Mann DA, Oakley F. *Serotonin paracrine signaling in tissue fibrosis.*
*Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 2013, 1832(7), 905-910.

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Link to published article:
http://dx.doi.org/10.1016/j.bbadis.2012.09.009

Date deposited: 2\textsuperscript{nd} December 2014

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1. Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is an ancient signaling molecule which is found in simple single cell eukaryotes such as Paramecium and Tetrahymena where it regulates growth and swim behavior [1]. 5-HT is also found in molds and plants as well as higher order multicellular organisms such as worms and insects where it controls diverse physiological functions such as swimming, ovulation and insulin secretion [2-4]. In humans 5-HT is perhaps best characterized for its role in the central nervous system (CNS) where it operates as a neurotransmitter at neuronal synapses in influencing a broad range of neurophysiological functions including learning and memory, mood, pain and appetite. However, the majority of 5-HT is found outside of the CNS with the major site of synthesis in enterochromaffin cells of the gut where it helps to control smooth muscle contraction and digestion of food. 5-HT can be taken up from the serum by platelets and mast cells via the serotonin transporter (SERT). These cells transport 5-HT to a wide number of tissues and importantly will accumulate within injured tissues releasing 5-HT upon appropriate stimulation. Functions of 5-HT outside the CNS include vasoconstriction/vasodilation, cardiac development and function, respiratory drive, metabolic rate and temperature control, mammary gland development and milk release, uterine contraction, Oocyte maturation and in males the control of penile flaccidity and detumescence [5].

The ability of 5-HT to influence such a wide variety of CNS and systemic physiological functions can be attributed to its diverse receptor system. The 5-HT receptor family consists of 13 distinct genes encoding G-protein coupled seven-transmembrane receptors (GPCRs) and in addition 1 ligand-gated ion channel (the 5-HT3 receptor). The GPCRs are divided into three major groupings according to whether they signal by coupling to Gαq (5-HT2A, 5-HT2B and 5-HT2C), Gαi/o (5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F and 5-HT5A/B) or Gαs (5-HT4, 5-HT6 and 5-HT7) G proteins [6]. However, it should be noted that GPCRs including the 5-HT receptors are able to couple to more than one G-protein and to a variety of other types of intracellular signaling molecules. Focusing on the three G-proteins alone, each is able to trigger distinct downstream signaling events, which explains the diverse range of physiological responses that are attributed to 5-HT, Gαq coupled receptors stimulate the formation of diacylglycerol (DAG) and inositol phosphates leading to activation of protein kinase C (PKC) and elevation of intracellular calcium [7,8]. In addition Gαi/o-coupled 5-HT receptors activate Rho resulting in formation of stress fibers and focal adhesions which influences cell migration and adhesion [9]. Activation of the Gαs class of 5-HT receptor causes suppression of adenyl cyclase and reduced levels of cAMP which in neurons results in suppression of neuronal firing [6]. Conversely the activation of Gαq-coupled 5-HT receptors stimulates adenylyl cyclases promoting the accumulation of cAMP and downstream activation of protein kinase A (PKA) and the cAMP response element binding protein (CREB). It is therefore critical to
take into account the importance of cellular context and the expression profile of the 5-HT receptors when considering the physiological impact of 5-HT on normal and pathological tissue processes.

2. Serotonin in wound healing

Wound healing is a highly complex coordinated process that can be separated into four distinct phases beginning immediately after injury with (i) coagulation and homeostasis, which is rapidly followed by (ii) inflammation and later by (iii) a fibroproliferative process characterized by scar tissue formation which can eventually resolve over weeks/months by (iv) full restorative regeneration of functional tissue depending on the severity of the wound and the tissue. Platelets obviously play a central role in coagulation and the aggregation of platelets at sites of tissue damage leads to the release of 5-HT which then contributes to homeostasis through its powerful vasoactive properties. Serotonin can either stimulate constriction or dilation of microvasculature depending on the tissue in question. In the liver, 5-HT appears to mainly promote constriction of hepatic sinusoids, since mice lacking peripheral serotonin (Tph−/−) display enhanced sinusoidal perfusion under both normal and diseased states [10]. By contrast, platelet-derived 5-HT coordinates the formation of gaps between endothelial cells in the joint microvasculature, which in arthritic conditions may contribute to inflammation [11]. Precisely how these differential responses are regulated is not well defined but presumably involves differential signaling via specific 5-HT receptors expressed on vascular endothelial and smooth muscle cells.

Beyond its homeostatic role which contributes to recruitment and retention of leukocytes at sites of injury, functions for 5-HT in the inflammatory response are not well defined; however it is clear that 5-HT can influence the behavior and function of many types of immune cell. 5-HT has chemotactic actions on mast cells and eosinophils [12,13] and mature dendritic cells (DCs) respond to 5-HT via 5-HT3, 5-HT4, and 5-HT7 receptors to increase their expression of IL-6 [14,15]. Human monocytes primed with LPS respond to 5-HT via 5-HT1F, 5-HT2A and 5-HT2F receptor subtypes to increase their secretion of IL-1β [16]. 5-HT also inhibits monocyte apoptosis via the 5-HT1A and 5-HT7 receptors allowing monocytes to persist in tissues and to promote inflammation [17]. Tph1−/− mice are less susceptible to experimental colitis, which is associated with depressed cytokine expression and macrophage infiltration to the bowel suggesting a key role for 5-HT in GI inflammation [18]. T cells display a concentration dependent response to 5-HT, with low levels stimulating proliferation and IL-2 expression [19], whereas high concentrations inhibit the mitogenic stimulation of T cell proliferation and IL-2 receptor expression [20]. Tph1−/− mice are less susceptible to steatosis-induced hepatic inflammation, although in this case there was no influence of 5-HT on cytokine expression and instead the inflammatory properties of 5-HT were attributed to production of intracellular ROS in hepatocytes following 5-HT uptake and degradation. As IL-1β and TNF-α can induce the expression of the serotonin transporter SERT [21], uptake of 5-HT and production of ROS may be part of an inflammatory positive feedback mechanism.

3. Serotonin, a stimulator of tissue fibrosis

Mechanistic links between fibrosis and 5-HT were first reported in the 1960s for the condition called carcinoid syndrome, which is caused by neuroendocrine carcinoid tumors that secrete vast quantities of 5-HT [22]. The syndrome is characterized by tissue fibrosis that particularly affects cardiac valves but also impacts on other organs including the lung and skin [23,24]. In the 1980s it was determined that retroperitoneal fibrosis caused by the ergot methysergide is due to the metabolism of this compound into methylergonovine which converts it from a 5-HT2B receptor antagonist to agonist [25]. Subsequently agonism of 5-HT2B has been implicated in fibrosis caused by fenfluramine used in the treatment of obesity [26] and psychiatric disorders [27] and the hallucinogen MDMA [28], both of which trigger 5-HT2B signaling. Dopamine agonists with structural similarity to 5-HT such as pergolide and cabergoline that are used in the treatment of Parkinson’s disease have also been associated with development of fibrosis in heart valves involving 5-HT2B agonism, thus limiting their clinical utility [29]. Studies with animal models have confirmed the necessity to screen serotonergic compounds for activation of 5-HT2B activation and promotion of valvulopathy [30].

4. Serotonin signaling in hepatic regeneration and fibrosis

The adult mammalian liver is a highly regenerative organ. It is capable of rapidly and effectively restoring lost liver mass and rebuilding complex tissue structures such as hepatic sinusoids and bile ducts that are vital for normal liver function. Recent investigations employing experimental rodent models of liver regeneration have led to the discovery of autocrine and paracrine hepatic 5-HT signaling pathways that help to regulate the growth and regeneration of parenchymal liver cells. Moreover there appears to be important cross-talk between these 5-HT-driven epithelial cell growth mechanisms and 5-HT signaling pathways that act on myofibroblasts to stimulate hepatic fibrosis. Since there is growing evidence of an inverse relationship between the efficiency of epithelial regeneration and development of tissue fibrosis, manipulating 5-HT pathways that cross-talk between these processes may offer therapeutic opportunities in chronic liver disease.

Seventy percent partial hepatectomy (PHx) provides a model of liver regeneration in which lost mass and tissue architecture is completely restored within 14 days [31]. Intestinal 5-HT is rapidly mobilized and accumulates in the remnant liver following PHx. Early studies demonstrated enhanced regeneration of hepatocyte proliferation following administration of 5-HT to PHx mice and indicated a requirement for 5-HT1A/1B and 5-HT2B receptors, although these early studies suffered from limited availability of receptor-specific agonists and antagonists. In 2006, Lesurtel et al. identified platelets as the primary source of 5-HT in the regenerating liver and demonstrated profound impairment of hepatocyte regeneration in Tph1−/− mice recovery from PHx [32]. Further experiments suggested a role for 5-HT2A, and to a lesser extent 5-HT2B receptors as mediators of 5-HT-driven hepatocyte regeneration. Subsequent studies by the same group of investigators have confirmed a pro-inflammatory and pro-regenerative role for 5-HT in post-ischemic liver repair [33], they discovered that serotonin agonism of 5-HT2B receptors improves animal survival in small liver graft transplantation [34] and age-associated impairments in regenerative capacity [35]. However, of note a different team of investigators working with SERT-deficient rats which lack platelet-derived 5-HT due to absence of uptake from the gut failed to demonstrate a role for this source of 5-HT in liver regeneration [36]. This latter finding may suggest that low levels of free 5-HT in the serum or possibly 5-HT produced by cells resident in the liver may be involved. In this regard a recent study by Omenetti and colleagues is of interest since it reported an unexpected source and role for 5-HT in the hepatic biliary tract [37]. Cholangiocytes are epithelial cells of the bile duct, which have a variety of roles in liver homeostasis and immunity. The study by Omenetti revealed that cholangiocytes express neural-restricted TPH2 and can produce 5-HT, which upon secretion represses cholangiocyte proliferation by a negative feedback mechanism. However, in response to biliary injury 5-HT triggers production of TGFβ1 by wound healing myofibroblasts and in turn soluble TGFβ1 acts on cholangiocytes to repress TPH2 expression and enable their proliferation. It will be interesting to learn which 5-HT receptor subtypes transmit the growth suppressive effects of 5-HT on cholangiocytes given that hepatocytes predominantly utilize 5-HT2A to stimulate their proliferation. It will
also be of interest to determine if cholangiocyte-derived 5-HT contributes to hepatocyte regeneration following liver damage.

In 2006 our group reported that sinusoidal hepatic stellate cells (HSC) strongly upregulate expression of 5-HT₂A and 5-HT₂B upon their transdifferentiation to a myofibroblast phenotype [38]. HSC are the major contributor to fibrogenesis in liver disease, producing vast quantities of extracellular matrix and the collagenase inhibitor TIMP-1, as such they are considered important targets for prevention and treatment of fibrosis in chronic liver disease. HSC also express SERT and are able to uptake, release and respond to 5-HT by this autocrine route as well as via paracrine routes such as platelet-derived and cholangiocyte-derived 5-HT [38]. 5-HT₄ receptor-selective antagonists inhibit HSC proliferation and induce apoptosis which implicates 5-HT/5-HT₂ signaling in the regulation of fibrosis, since the balance between myofibroblast proliferation and apoptosis is an important determinant of fibrosis progression. Taking this work further, we have more recently proposed that signaling through the 5-HT₂B receptor on HSC-derived myofibroblasts is both pro-fibrogenic and anti-regenerative in the diseased liver [39]. Specific genetic (5-HT₂B knockout) or pharmacological blockade of 5-HT₂B stimulates hepatoocyte proliferation and suppresses fibrosis in multiple distinct injury models. A common pathway may be responsible for these dual functions of 5-HT/5-HT₂ signaling since it triggers ERK- and JunD-dependent activation of TGFβ₁ expression, the latter being a potent suppressor of hepatocyte proliferation as well as being a powerful stimulator of fibrogenic gene expression [40,41].

As illustrated in Fig. 1, based on current available knowledge there is substantial complexity in the mechanisms by which 5-HT influences liver injury and repair, with multiple effects on different cell types and the activation of cell-cell signaling pathways that link epithelial cell regeneration and fibrosis. At present, the available data suggest that 5-HT/5-HT₂ signaling in hepatocytes is pro-regenerative whereas 5-HT/5-HT₂ signaling via HSC in a fibrogenic microenvironment is anti-regenerative. However, this current model may be refined by the future availability of more specific 5-HT receptor agonists and antagonist, and conditional genetic targeting systems that allow 5-HT receptor genes to be deleted in each of the relevant parenchymal and non-parenchymal liver cells.

5. Serotonin signaling in the lung

5-HT has been linked to pulmonary fibrosis since the 1960s when it was documented that the headache medicine methysergide, which has close structural similarities with 5-HT, caused pleuro-pulmonary fibrosis [42]. Fibrosis is a feature of many different types of chronic respiratory diseases including idiopathic pulmonary fibrosis (IPF), pulmonary arterial hypertension (PAH), chronic obstructive pulmonary disease (COPD), post-transplant bronchial obliterator syndrome (BOS) and asthma [43]. In addition, lung fibrosis is a serious pathological component of systemic sclerosis [44] and can also be caused by a variety of medications (e.g. methotrexate) [45] as well as radiation therapy to the chest [46].

The role of 5-HT in these different respiratory pathologies has not been fully investigated; however evidence is emerging that it can be secreted locally by many different cell types and in addition to its powerful vasoactive effects on pulmonary arteries, 5-HT can stimulate the proliferation and fibrogenic actions of lung (myo)fibroblasts [47,48]. Importantly, platelets and mast cells are not the only source of 5-HT in the respiratory system. In addition, to recruited platelets and mast cells resident pulmonary neuroendocrine cells synthesize and secrete 5-HT [49]. These neuroendocrine cells become proliferative in PAH and this correlates with proliferation of myofibroblasts in the pulmonary arteries. In some conditions, such as acute post-operative PAH in children it is suggested that proliferating pulmonary neuroendocrine cells may be the major source of 5-HT. Expression of Tph1 is found in pulmonary epithelial cells and levels are increased in patients with idiopathic PAH [50]. Noteworthy is that SERT is particularly highly expressed in the lung and has been postulated to be involved in promoting the proliferation of pulmonary arterial fibroblasts and pulmonary arterial smooth muscle cells via the activation of ERK and ROS following the internalization of 5-HT [51].

The effects of 5-HT in the respiratory systems are pulmonary arterial constriction, bronchoconstriction and stimulation of hyperplastic and hypertrophic alterations in smooth muscle cells and myofibroblasts [52]. This stimulates sclerotic remodeling of the pulmonary vasculature and/or airways, with the end result being increased pulmonary vascular resistance and/or lung fibrosis. There remains uncertainty regarding the precise contributions to specific 5-HT receptors in PAH and lung fibrosis. Prior to 1993 it was assumed that 5-HT₂A mediated vasoconstriction of pulmonary arteries and its antagonist ketanserin were found to have some clinical utility, particularly in the elderly [53]. However, more recent work has suggested contributions of 5-HT₁B and 5-HT₂B receptors [54–56]. The Eckelberg group recently reported increased expression of 5-HT₁A/B and 5-HT₂B in the lungs of patients with IPF and in sufferers of non-specific interstitial pneumonia [57]. 5-HT₂A was also increased selectively in IPF lungs and was localized to fibroblasts, whereas 5-HT₂B was chiefly found at the lung epithelium. The authors also demonstrated that the 5-HT₁A/B antagonist terguride is a potent repressor of fibroblast TGFβ₁ expression and when administered in vivo the drug improved lung function and decreased fibrosis when applied in a therapeutic regimen to mice with established bleomycin-induced lung disease. This latter finding confirmed earlier observations by Fabre et al. that bleomycin-induced fibrosis is associated with increased serotonin in the lung and can be attenuated by blockade of either 5-HT₁A (by ketanserin) or 5-HT₂B (by SB215505) [58]. Although Pfreidome has recently been approved for the treatment of IPF it remains a deadly disease with rising incidence and as such clinical studies in IPF with 5-HT₂A and 5-HT₂B antagonists are certainly warranted.

6. Serotonin signaling in the heart

In addition to the impact of 5-HT-like drugs and carcinoid tumors on valvular fibrosis, 5-HT receptors and transporters may also contribute to cardiac hypertrophy which is characterized by the loss of cardiac myocytes, accumulation of interstitial fibroblasts and collagen deposition [59]. The importance of 5-HT in the regulation of heart structure was demonstrated in mice lacking the 5-HT₂A receptor, which are susceptible to embryonic and neonatal death due to lack of trabeculae in the heart [60]. Furthermore, targeted over-expression of 5-HT₂B in cardiomyocytes induces cardiac hypertrophy [61]. Collectively these observations suggest that 5-HT/5-HT₂B signaling may act either directly on cardiomyocytes or indirectly through other cell types via the release of paracrine regulators.

As cardiac hypertrophy can be initiated by processes resulting from ligands of a variety of other G protein-coupled receptors such as the AT₁ angiotensin II receptor, ET₄ endothelin 1 receptor and adrenergic receptors, there is potential for 5-HT/5-HT₂B signaling to cross-talk with other hypertrophic pathways. Jaffré and colleagues have discovered direct effects of 5-HT on ventricular fibroblasts including production of IL-6, IL-1β and TNF-α in a 5-HT₂B-dependent manner. Adrenergic receptor stimulation has also been shown to induce these hypertrophic cytokines from cardiac fibroblasts, which are found at elevated levels in the diseased heart [62]. The study by Jaffré modeled β-adrenergic stimulation of cardiac hypertrophy by perfusion with isoproterenol (ISO) [63]. As expected ISO perfusion elevated plasma levels of IL-6, IL-1β and TNF-α, which was not seen in 5-HT₂B knockout mice or wild type mice co-administered the 5-HT₂B antagonist SB206553. Moreover, SB206553 treatment or absence of the 5-HT₂B gene protected mice from ISO-induced hypertrophic remodeling of the heart muscle. Whether these effects result from
signaling cross-talk between adrenergic ligand/receptor and 5-HT/5-HT2B pathways in fibroblasts or may instead be due to direct signaling emerging from heterodimer complexes formed between adrenergic and 5-HT2B receptors is still to be determined. A potential mechanism by which 5-HT2B receptors may cross-talk with other G protein-linked receptors is through pathways leading to ROS generation. 5-HT2B is coupled to NAD(P)H oxidase [64] which also plays a pivotal role in physiological responses to angiotensin II/AT1 signaling. Monassier et al. recently reported that selective antagonism of 5-HT2B with SB215505 prevented cardiac superoxide generation and hypertrophy caused by infusion of either angiotensin II or ISO [65]. Hence, these studies support the emerging role of the 5-HT2B receptor in the control of (myo)fibroblast function and add to the growing evidence that 5-HT2B antagonists may be useful in the treatment or prevention of pathological cardiac remodeling.

7. Serotonin signaling in systemic sclerosis (scleroderma)

Systemic scleroderma (SSc) is a rare autoimmune disease of unknown cause mainly affecting females with an onset between the ages of 30–50. The disease is characterized by deposition of fibrillar collagen in the skin, lungs, stomach, heart and the kidneys, with the latter being a poor prognostic factor [44]. Most SSc patients also have vascular disease and Raynaud’s phenomenon [66]. Experimental studies in the 1950s revealed that 5-HT can stimulate the proliferation of skin fibroblasts and that when injected subcutaneously in rodents causes remodeling of skin in a manner that resembles skin pathology in SSc [67]. Roddie et al. also reported in the mid-1950s that infusion of 5-HT into brachial arteries of man induces features of Raynaud's [68]. Scleroderma is reported in patients with carcinoid tumors [23] and was observed following the treatment of intention myoclorius with L-5-hydroxytryptophan and carbopidopa, which was associated with high serotonin levels [69].

The pathophysiological basis for involvement of 5-HT in SSc is unclear; however patients suffer progressive endothelial cell damage and this is evident before fibrosis is observed. It has been proposed that loss of anticoagulant properties of the endothelium may trigger platelet activation and release of 5-HT; this idea being supported by elevated plasma levels of 5-HT in SSc [70], although others failed to reproduce this finding [71]. A recent elegant study by Dees and colleagues provided stronger experimental evidence for a causative role of platelet-derived 5-HT in scleroderma [72]. They showed that cultured dermal fibroblasts from SSc patients and healthy individuals respond to 5-HT by increasing their expression of collagen Iα1, collagen 1α2 and fibronectin. These effects of 5-HT on matrix synthesis were blocked by the 5-HT2B antagonist SB 204741 or by transfected 5-HT2B siRNAs, whereas the 5-HT2A antagonist ketanserin was without effect. The authors also showed that broad specificity 5-HT2 inhibitors as well as the more selective 5-HT2B antagonist SB 204741 prevent bleomycin-induced dermal fibrosis, this being associated with reduced numbers of myofibroblasts. Furthermore, SB 204741 also reduced fibrosis and myofibroblast transdifferentiation in the genetic tight skin1 (Tsk-1) model and similar effects were observed when Tsk-1 mice were crossed with either 5-HT2B or Tph1 knockout mice. Intriguingly, 5-HT/5-HT2B stimulation of matrix synthesis by dermal fibroblasts was shown to be dependent on activation of TGFβ1 gene transcription and subsequent TGFβ1 signaling. The same group later reported that the AP-1 transcription factor JunB, which is over-expressed in SSc skin and cultured fibroblasts, mediates TGFβ1-induced fibroblast activation and bleomycin-induced fibrosis [73]. These data resemble our findings.
in the diseased liver and indicate the 5-HT/5-HT(2)R-ERK-JunD-TGFB pathway in fibroblastic wound healing cells may be a core fibrogenic signaling route in multiple organs.

8. Summary

There is now a clear pattern emerging from independent studies in distinct organ systems that the 5-HT system activated during the earliest phases of wound repair has a major influence on fibrogenesis. In the context of acute injury the pro-fibrogenic and pro-regenerative influences of 5-HT will combine to ensure optimal repair and restoration of tissue architecture and function. However, in the context of chronic disease it may be desirable to tone down the fibrogenic actions of 5-HT. The latter may be achieved by modulating the activities of specific 5-HT receptors that trigger the activation of fibrogenic signal transduction. While there is still much more to be learned about the way in which the different 5-HT receptors combine to regulate tissue repair there is already sufficient pre-clinical data to warrant clinical investigations with antagonists of the 5-HT(2) receptor. As discussed, 5-HT(2) receptors have been strongly implicated as drivers of fibrosis in heart valves, lung, skin and liver, plus they are stimulators of the expression of TGFB. 5-HT(2) receptor antagonists are safe in man, are already in clinical trials for PAH [74] and should now be advanced into trials for fibrosis.

Acknowledgments

Work in the laboratories of D.A.M. and F.O. relevant to this review is funded by grants from the UK Medical Research Council, the Wellcome Trust, The European Council and Newcastle University. The authors additionally wish to acknowledge the work of many contributors who for reasons of brevity may not have been cited in this review.

References

The serotonin hypothesis of pulmonary hypertension


