in PDGF-BB signaling and PAH PASMC proliferation. Furthermore, co-immunoprecipitation experiments showed that SERT and PDGFRβ become physically associated upon PDGF stimulation (Ren et al., 2011).

Links between 5-HT and genetic disease association have also been identified using transgenic mice with a heterozygous deletion of bone morphogenetic protein (BMP) type II receptor (BMPR-II). Mutations in BMPR-II underlie most cases of heritable PAH and a significant proportion of sporadic cases (Hong et al., 2008). PASMCs from PAH patients not only exhibit attenuated growth suppression by BMPs, but an abnormal mitogenic response to transforming growth factor (TGF)-β1. BMPR-II mutation reduces Smad1/5/8-driven expression of the inhibitor of DNA binding protein 1 (Id1) and loss of the suppressive effects of BMPs, thus contributing to pulmonary vascular remodeling (Davies & Morrell, 2008). However, BMPR-II mutant mice fail to spontaneously develop PAH, indicating the need for a second stimulus — also proposed to explain the low disease penetrance in human mutation carriers. Chronic infusion of serotonin caused increased pulmonary artery systolic pressure, right ventricular hypertrophy, and pulmonary artery remodeling in BMPRII$^{+/-}$ mice compared with wild-type littermates, an effect that was exaggerated under hypoxic conditions (Long et al., 2006). Furthermore, pulmonary, but not systemic, resistance arteries from BMPRII+/− mice