EDITORIAL

Fenfluramine: riddle or Rosetta stone?

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Idiopathic pulmonary arterial hypertension (IPAH) used to be called primary pulmonary hypertension. Both terms indicate that we do not yet fully understand the aetiology of the disease. A clue to the underlying mechanisms lies in the observation that the appetite-suppressant drugs aminorex [1, 2] and fenfluramine [3] were both associated with a marked increase in the incidence of pulmonary arterial hypertension (PAH) while they were available in Europe. Although they are no longer prescribed, it is important to study them to gain an understanding of IPAH and to ensure that new drugs do not have the potential to cause pulmonary hypertension. In general, there is no clinical or pathological way to distinguish PAH associated with these anorectic agents from IPAH independent of them. This point is clearly made in a study by SOUZA *et al.* [4] in the present issue of the *European Respiratory Journal*. SOUZA *et al.* [4] describe the follow-up of 109 patients diagnosed with PAH in association with exposure to fenfluramine between 1986 and 2004. In virtually every respect, the presentation and clinical course of these patients was the same as that of patients with IPAH who were diagnosed over the same period. Interestingly, 18% of the fenfluramine PAH patients had a bone morphogenetic protein receptor 2 (BMPR2) gene mutation, a very similar incidence to that observed in sporadic IPAH [5]. This suggests the likelihood of underlying genetic susceptibility that renders both groups vulnerable, perhaps to a similar second hit.

FENFLURAMINE-INDUCED VALVE DISEASE

There is one marked difference in the spectrum of disease caused by fenfluramine and that seen in IPAH. Although fenfluramine was recognised to cause PAH it was actually withdrawn from the market in 1997 because of an associated cardiac valvulopathy [6]. The valvulopathy does not occur in IPAH but resembles that seen in clinical carcinoid heart disease, where high levels of 5-hydroxytryptamine (5-HT) are primarily responsible. Indeed, chronic systemic administration of 5-HT in rats has been demonstrated to cause valvulopathy and myofibroblast proliferation [7]. The fenfluramine-related valvulopathy is thought to be caused by stimulation of the

5-HT_{2B} receptor by the metabolite norfenfluramine [8]. 5-HT acts on cells not only by binding to receptors on the cell surface but also by being taken up into the cell through the 5-HT transporter (5-HTT). Genetically induced deficiency of the 5-HTT in mice also leads to valvulopathy and fibrosis [9]. Fenfluramine inhibits the 5-HTT, in addition to stimulating the 5-HT_{2B} receptor, so both activities may play a role.

In general, patients with PAH associated with fenfluramine did not develop valvulopathy. Conversely, patients who had valvulopathy associated with fenfluramine usually had pulmonary artery pressures compatible with the valve defect, rather than severe PAH. The question then arises, why did some patients who took fenfluramine develop valvulopathy, some develop PAH and most have no clinical sequelae?

ELEVATION OF PLASMA 5-HT LEVELS

Plasma 5-HT is increased in rats treated with fenfluramine, or its D-isomer dexfenfluramine [10–12]. This may occur because dexfenfluramine blocks uptake of 5-HT by the 5-HTT but if that were the only mechanism of the associated PAH in patients, then the serotonin reuptake inhibitors (SSRIs), such as fluoxetine, which are widely used, would cause PAH. They do not. In fact, the SSRIs appear to have a beneficial effect in animal models of pulmonary hypertension [13] and possibly in patients [14]. Most of the 5-HT in the blood is in the platelets, but the increased plasma level caused by dexfenfluramine is not the result of 5-HT release from the platelets, as the concentration of dexfenfluramine required for release is far above that achieved clinically [15]. In IPAH, 5-HT levels are increased, even after lung transplantation [16]. This observation, made in 1995, indicates that 5-HT plays an aetiological role in IPAH or is linked to the aetiological mechanism. Although 5-HT is involved in the mechanism of IPAH and in the carcinoid valvulopathy, patients with IPAH do not have valvulopathy. Conversely, any pulmonary hypertension in carcinoid valvulopathy is in keeping with the severity of the valve disease, rather than suggesting concomitant IPAH. It is hard to explain these differences on the basis of the duration of exposure or the level of plasma serotonin. It seems likely that the difference lies in the genetic substrate of IPAH, including differences in the function and/or expression of BMPR2, 5-HTT, 5-HT receptors and possibly potassium (K⁺) channels.

5-HT AND 5-HTT IN ANIMAL MODELS

The mechanism of chronic hypoxic pulmonary hypertension clearly involves 5-HT. Genetic deletion of the enzyme responsible for the synthesis of 5-HT, tryptophan hydroxylase, reduces pulmonary hypertension and pulmonary vascular remodelling caused by chronic hypoxia in mice [17, 18]. The tryptophan hydroxylase inhibitor, p-chlorophenyladenine, has

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similar effects [18]. Comparable evidence demonstrates the role of the 5-HTT. Hypoxia increases expression of 5-HTT in pulmonary artery smooth muscle (PASM) [19]. Mice that are genetically deficient for 5-HTT develop less pulmonary hypertension and pulmonary vascular remodelling in chronic hypoxia than wild-type mice [20]. Conversely, overexpression of the gene for 5-HTT in mice increases right heart pressures and vascular remodelling in response to chronic hypoxia [21]. The observation that the ability of 5-HT to cause proliferation of PSMCs is blocked by 5-HTT uptake inhibitors, such as fluoxetine, also illustrates the potential importance of the 5-HTT [22]. Assessing the role of the 5-HTT in the mechanisms underlying PAH is particularly relevant in the case of fenfluramine. Both the anorectic drugs that have been associated with outbreaks of PAH, aminorex and fenfluramine, are avidly taken up by the 5-HTT compared with other drugs [23]. All the observations cited suggest that in chronic hypoxia the uptake of 5-HT by the 5-HTT will lead to PASM proliferation. Similarly, the uptake of fenfluramine by the 5-HTT, like 5-HT, leads to smooth muscle proliferation and pulmonary vascular remodelling [24].

5-HT IN IPAH

5-HTT expression is increased in the PASM of IPAH patients [25]. These smooth muscle cells proliferate more rapidly than controls when stimulated with 5-HT. A genetic basis for the increased expression of the 5-HTT exists in the finding that the homozygous form of the L allele of the 5-HTT gene promoter is more common in patients with severe IPAH than in controls [25]. Human pulmonary artery endothelial cells (PAECs) synthesise 5-HT [26] and, when stimulated, PAECs from IPAH patients generate more 5-HT than PAECs from control subjects. The tryptophan hydroxylase responsible for this 5-HT synthesis can be demonstrated on immunostaining to be confined to the intima. These observations indicate that while most of the 5-HT in the body may be produced by the enterochromaffin cells of the small bowel, some is formed in the lung vessels.

Immunostaining of lungs from patients with IPAH, secondary PAH and pulmonary veno-occlusive disease shows the presence of 5-HT receptors (5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}), as well as 5-HTT in the vessels [27]. However, the proliferation caused by 5-HT in the cultured PSMCs from these patients appears to be related primarily to the activity of the 5-HTT, as it can be totally prevented by 5-HTT blockers but not by 5-HT receptor antagonists. In trying to decide whether the 5-HTT or the 5-HT receptors play a more important role in IPAH, this experiment has to be reconciled with the observation that genetic deficiency of 5-HT_{2B} in mice reduces their pulmonary hypertension and pulmonary vascular remodelling in response to chronic hypoxia [28]. Fortunately, it has been suggested that both 5-HTT activity and 5-HT receptor stimulation are necessary for the full mitogenic activity of 5-HT on PSMCs [29]. Proliferation is induced by 5-HT which is taken up by the 5-HTT and in turn activates extracellular signal-regulated kinase (ERK)1/ERK2, but translocation of ERK1/ERK2 to the nucleus requires Rho kinase activity that is triggered by 5-HT interaction with the 5-HT_{1B}/1D receptors. Earlier work makes it likely that dexfenfluramine also uses both 5-HTT and 5-HT receptor pathways [25].

K⁺ CHANNELS AND 5-HT

There is a parallel mechanism to the 5-HT pathway that has to be put in perspective and it relates to K⁺ channels. The anorectic agents fenfluramine and aminorex both inhibit K⁺ channels in PSMCs, causing membrane depolarisation and calcium entry through L-type calcium channels [30]. The metabolite nordexfenfluramine stimulates calcium release from the sarcoplasmic reticulum and calcium entry through store-operated channels [31]. Decreased K⁺ channel expression or function in PSMCs is also reported in chronic hypoxic pulmonary hypertension [32], spontaneous pulmonary hypertension in the fawn-hooded rat [33] and in IPAH patients [34]. K⁺ channel inhibition leads to decreased apoptosis through a rise in cytosolic K⁺ and to increased proliferation through a rise in cytosolic Ca⁺⁺ [35]. There are several interactions between the K⁺ channels and 5-HT. Dexfenfluramine increases 5-HT release from the enterochromaffin cells of the small intestine [36] and this effect is mimicked by the classical K_v channel inhibitor, 4-aminopyridine. This may help to explain the increased plasma 5-HT levels in fenfluramine-induced PAH. If the K⁺ channel abnormality seen in IPAH in PSMCs is more widely distributed and occurs in enterochromaffin cells, it might also explain the increased plasma 5-HT seen there. The fawn-hooded rat, which develops spontaneous pulmonary hypertension, has both increased plasma 5-HT and decreased K⁺ channel activity in the PSMCs [33]. Whether one abnormality precedes the other is not clear. However, the final clues to the riddle are as follows. First, that overexpression of the 5-HTT results in decreased smooth muscle levels of mRNA for K_v 1.5 and K_v 2.1 channels [37], and secondly, that the inhibition of K_v 1.5 mRNA can be achieved by incubating PSMCs with 5-HT. The latter action can be inhibited by pre-treatment with the 5-HTT blocker fluoxetine. Thus, on the one hand, fenfluramine and K⁺ channel blockers can increase 5-HT release, while on the other, 5-HT can inhibit K⁺ channel mRNA [37] and activity [38]. Consistent with this information is the observation that BMP2, the ligand for the BMPR2 receptor, which tends to downregulate 5-HT signalling, increases both K⁺ channel function and expression in PSMCs [39].

The report by SOUZA *et al.* [4] reminds us of the severe, life-long consequences that can be triggered by the relatively short use of some anorectic agents. In the case of pulmonary arterial hypertension associated with fenfluramine, the pathophysiological features that it shares with idiopathic pulmonary arterial hypertension are increased plasma 5-hydroxytryptamine, abnormal function of the 5-hydroxytryptamine transporter (possible involvement of 5-hydroxytryptamine receptors) and a decrease in K⁺ channel function/expression. New drugs should be screened for these characteristics. We are beginning to understand the hieroglyphics on our Rosetta stone.

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