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REVIEW

5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension

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Abbreviations: cyclic AMP, adenosine 3′ 5′ cyclic monophosphate; cyclic GMP, guanosine 3′ 5′ cyclic monophosphate; ET-1, endothelin-1; HIF, hypoxia inducible factor; HPV, hypoxic pulmonary vasoconstriction; 5-HT, 5-hydroxytryptamine; 5-HTT, 5-hydroxytryptamine transporter; MAO-B, monoamine oxidase-B; PAH, pulmonary arterial hypertension; PLC, phospholipase C; PPH, primary pulmonary hypertension; SMC, smooth muscle cell

Introduction

Circulating 5-hydroxytryptamine (5-HT) is produced mainly in the enterochromaffin cells of the intestine. 5-HT is also, however, locally released from pulmonary neuroendocrine cells and neuroepithelial bodies distributed throughout the airways. Secretion of large amounts of 5-HT from these cells occurs in response to airway hypoxia and increased local 5-HT may contribute to secondary pulmonary arterial hypertension [PAH] (Johnson & Georgieff, 1989). Normally, plasma levels of free 5-HT are extremely low as circulating 5-HT is stored within the platelets. Human blood platelets contain a relatively specific uptake mechanism for 5-HT (the 5-HT transporter [5-HTT]) at the plasma membrane, intracellular storage organelles (dense bodies) and a metabolizing enzyme (monoamine oxidase B). The 'Serotonin hypothesis of PAH' was developed in the 1960's after an outbreak of PAH was observed in patients taking aminorex, a diet pill that increases 5-HT availability by inducing platelet release of 5-HT, inhibiting its reuptake and inhibiting monoamine oxidase activity. Since then there has been increasing interest in the role of 5-HT in the development of PAH. Here we review pharmacological evidence that suggests changes in 5-HT availability, 5-HT-induced vasoconstriction, 5-HT-induced mitogenesis and 5-HT transporter activity are associated with the development of PAH.

The normal pulmonary circulation

There are major structural and functional differences between the systemic and pulmonary circulations which predict differential pharmacology. In the systemic circulation, 75–80% of vascular resistance is maintained by small muscular arterioles whilst resistance is relatively evenly distributed throughout the normal pulmonary circulation. In man, pulmonary arteries >1 mm i.d. are elastic in nature and essentially similar to large systemic conducting arteries. These pulmonary arteries have well developed internal and external laminae and a less distinct medial layer than that of systemic arteries. Most pulmonary arteries follow, and are adjacent to, the airways. Distal to the respiratory bronchioles the smooth muscle layers are abruptly reduced and the arteries are only partially muscularized or non-muscular (Meyrick & Reid, 1983). These are the vessels most affected by pulmonary hypertension.

Vascular tone is a major determinant of pulmonary vascular reactivity to endogenous vasoconstrictors and hypoxia (McMurtry *et al*., 1977; Barer *et al*., 1993; MacLean, 1999b). Initial vascular tone is extremely low in the normal lung unlike in the systemic circulation where basal tone is maintained by tonic activity of the sympathetic nervous system. Sympathetic innervation of the pulmonary circulation does exist and its activation has similar effects as in the systemic circulation but this does not contribute to basal vasomotor tone.

Pulmonary arteries exhibit a vasoconstrictor response to hypoxic conditions (Von Euler & Liljestrand, 1946) unlike the vasodilator response to hypoxia exhibited by the systemic circulation. In the foetus, this hypoxic pulmonary vassoconstriction (HPV) serves to increase pulmonary vascular resistance and divert the circulation through the ductus arteriosus. The foetal pulmonary circulation therefore only receives $\sim$10% of the cardiac output unlike the situation after birth where exposure to atmospheric oxygen fully dilates the pulmonary circulation which henceforth receives 100% of the cardiac output. After birth, HPV is an important negative feedback mechanism required for ventilation-perfusion matching. Since its first description the mechanism of HPV has been investigated but still remains largely unsolved. Recent hypotheses include hypoxia-induced inactivation of voltage activated potassium (Kv) channels in the pulmonary circulation (Smirnov *et al*., 1994; Weir & Archer, 1995).

There is great heterogeneity in the phenotype of pulmonary vascular SMCs (Frid *et al*., 1994). This varies with the size and location of the pulmonary artery. Different phenotypes respond differentially to vasoactive factors and exhibit differential pharmacology. For example, K^+ channels are differentially distributed (Michelakis, 1997) and there are cell-specific differences in the endothelin-1 (ET-1) system (Tchekneva *et al*., 2000). The vasoconstrictor response to ET-1 is mediated by ET_A receptors in large pulmonary arteries but by ET_B-like receptors in smaller muscular pulmonary arteries (MacLean *et al*., 1994a; McCulloch *et al*., 1996).

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Hence there are many differences between the normal pulmonary and systemic arterial ciculations which should predict differential pharmacology and vascular reactivity. Changes occur in the pulmonary hypertensive lung which further alters pulmonary arterial pharmacology and reactivity.

Pulmonary arterial hypertension

Primary PAH (PPH) is the clinical term used to describe a rare condition associated with progressive elevation in pulmonary arterial pressure (by 10–15 mmHg), associated with pulmonary vascular remodelling, for which no underlying cause can be found. PAH occurs more commonly secondary to collagen vascular disease, congenical systemic to pulmonary shunt, portal hypertension, human immunodeficiency virus infection, chronic obstructive lung diseases, interstitial fibrosis and high left-sided filling pressures. A familial form of PAH has also been described and characterized with linkage to chromosome 2q31-q32. It is an autosomal dominant disease with incomplete penetrance and genetic anticipation (Nichols *et al*., 1997).

Both secondary PAH and PPH share common pathobiologies. The earliest change is the muscularization of the terminal portion of the pulmonary arterial vascular tree (Figures 1 and 2). This is caused by hyperplasia of smooth muscle cells (SMCs) which extend distally in a layer to the original elastic lamina (Heath *et al*., 1987). The chronic hypoxic rat is a commonly studied model of pulmonary hypertension where rats are maintained in hypoxic conditions for, typically, up to 3 weeks. Figure 2 demonstrates muscularization of small pulmonary arteries in the chronic hypoxic rat lung. It should be noted, however, that the pattern of SMC migration observed in hypoxic PAH is different from that observed in plexogenic PAH (characteristic of PPH; Heath, 1992). In PPH, the migration is more widespread and plexogenic lesions occlude the vascular lumen. In hypoxic PAH, there is more limited migration and occlusive lesions are rare. However cellular intimal proliferation exists in both hypoxic PAH and PPH. Importantly, regardless of the aetiology, the vascular changes render the pulmonary circulation resistant to standard vasodilators relative to the systemic circulation. Hence, the use of these for PAH is limited by systemic hypotension.

PAH exhibits a very complex pathobiology with many other factors influencing both vascular remodelling and reactivity. Inactivation of voltage regulated potassium channels (K_V) by hypoxia or anorexigens can cause vasoconstriction and defects in K_V channels have been implicated in PAH (Yuan *et al*., 1998). Altered endothelial cell function is also thought to play a role in PAH. For example, there is a loss in the prostacyclin synthase enzyme in PAH patients (Tuder *et al*., 1999) and there may also be a decreased production of nitric oxide in severe PAH (Giaid & Saleh, 1995). Phenotypically altered endothelial cells may lead to 'misguided angiogenesis' resulting in hypertrophy and hyperplasia (Lee *et al*., 1998). Endothelial cells, aggravated by increased shear stress, may also release mediators that induce vascular SMC growth, such as vascular endothelial growth factor (Cool *et al*., 1999). Persistent matrix protein synthesis is observed in PAH and there is an increase in the production of endogenous vascular elastase in pulmonary vascular SMCs which may also lead to SMC migration (Rabinovitch, 1999). Mediators of inflammation can also cause pulmonary artery cell growth and vasoconstriction. In patients with PPH there are increased levels of transforming growth factor-β, interleukin-1 and 6 and over expression of 5-lipoxygenase activating protein leading to production of inflamatory leukotrienes (Wright *et al*., 1998). Abnormalities in platelet activation and function in PAH also support a potential role of thrombosis in the initiation of PAH. Hence PAH exhibits a multifactorial and complex pathobiology.

In the normal lung, low pulmonary vascular tone is regulated by a balance between the effects of vasodilators/antiproliferative agents such as prostacyclin and nitric oxide and vasoconstrictors/co-mitogens such as 5-HT and ET-1 (Fishman, 1998; MacLean, 1999a). The increase in vascular tone observed in PAH may be due to decreased levels of cyclic GMP and cyclic AMP caused by increased phosphodiesterase activity (MacLean *et al*., 1996b; 1997), depolarization by inactivation of K^+ channels (Osipenko *et al*., 1998) as well as increased levels of endogenous vasoconstrictors such as angiotensin II (Cargill & Lipworth, 1995), thromboxane A_2 (Christman, 1998), ET-1 (Stewart *et al*., 1991) and 5-HT.

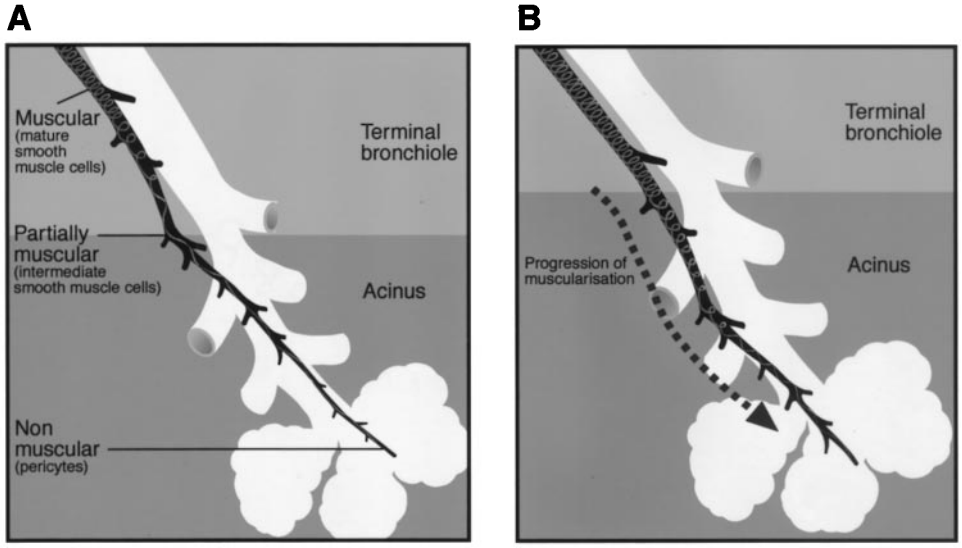

Figure 1 Diagrammatic representation of the pulmonary arteries within the lung. (A) In the normal lung there is an uneven distribution of smooth muscle phenotypes and numerous non-muscular precapillary vessels (see Figure 2). (B) In the pulmonary hypertensive lung there is progression of muscularization into the non-muscular terminal portion of the arterial tree. This is due to hyperplasia and redistribution of smooth muscle cell phenotypes (see text and Figure 2 for details).

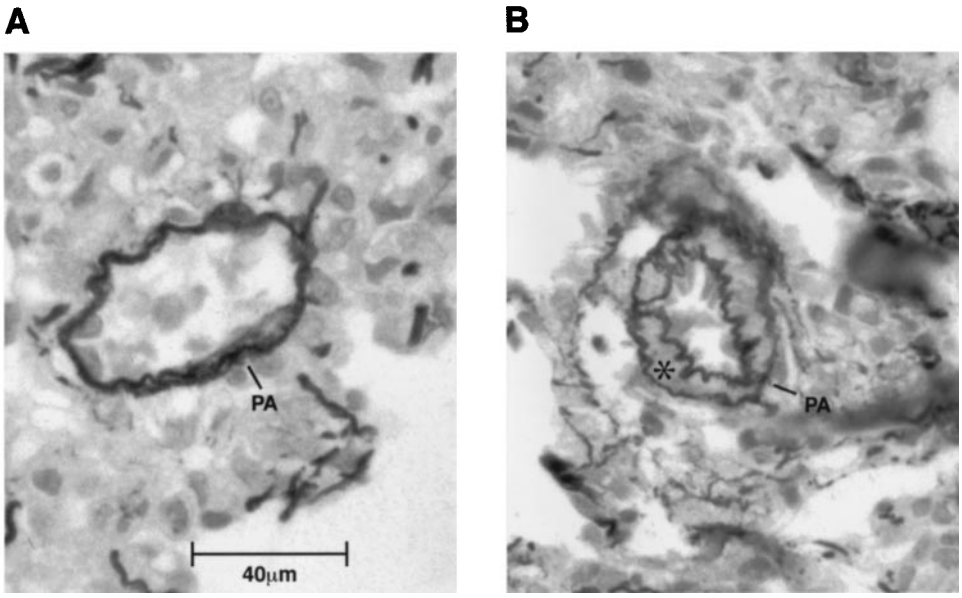

Figure 2 Remodelling of precapillary pulmonary arterioles (PAs) in rat lung following development of pulmonary hypertension. (A) Normal rat lung. Note absence of medial layer. (B) In rats exposed to 2 weeks of hypobaric hypoxia with associated pulmonary hypertension. *Note two elastic laminae separated by distinct medial layer.

5-HT and pulmonary arterial hypertension

5-HT may promote pulmonary vascular SMC proliferation, pulmonary arterial vasoconstriction, and local microthrombosis (Fanburg & Lee, 1997). Alterations in 5-HT turnover leading to an increased availability of free 5-HT in the vicinity of the pulmonary artery wall has been proposed as a potential important pathophysiologic process (see *The World Symposium on Primary Pulmonary Hypertension*, 1998, http://www.who.int/ncd/cvd/pph/html).

Under normal conditions, the lung vascular bed is not exposed to excessive 5-HT levels, because of its position as a secondary filter located downstream from the liver and because of the ability of platelets to store large amounts of 5-HT. There is evidence that alterations in platelet 5-HT storage and/or increased platelet consumption by the lung may, however, trigger the development of primary PAH: (1) Decreased platelet 5-HT storage with enhanced plasma concentration of free 5-HT has been reported in platelet storage pool disease (Hervé *et al*., 1990), and in numerous disorders occasionally associated with PAH, including anorexigen intake, portal hypertension (Laffi *et al*., 1992; Liu & Lee, 1999), Raynaud's phenomenon and collagen vascular disease (Hervé *et al*., 1995). However, no link between plasma 5-HT levels and PAH has been definitively proven. Indeed, in mice deficient for the 5-HT transporter gene, where platelets are depleted of 5-HT, the development of chronic hypoxia-induced pulmonary vascular remodelling is reduced (Eddahibi *et al*., 2000a). (2) Platelet 5-HT storage remains impaired in PPH patients after heart-lung transplantation (Hervé *et al*., 1995), whereas it is normal in patients with secondary PAH (Breuer *et al*., 1996), or after long term prostacyclin treatment (Humbert *et al*., 1998), indicating that this platelet dysfunction is not secondary to the pulmonary vascular disease. (3) The fawn-hooded rat, which has a genetic defect in 5-HT platelet storage, develops severe PAH upon exposure to modest hypoxia (Ashmore *et al*., 1991). (4) Platelet count is low in patients with PPH (Eddahibi *et al*., 2000b).

Diet pill-induced PAH

An association between the anorexigen aminorex and PAH was observed in the 1960s and aminorex was later withdrawn (Kay *et al*., 1971). Later, In the 1980s, primary PAH was associated with fenfluramine use and it was subsequently shown that PAH occurs approx 30 times more frequently in patients receiving these anorectic agents for more than 3 months compared to the general population (Abenhaim *et al*., 1996; Fishman, 1999). In the US, dexfenfluramine was widely co-prescribed with phentermine, a combination which became known as 'fen/phen'. Phentermine can inhibit monoamine oxidase B, an action which could potentially inhibit the metabolism of 5-HT hence increasing local and plasma levels of 5-HT. In addition, phentermine has been shown to prolong the vasoconstrictor effects of 5-HT in rat lung (Seiler *et al*., 1976). Fenfluramine, in association or not with phentermine, has been shown recently to induce valvular heart disease very similar to those observed after exposure to 5-HT-like drugs such as ergotamine and methysergide, and with increased 5-HT levels associated with carcinoid disease (Connolly *et al*., 1997). There is certainly evidence suggesting a synergistic interaction between fenfluramine and phentermine to favour PAH and cardiac valve disease (Wellman & Maher, 1999). In addition, fenfluramines may precipitate secondary forms of PAH (Rich *et al*., 2000). The epidemy of PAH and valvular heart disease associated with fenfluramine intake led to its withdrawal by both the FDA and European drug agency in 1998.

By interacting with the 5-HTT, fenfluramine releases 5-HT from platelets and inhibits its reuptake into platelet and pulmonary endothelial cells (Fristrom *et al*., 1977; Buczko *et al*., 1975). As a consequence circulating free 5-HT concentration increases with fenfluramine treatment (Martin & Artigas, 1992). Plasma (platelet poor) 5-HT values increase after dexfenfluramine treatment in rats (Eddahibi *et al*., 1998). All these observations suggest that fenfluramine may trigger PAH by aggravating or inducing an impairment in the platelet 5-HT storage.

5-HT may be implicated in both the vasoconstrictor and remodelling aspects of the disease.

5-HT and pulmonary vascular remodelling

As discussed, proliferation of SMCs is an important component of pulmonary arterial remodelling, which accounts for the increased thickness of the medial muscular coat in normally muscularized arteries and extension of muscle into smaller and more peripheral arteries (Rabinovitch *et al*., 1979). The mechanisms leading to pulmonary SMCs proliferation, however, are not well understood.

5-HT is a known mitogen for SMCs isolated from bovine, porcine, and rat aorta as well as rat and bovine pulmonary arteries (Lee *et al*., 1991; Nemecek *et al*., 1986; Pitt *et al*., 1994). The mechanism by which 5-HT causes SMC proliferation varies with cell types and species. Several studies have concluded that the mitogenic action of 5-HT is initiated through its binding to a cell surface receptor, notably the 5-HT_{2A} type (Pitt *et al*., 1994), whereas evidence has also been provided that it results from an energy-dependent transport of 5-HT into the cell (Lee *et al*., 1991). 5-HT is a potent inducer of bovine and rat pulmonary artery SMC proliferation and this effect is dose-dependently inhibited by highly selective inhibitors of 5-HT transport such as paroxetine and fluoxetine (Lee *et al*., 1991; Eddahibi *et al*., 1999b), but not by the 5-HT_{2A} receptor antagonist ketanserin. In rat vascular SMCs, fluoxetine and paroxetine inhibited [^{3}H]5-HT uptake and 5-HT-induced cell proliferation at similar concentrations suggesting that both phenomena are tightly related (Eddahibi *et al*., 1999b). The proliferative response of bovine pulmonary vascular SMCs to 5-HT is also inhibited by agents that block the transport of 5-HT but not by 5-HT receptor antagonists (Lee *et al*., 1991).

At this time, the mechanisms by which 5-HT exerts its mitogenic effect after being transported inside SMCs, remain speculative. Lee *et al*. (1997) observed that 5-HT-induced DNA synthesis is associated with tyrosine phosphorylation of GTPase-activating protein and that both effects are blocked by 5-HT transport or tyrosine kinase inhibitors (Lee *et al*., 1997). Therefore, although 5-HT induced mitogenesis in SMCs requires cellular internalization through the 5-HTT rather than binding to a membrane receptor, tyrosine phosphorylation of GTPase-activating protein appears as a downstream intermediate in the signalling pathway. Recently, involvement of superoxide anions formation in association with 5-HT transport has also been suggested to play a role in the mitogenic effects of 5-HT (Lee *et al*., 1998). 5-HT_{2A} receptors have been shown to induce proliferation *via* the phosphatidylcholine-specific phospholipase C/mitogen activating protein kinase pathway in rat mesangial cells (Goppelt-Struebe & Stroebel, 1998) but this has not been confirmed in pulmonary vascular SMCs.

Hypoxia-induced 5-HTT gene expression in vitro *and* in vivo

Exposure of pulmonary artery SMCs to hypoxia results in a rapid and transient increase in the level of 5-HTT mRNA, followed by a prolonged 2.5–3.0-fold increase in 5-HT transport activity (Eddahibi *et al*., 1999b). The increased 5-HTT mRNA accumulation is directly related to an increased transcription rate of the 5-HTT gene. Thus, in cells transfected with a luciferase-reporter gene construct containing the human 5-HTT promoter, exposure to hypoxia is associated with a marked increase in luciferase activity. This effect on the 5-HTT promoter is specific, as when the cells are transfected with the same luciferase reporter gene under the dependence of the SV40 promoter, hypoxia did not change luciferase activity (Eddahibi *et al*., 1999b). One mechanism of hypoxia-induced gene expression involves the transcription factor 'hypoxia inducible factor' or HIF-1, that binds to identified hypoxia-sensitive elements in the promoter of several hypoxia-inducible genes (Semenza *et al*., 1996; Wang & Semenza, 1993). The consensus sequence of these elements is 5′-TACGTGCT-3′. Interestingly, two core sequences 5′-CGTG-3′ are present in the promoter region of the 5-HTT gene. Accordingly, it can be inferred that hypoxia increases the transcription rate of the 5-HTT gene through binding of HIF-1 to one of these hypoxia-sensitive elements.

An increase in 5-HTT mRNA levels has also been observed in the lung from rats exposed to chronic hypoxia. Hybridization studies showed that the 5-HTT transcript was predominantly located in the media of newly remodeled distal pulmonary arteries in pulmonary hypertensive rats (Eddahibi *et al*., 1999b). Basal 5-HTT expression was absent in proximal pulmonary arteries of control normoxic rats whereas 5-HTT transcripts could be easily detected in the same tissues of hypoxic rats. These data suggest that *in vivo* pulmonary artery SMC from large pulmonary arteries do not phenotypically express 5-HTT under normoxic conditions, and that induction occurs in response to hypoxia. In that case, one would infer that 5-HT may behave as a mitogenic factor for pulmonary artery SMC only during conditions of increased 5-HTT mRNA expression.

5-HTT and pulmonary vascular remodelling

The fact that 5-HT favours the development of pulmonary hypertension through 5-HTT activity is supported by several observations: (1) continuous intravenous infusion of the 5-HT during a 2-week exposure to hypoxia aggravates pulmonary hypertension in rats (Eddahibi *et al*., 1997). This aggravating effect of 5-HT infusion on hypoxic pulmonary hypertension is prevented when rats are treated short term with dexfenfluramine, an inhibitor of 5-HT transport (Eddahibi *et al*., 1998). Moreover, the mitogenic effects of 5-HT in cultured pulmonary artery SMCs are prevented by various inhibitors of 5-HT uptake such as fluoxetine, paroxetine and citalopram (Eddahibi *et al*., 1999b). Hence, evidence suggests that internalization of 5-HT through 5-HTT is essential for its *in vivo* mitogenic effect in hypoxia; (2) 5-HTT is encoded by a single gene expressed in several cell types such as neurons, platelets, pulmonary vascular endothelial and SMCs (Lesch *et al*., 1994). In mice lacking the 5-HTT gene and exposed to hypoxia for 2 weeks, the number and the wall thickness of muscularized pulmonary arteries were decreased as compared with wild-type controls (Eddahibi *et al*., 2000a). Concomitantly, the pulmonary artery pressure was lower and the right ventricle less hypertrophied in hypoxic 5-HTT$-/-$ mutants than in wild-type mice. These preliminary observations that hypoxic pulmonary hypertension is impaired in 5-HTT$-/-$ mice provides further evidence that 5-HTT plays a major role in hypoxia-induced vascular remodelling through its ability to mediate the mitogenic action of 5-HT.

5-HTT: target for drugs that increase the risk of PAH

Chronic dexfenfluramine treatment may affect lung expression of the 5-HT transporter. Previous studies have shown

that 5-HTT levels and activity in 5-HTergic neurons can be modulated by hormones and pharmacological agents. 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 (Semple-Rowland *et al*., 1996). However, the effect of chronic dexfenfluramine treatment on 5-HTT expression in lung tissue has not been investigated. Preliminary results obtained in rats showed that discontinuation of a 30-day dexfenfluramine treatment (2 mg kg^{-1} day) is followed by a transient increase in 5-HTT mRNA levels in lung tissues, whereas no alterations in the levels of this transcript were noted during the treatment (Eddahibi *et al*., 2000c). Therefore, one reasonable hypothesis is that over-expression of 5-HTT may result from chronic treatment with dexfenfluramine, thereby favouring or

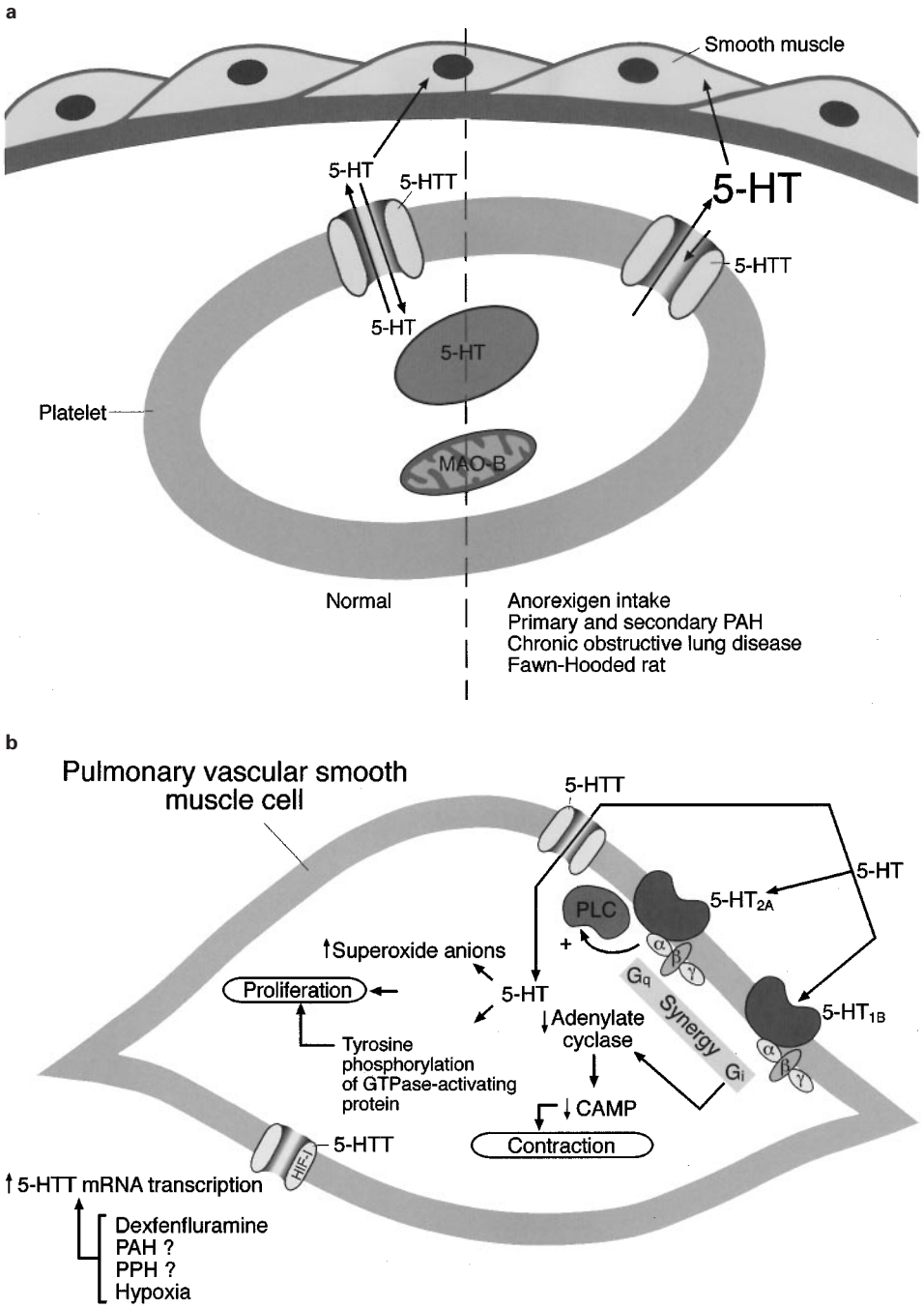

Figure 3 The 5-HT Hypothesis of pulmonary arterial hypertension. (A) Platelet handling of 5-HT. 5-HT uptake by platelets through the 5-HTT may be compromised after anorexigen intake, in primary and secondary pulmonary arterial hypertension, in chronic obstructive lung disease and in the fawn-hooded rat which is genetically pre-disposed to pulmonary arterial hypertension. Release of platelet 5-HT may be enhanced by certain anorexigens. This, and perhaps increased accumulation of platelets in the lung, would lead to increased exposure of pulmonary vascular smooth muscle cells to 5-HT. (B) 5-HT and human pulmonary vascular smooth muscle cells. 5-HT can cause pulmonary vascular smooth muscle cell contraction and proliferation. Proliferation occurs through 5-HT uptake by the 5-HTT and subsequent stimulation of superoxide anions and increased tyrosine phosphorylation of GTPase-activating protein. Increased 5-HT exposure combined with increased 5-HTT would potentiate these effects. Increased 5-HTT activity may be secondary to increased 5-HTT transcription stimulated by anorexigens, hypoxia (*via* HIF-1 activation) and in PPH and secondary PAH. Contraction occurs through 5-HT_{1B} receptor (G_i-coupled) activation which can be enhanced by decreased cyclic GMP levels and through synergy with G_q-coupled receptor activation.

potentiating pulmonary artery SMC proliferation in response to various stimuli.

5-HTT and human pulmonary hypertension

Recently, marked increases in 5-HTT binding and 5-HTT activity in platelets from patients with primary and secondary forms of pulmonary hypertension as well as in patients with chronic obstructive lung disease have been observed (Eddahibi *et al*., 1999a). Moreover, increased 5-HTT mRNA were observed in lung specimens from patients with primary PAH who underwent lung transplantation. Although these results need to be confirmed by further studies, they suggest that human pulmonary hypertension is associated with platelets and/or pulmonary artery SMC 5-HTT overexpression.

5-HT and pulmonary vasoconstriction

Until recently it was assumed that the 5-HT_{2A} receptor mediates pulmonary arterial contraction. Indeed, under control conditions, the 5-HT_{2A} receptor mediates contraction in the rat, cow and dog pulmonary arteries (Chand & Altura, 1980; MacLean *et al*., 1994b; 1996b). However, emphasizing the importance of comparative studies in human tissues, recent evidence suggests that the 5-HT_{1B} receptor mediates contraction in human pulmonary arteries. The 5-$HT_{1D/1B}$ receptor agonist sumatriptan demonstrates a potent vasopressor response in the pulmonary circulation in man (McIntyre *et al*., 1992) and constricts human large isolated pulmonary arteries where vasoconstriction is mediated by the 5-$HT_{1B/1D}$ receptor (MacLean *et al*., 1996a). Recently, it has also been shown that the 5-HT_{1B} receptor that plays a major role in mediating vasoconstriction in the human isolated small muscular pulmonary arteries (Morecroft *et al*., 1999). These findings explain why ketanserin, the 5-HT_{2A} receptor antagonist, has been of limited use in the treatment of PAH. For example in PAH secondary to platelet storage pool disease or chronic obstructive pulmonary disease ketanserin is ineffective or its use limited due to the induction of systemic hypotension (Hamet *et al*., 1985; Hervé *et al*., 1995; Domenighetti *et al*., 1997). 5-HT_{1B} receptor antagonists may prove to be more pulmonary selective as it is the 5-HT_{2A} receptor that mediates vasoconstriction in most systemic arteries.

Pulmonary arterial responses to 5-HT are enhanced in the chronic hypoxic rat model of PAH (MacLean *et al*., 1996b). Pulmonary vasoconstriction to 5-HT is normally mediated entirely by the 5-HT_{2A} receptor in the rat. However, in chronic hypoxic rats, the increased vasoconstriction to 5-HT is mediated by both the 5-HT_{2A} and 5-HT_{1B} receptor (MacLean *et al*., 1996b). Indeed, the mRNA for the 5-HT_{1B} receptor is increased in the pulmonary arteries from these rats (Heeley *et al*., 1998). Chronic treatment with the 5-$HT_{1B/D}$ antagonist GR127935 significantly reduces the development of right ventricular hypertrophy in these chronic hypoxic rats (Keegan *et al*., 2000). Collectively, therefore, evidence accumulated from both rat and human studies suggests that the 5-HT_{1B} receptor is important in mediating pulmonary vasoconstriction and it may contribute significantly to the increased vasoconstrictor response to 5-HT observed in PAH.

Vasoconstrictor responses to 5-HT_1 receptor stimulation are often only observed in the presence of agonist-induced vascular tone or decreased cyclic GMP levels, a phenomenon often termed 'pharmacological synergy' (MacLean *et al*., 1994b; Sweeney *et al*., 1995). This phenomenon may contribute to the increased vasoconstrictor response to 5-HT_{1B}-receptor stimulation in PAH where the increased endogenous tone, G_i-/G_q-protein synergy and decreased cyclic GMP levels prevail. This has been described recently in a recent review (MacLean, 1999b).

Taken together, recent studies therefore suggest that selective 5-HT_{1B}-receptor antagonists may be useful in reducing the pulmonary vasoconstriction associated with PAH, avoiding the systemic hypotension which so often limits vasodilator therapy.

Conclusion

In summary and conclusion, evidence suggests that there are critical changes in platelet handling of 5-HT, and perhaps handling of platelets by the lung, combined with changes in the pulmonary vascular smooth muscle response to 5-HT, which can lead to PAH. In PPH, PAH, or after anorexigen intake, there may be deficiencies in 5-HT re-uptake into platelets which would lead to increased circulating or local 5-HT levels. Where 5-HT release from platelets is stimulated (for example in the presence of dexfenfluramine) or monoamine oxidase-B activity is compromised, this effect may be amplified. Increased local levels of 5-HT, induced by platelet release or increased accumulation of platelets in the lung, would expose pulmonary vascular SMCs to elevated levels of 5-HT. The effect of this would be to increase vascular pulmonary smooth muscle contraction and proliferation. Contraction would result from increased stimulation of the 5-HT_{1B} receptor, an effect which would be potentiated in PAH by increased vascular tone, increased G_q-/G_i- protein synergy and decreased cyclic GMP levels. Current evidence suggests that 5-HT-induced proliferation occurs primarily through 5-HT uptake by the 5-HTT. In hypoxia, after anorexigen intake, and perhaps in genetically predisposed PPH patients and in secondary PAH, increased expression of 5-HTT may lead to further increases in the transport of 5-HT into the SMCs and increased proliferation. This current hypothesis is illustrated in Figure 3.

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