Published in final edited form as:
Pharmacol Ther. 2011 November ; 132(2): 146–157. doi:10.1016/j.pharmthera.2011.03.008.

Serotonin Receptors and Heart Valve Disease – it was meant 2B

Joshua D. Hutcheson[1], **Vincent Setola**[2], **Bryan L. Roth**[2], and **W. David Merryman**[1]
[1]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN

[2]Department of Pharmacology, University of North Carolina, Chapel Hill, NC

Abstract

Carcinoid heart disease was one of the first valvular pathologies studied in molecular detail, and early research identified serotonin produced by oncogenic enterochromaffin cells as the likely culprit in causing changes in heart valve tissue. Researchers and physicians in the mid-1960s noted a connection between the use of several ergot-derived medications with structures similar to serotonin and the development of heart valve pathologies similar to those observed in carcinoid patients. The exact serotonergic target that mediated valvular pathogenesis remained a mystery for many years until similar cases were reported in patients using the popular diet drug Fen-Phen in the late 1990s. The Fen-Phen episode sparked renewed interest in serotonin-mediated valve disease, and studies led to the identification of the 5-HT_{2B} receptor as the likely molecular target leading to heart valve tissue fibrosis. Subsequent studies have identified numerous other activators of the 5-HT_{2B} receptor, and consequently, the use of many of these molecules has been linked to heart valve disease. Herein, we: review the molecular properties of the 5-HT_{2B} receptor including factors that differentiate the 5-HT_{2B} receptor from other 5-HT receptor subtypes, discuss the studies that led to the identification of the 5-HT_{2B} receptor as the mediator of heart valve disease, present current efforts to identify potential valvulopathogens by screening for 5-HT_{2B} receptor activity, and speculate on potential therapeutic benefits of 5-HT_{2B} receptor targeting.

1. Serotonin and its receptors

1.1. Serotonin

Serotonin or 5-hydroxytryptamine (5-HT; Fig. 1) is enzymatically transformed from the essential amino acid tryptophan following hydroxylation and decarboxylation. Serotonin was discovered and isolated from serum 60 years ago (Rapport, Green et al. 1948), and shortly after, the molecule was determined to originate from the enterochromaffin (or Kulchitsky) cells that are found throughout the gastrointestinal and bronchopulmonary system (Erspamer and Asero 1952). High concentrations of 5-HT are found in blood platelets and enterochromaffin cells of the gut; lesser amounts are found around neurons located along the raphé nuclei of the brainstem. The human brain has evolved a sophisticated arrangement of axons stemming from the raphé nuclei to innervate nearly every brain region.

For Correspondence: W. David Merryman, Ph.D. Department of Biomedical Engineering, Vanderbilt University, Room 9445D MRB IV-Langford, 2213 Garland Avenue, Nashville TN 37232-0493, Office: 615-322-7219, Fax: 615-322-6541, david.merryman@vanderiblt.edu.

5-HT is involved in a diverse array of physiologic and biologic processes. In the brain, 5-HT has been found to affect sleep, mood, appetite, anxiety, aggression, perception, pain, and cognition (Roth, Willins et al. 1998; Edited by Roth 2006; Berger, Gray et al. 2009). Systemically, 5-HT contributes to vascular and non-vascular smooth muscle contraction and platelet aggregation. Serotonin released from neurons is recaptured by an active reuptake pump (serotonin transporter), and is then inactivated by monoamine oxidase and converted to 5-hydroxyindoleacetic acid (Guyton and Hall 1996). *In vivo*, the rate of urinary 5-hydroxyindoleacetic acid output is commonly used as an index of 5-HT metabolism.

1.2. Serotonin receptors

Signaling of 5-HT is mediated by receptors that are located on the cell membrane of neurons and most other cells in the body (Edited by Roth 2006; Berger, Gray et al. 2009). There are six classes of G protein-coupled 5-HT receptors (5-$HT_{1,2,4,5,6,7}$) that can be subdivided into 14 unique subtypes. The 5-HT_3 receptor is unique among 5-HT receptors in that it is a ligand gated ion channel (Maricq, Peterson et al. 1991; Edited by Roth 2006). Heterotrimeric guanine nucleotide-binding protein G protein-coupled receptors (GPCRs) are well characterized and have been described extensively (Kroeze, Kristiansen et al. 2002; Kroeze, Sheffler et al. 2003). Briefly, GPCRs are transmembrane proteins consisting of seven membrane-spanning α-helical segments with an extracellular N-terminus and an intracellular C-terminus. The binding of 5-HT to one of its receptors is thought to elicit a conformational change that activates associated heterotrimeric G proteins and recruits other downstream signaling/scaffolding molecules, such as GPCR kinases and β-arrestins (Armbruster and Roth 2005; Allen and Roth 2010). Upon activation by an agonist-occupied GPCR, G proteins release guanosine diphosphate, which is constituitively bound to the α subunit of the heterotrimer, and bind guanosine triphosphate (GTP). Binding of GTP to the α subunit causes it to dissociate from the βγ subunits (which remain associated to each other); free Gα then interacts with nearby, downstream effectors (*e.g.*, adenylate cyclase for Gαs/olf- and Gαi/o/z-types or phospholipase C for Gαq/11-types), generating second messengers (*e.g.*, cAMP produced by adenylate cyclase or inositol 1,4,5-trisphosphate and diacylglycerol produced by phospholipase C) that modulate downstream effectors inside the cell (*e.g.*, protein kinases A and C activated by cAMP and diacylglycerol) [see (Raymond, Mukhin et al. 2001) for review of 5-HT signaling pathways]

Because of the systemic presence of 5-HT and the multitude of receptor types found throughout the body that can elicit a myriad of cellular responses, drugs targeting 5-HT receptors are effective treatments for a variety of conditions. Each 5-HT receptor subtype contains at least one important therapeutic target. For instance, antimigraine medications (*e.g.*, ergotamine, sumatriptan) activate 5-$HT_{1B/D}$ receptors. Clinically effective antipsychotics block the activation of 5-HT_{2A}, 5-HT_{2C} (Roth et al, 1992), 5-HT_6 (Roth, Craigo et al. 1994), and 5-HT_7 (Roth, Craigo et al. 1994; Abbas, Hedlund et al. 2009) receptors [see (Roth and Xia 2004) for review]. Antagonism of 5-HT_6 receptors has been postulated to enhance memory and learning in healthy individuals (Lindner, Hodges et al. 2003). There are many pharmaceuticals used to target the multitude of serotonergic GPCRs; however the 5-HT_2 receptors are among the most frequently targeted, highlighting their important role in physiological and pathophysiological processes see [(Roth 2010) for recent review].

1.3. 5-HT_2 receptors

The 5-HT_2 family consists of three GPCRs: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. 5-HT induces an increase in inositol 1,4,5-trisphosphate (which leads to release of intracellular calcium stores and diacylglycerol production (Conn and Sanders-Bush 1984; Roth, Nakaki et al. 1984; Kursar, Nelson et al. 1994). In addition to these known signaling mechanisms, 5-HT_2

receptors also generate second messenger signaling that leads to cell-type specific responses depending on the organ under consideration. Some of the most notable effects of 5-HT_2 receptor-preferring drugs involve the brain, and these activities are exploited therapeutically. Two important, common examples are atypical antipsychotics and anorexigens. These drugs —and/or their metabolites—display activity at 5-HT_{2A} (viz: atypical antipsychotics are inverse agonists) (Meltzer, Matsubara et al. 1989; Weiner, Burstein et al. 2001) and 5-HT_{2C} receptors (anorexigens and putative atypical antipsychotic drugs are agonists) (Kozikowski, Cho et al. 2010). 5-HT_{2A} and 5-HT_{2C} receptors are highly abundant in various human brain regions with 5-HT_{2A} being highly concentrated in cortical regions and 5-HT_{2C} more broadly distributed (Pazos, Cortes et al. 1985; Abramowski, Rigo et al. 1995). The 5-HT_{2B} receptor subtype displays a lower expression in the brain (Kursar, Nelson et al. 1994), and thus, it plays a lesser role in the effects of psychoactive agents. Nevertheless, recent genetic and pharmacologic studies have implicated 5-HT_{2B} receptors in the biological activities of the recreational psychostimulant 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) (Doly, Valjent et al. 2008; Doly, Bertran-Gonzalez et al. 2009) and the anorexigen fenfluramine (Banas, Doly et al. 2010).

The putative cardiovascular action of the 5-HT_{2A} and 5-HT_{2B} receptors may be similar. Both of these receptors have been shown to elicit mitogenic and secretory responses in ventricular and heart valve fibroblasts (Xu, Jian et al. 2002; Setola, Hufeisen et al. 2003; Jaffre, Callebert et al. 2004; Yabanoglu, Akkiki et al. 2009), indicating a possible role for each in cardiac development and disease. An issue in isolating functional differences between the 5-HT_2 receptors has been the lack of specificity in pharmacological agents used to target the receptors. In fact, many clinically used agents, particularly antipsychotics and anorexigens, display some activity at all three 5-HT_2 receptor subtypes. One reason for low selectivity among 5-HT_2 receptor-active compounds is the high degree of amino acid sequence homology among the three subtypes (Roth, Willins et al. 1998; Barnes and Sharp 1999) with a structural similarity of 45–50% between the receptors (Pytliak, Vargova et al. 2010). This homology is of significant consequence as drugs intended for the 5-HT_{2A} or 5-HT_{2C} receptors located in the brain may also bind to the 5-HT_{2B} receptors expressed in the brain or in other tissues. Therefore, special attention should be given to differential properties of these receptor subtypes to identify functional differences and enhance understanding of target specificity.

In 2005, we examined non-conserved residues in the transmembrane helices of the 5-HT_2 receptors to identify ones that might participate in the preferential binding of (+)-norfenfluramine to 5-HT_{2B} receptors. We generated a series of 5-HT_{2B} receptor point mutants that contained one 5-HT_{2A}- and/or 5-HT_{2C}-like putative ligand binding residue, and we determined whether any of the point mutations affected (+)-norfenfluramine binding affinity. Mutation of a valine in TM2, V2.53, to leucine (the analogous residue in the 5-HT_{2A} receptor, caused a 17-fold decrease in the K_i of (+)-norfenfluramine. Residue 2.53 in the 5-HT_{2C} receptor is also a valine, and the V2.53L point mutation caused a 12-fold decrease in the K_i of (+)-norfenfluramine. The reciprocal point mutation (L2.53V) in the 5-HT_{2A} receptor had no effect on the K_i of (+)-norfenfluramine. The preceding observations suggest that V2.53 in the 5-HT_{2B} receptor contributes to the high-affinity binding of (+)-norfenfluramine.

Using a rhodopsin-based 5-HT_{2B} receptor homology model, we performed *in silico* ligand docking, and molecular dynamics simulations to predict how V2.53 might contribute to (+)-norfenfluramine binding. One result suggested that both terminal methyl groups of V2.53 formed stabilizing van der Waals (vdW) interactions with the α-methyl group of (+)-norfenfluramine, and that the V2.53L mutation resulted in the loss of one of these interactions. To test that prediction, we generated additional point mutants and

norfenfluramine analogs. First, we reasoned that a V2.53A mutation would eliminate both vdW interactions, further decreasing (+)-norfenfluramine affinity. In fact, the mutation caused a 150-fold reduction in the K_i of (+)-norfenfluramine. Second, we synthesized a norfenfluramine analog lacking an α-methyl group. The affinity of α-desmethyl-norfenfluramine for the wild type 5-HT_{2B} receptor was reduced three-fold compared with (+)-norfenfluramine. Further, α-desmethyl-norfenfluramine binding was less sensitive to the V2.53L mutation than was (+)-norfenfluramine. Our molecular dynamics simulations also predicted that a V2.53I mutation would permit two vdW interactions between the terminal γ- and δ-methyl groups of I2.53 and the α-methyl group of (+)-norfenfluramine. Upon experimental validation, the K_i of (+)-norfenfluramine binding to the V2.53I 5-HT_{2B} receptor was 35 nM, compared with 22 at the wild type 5-HT_{2B} receptor. Together, our *in vitro* and *in silico* studies of the 5-HT_{2B} receptor (+)-norfenfluramine binding provide evidence linking V2.53 to the high-affinity and subtype-selective binding of the valvulopathogenic anorexigen.

1.4. 5-HT_{2B} receptors

What is now called the 5-HT_{2B} receptor (Fig. 2) was first recognized 50 years ago relating to the putative role of a specific 5-HT receptor subtype in the contraction of the gastric fundus from rat stomach (Vane 1959). Although there was controversy prior to the cloning of the 5-HT_{2B} receptor whether the stomach fundus receptor was pharmacologically distinct from the 5-HT_{2A} and 5-HT_{2C} receptors (Kaumann and Levy 2006), this disappeared once all three were cloned and their tissue distribution illuminated. Since then, 5-HT_{2B} receptors have been found to be present in both rodent and human tissues, particularly in the cardiovascular system, gastrointestinal tract, bone, and central nervous system (Ullmer, Schmuck et al. 1995; Choi and Maroteaux 1996; Fitzgerald, Burn et al. 2000). Importantly, the tissue distribution of 5-HT_{2B} receptor protein in rodents and humans is similar, as are their pharmacologies (Manivet, Schneider et al. 2002); this observation facilitates the extrapolation of physiological and pharmacological results from rodent studies of the 5-HT_{2B} receptor to humans. Accordingly, Sprague-Dawley rats have been used in several studies to gain insight into the mechanism of 5-HT-induced valvular alterations (Elangbam, Lightfoot et al. 2005; Gustafsson, Tommeras et al. 2005; Elangbam, Wehe et al. 2006; Hauso, Gustafsson et al. 2007; Elangbam, Job et al. 2008). Long-term serotonin administration in these rats has been shown to induce aortic valve insufficiency and histopathological changes similar to those observed in human carcinoid patients (discussed in Section 3.1) (Gustafsson, Tommeras et al. 2005). The valvular changes seem to be associated with an increase in 5-HT_{2B} receptor expression and a decrease in the expression of 5-HT transporter in both mitral and aortic valves (Elangbam, Job et al. 2008). These observations may indicate an indirect interaction between these two membrane proteins; whereby, in normal physiology, the 5-HT transporter may control homeostatic 5-HT levels, preventing 5-HT_{2B} receptor over-stimulation (Elangbam 2010). In the disease state, the cooperation between these receptors may be lost, leading to persistent 5-HT_{2B} receptor stimulation and the resultant valvulopathies discussed in Section 3.

The complexity and variety of 5-HT_{2B} receptor expression is paralleled by its signal transduction. Activation of 5-HT_{2B} has been found to stimulate phospholipase C (Kellermann, Loric et al. 1996) and phospholipase A2 (Tournois, Mutel et al. 1998), both of which increase intracellular calcium levels. Activation of 5-HT_{2B} receptors in some cell types has also been shown to stimulate nitric oxide synthase (Manivet, Mouillet-Richard et al. 2000). In fibroblasts and smooth muscle cells, the biological result of activating 5-HT_{2B} receptors is mitosis (Fitzgerald, Burn et al. 2000; Nebigil, Choi et al. 2000; Nebigil, Launay et al. 2000; Setola, Hufeisen et al. 2003) and secretion of inflammatory cytokines and extracellular matrix (ECM) components (Jaffre, Bonnin et al. 2009). As such, 5-HT_{2B}

receptors appear to play a crucial role in allowing these cells to maintain the structural homeostasis of the tissues comprising them (*e.g.*, myocardium, heart valves, blood vessels). For example, over-expression of 5-HT_{2B} receptors in hearts of transgenic mice results in cardiac hypertrophy and decreased ventricular function due to enhanced ECM and remodeling (Nebigil, Jaffre et al. 2003). Likewise, genetic deletion of 5-HT_{2B} receptors was shown to lead to ventricular dilation and incomplete cardiac development (Nebigil, Choi et al. 2000).

The molecular signaling pathways associated with these matrix remodeling responses appears to involve typical mitogenic signal transduction as well as coordination between the signaling of 5-HT_{2B} receptors and various growth factor and cytokine receptors (Nebigil, Choi et al. 2000; Nebigil, Launay et al. 2000; Jaffre, Callebert et al. 2004). Specifically, 5-HT_{2B} activation in mouse fibroblasts was initially shown to lead to the $p21^{Ras}$- and heterotrimeric G protein-dependent activation of mitogen activated protein kinase (MAPK) (Launay, Birraux et al. 1996). Further studies in fibroblasts revealed that 5-HT_{2B} activation also leads to phosphorylation of the cytoplasmic tyrosine kinase Src (Nebigil, Launay et al. 2000). Src phosphorylation appears to enhance signaling of the platelet derived growth factor and epidermal growth factor (Nebigil, Launay et al. 2000; Li, Zhang et al. 2008); Src inhibitors have been found to negatively affect signaling at both these receptors (Hsu, Persons et al. 1991; Nebigil, Launay et al. 2000). A more recent study connected these 5-HT_{2B} signaling pathways using pharmacological agents to selectively inhibit key signal transduction proteins in cardiac fibroblasts (Jaffre, Bonnin et al. 2009). Accordingly, 5-HT_{2B} receptor-mediated Src activation was found to lead to an increase in matrix metalloproteinase activity, which leads to a release of heparin-bound epidermal growth factor. In turn, epidermal growth factor signaling leads to MAPK activation and subsequent upregulation of various cytokines. In addition, Jaffré et al. (Jaffre, Bonnin et al. 2009) demonstrated that 5-HT_{2B} receptors work in concert with the angiotensin II type 1 receptor (AT_1R) to mediate hypertrophic signaling in cardiac fibroblasts. These results indicate that, in cardiac fibroblasts at least, the function of both GPCRs is required for the activity of either one. Consequently, by inhibiting one receptor the action of the other receptor is likewise inhibited.

Many of the cardiac-related studies into the molecular signaling pathways of the 5-HT_{2B} receptors have focused on the role of this receptor in ventricular fibroblasts. In this review, we will highlight the role 5-HT_{2B} receptors may play in mediating changes in heart valves. Therefore, to make the connection between the action of 5-HT_{2B} receptors in ventricles and heart valves, it is interesting to note that the signaling pathways discussed previously seem to play a major role in both regulating and responding to the biomechanical properties of ventricular tissue, and as noted below, biomechanical integrity is crucial to the appropriate function of heart valves. Thus, both AT_1 and 5-HT_{2B} receptors demonstrate a mechano-dependent upregulation and signaling activation during ventricular pressure overload that results in an increase in tissue stress levels (Rosenkranz 2004; Liang, Lai et al. 2006). Moreover, agonist signaling at these GPCRs has been shown to lead to an increase in expression of the cytokine transforming growth factor-β1 (TGF-β1) (Jian, Xu et al. 2002; Rosenkranz 2004), which has also been shown to exhibit mechano-responsive signaling (Wipff, Rifkin et al. 2007) and seems to be a key mediator in the tissue changes that lead to heart valve disease (Walker, Masters et al. 2004; Merryman, Lukoff et al. 2007).

2. Degenerative heart valve disease

2.1. Heart valve overview

Heart valves (HVs) control the direction of blood flow through the heart during the cardiac cycle by directing oxygen-poor blood to the lungs and oxygen-rich blood to the vital organs

via the systemic circulation. There are four valves in the heart: two on pulmonic side and two on the systemic side (Fig. 3). Progressing through the heart from the vena cava, deoxygenated blood enters the right atrium and passes through the tricuspid valve to the right ventricle, and then from the right ventricle through the pulmonary valve into the pulmonary artery, which directs blood flow to the lungs. Upon reoxygenation by perfusion through the lungs, the blood returns to the left atrium of the heart via the pulmonary vein, passes through the mitral valve into the left ventricle, and then enters the aorta through the aortic valve. The anatomies of the pulmonary and aortic valves are very similar, as are the anatomies of the tricuspid and mitral valves. The pulmonary and aortic valves are referred to as semilunar valves because the three leaflets of the valves look like a half-moon when excised (Fig. 4a). Semilunar valves open and close solely due to inertial forces from the blood during the cardiac cycle; whereas, the structure and function of the tricuspid and mitral valves, referred to as atrioventricular valves, are more complex than the semilunar valves (Fig. 4b). Briefly, their leaflets are tethered to the papillary muscles of ventricular wall via chordae tendineae, which act as anchors to prevent the leaflets from prolapsing into the atria during ventricular contraction. The primary difference between the tricuspid and mitral valve is the number of leaflets: the tricuspid has three and the mitral only two. The atrioventricular valves ensure that blood does not back-flow during myocardial contraction (systole), and the semilunar valves keep blood from re-entering the heart during relaxation (diastole).

All of the valves rely on hemodynamic forces to regulate the opening and closing of the HV leaflets. As such, the biomechanical integrity of the leaflet tissues is crucial to appropriate function of the HVs. Two distinct cell types have been shown to play a role in regulating tissue homeostasis within the HV: valve endothelial cells and valve interstitial cells (VICs) (Chester and Taylor 2007). Valve endothelial cells exhibit regional heterogeneity along the surface of the HV leaflets and may function to direct spatial-specific extracellular matrix (ECM) production through paracrine signaling to the VICs (Simmons, Grant et al. 2005). During HV development, activated VICs synthesize ECM components to form the leaflet structure that is crucial to correct HV function; however, during disease, over-activation of VICs can lead to inappropriate ECM accumulation and/or valvular architecture and subsequent loss of HV function that leads to heart valve disease (HVD). Under healthy physiologic conditions, the left side VICs are much more active and dynamic with greater levels of cytoskeletal proteins and ECM biosynthesis, likely due to the increased pressures imposed on the leaflets (Merryman, Huang et al. 2006; Merryman, Youn et al. 2006; Merryman, Liao et al. 2007).

2.2. Disease etiology and pathology

There exist two types of HVD: congenital and acquired (Otto 2004). Congenital valve disease is an abnormality that develops before birth and may be related to improper valve size, malformed leaflets, or an irregularity in the way the leaflets are attached. This most often affects the pulmonary and aortic valves. A very common congenital defect is bicuspid aortic valve disease where instead of the normal three leaflets or cusps, the bicuspid aortic valve has only two. Without the third leaflet, the valve may be stenotic (narrowing, obstructing flow) or regurgitant (‘floppy,’ allowing backward flow). It is estimated that some form of bicuspid aortic valve disease affects about 1.4% of the population (Lloyd-Jones, Adams et al. 2010), but this is difficult to verify since in some cases it goes undetected and does not result in significant changes in cardiovascular function. Acquired HVD pertains to problems that develop within valves that were at one time normal. This is typically referred to as age-related degenerative valve disease. In the early part of the 20th century, the primary cause of acquired valve disease stemmed from rheumatic valve disease;

however, this trend has changed dramatically with a decrease in rheumatic valve disease and a concomitant increase in age-related degenerative valve disease (Otto 2004).

The risk factors for HVD are similar to traditional clinical risk factors for atherosclerosis and coronary artery disease and include age, male gender, hypertension, diabetes, triglycerides, and smoking (Mohler, Sheridan et al. 1991; Stewart, Siscovick et al. 1997; Agmon, Khandheria et al. 2001). In fact, the similarities between the two have led to the hypothesis that acquired HVD is primarily a manifestation of atherosclerosis (O'Brien 2006). The hallmark of HVD pathogenesis is the formation of lesions containing cell types that are characteristic of chronic inflammation (Olsson, Dalsgaard et al. 1994; Olsson, Rosenqvist et al. 1994; Otto, Kuusisto et al. 1994). These include macrophages, T lymphocytes, and mast cells. Additionally, there are lipoproteins [LDL and Lp(a)] found in human diseased valve lesions, and accumulation of these lipoproteins is mediated, in part, by ECM proteoglycans (Walton, Williamson et al. 1970; O'Brien, Reichenbach et al. 1996). Besides lipoproteins, recent evidence has shown that the renin-angiotensin system, particularly angiotensin converting enzyme and angiotensin II, may play a role in HVD pathogenesis (O'Brien, Shavelle et al. 2002). Angiotensin II has a number of potential lesion-forming effects, including inflammation and macrophage and cholesterol accumulation. Therefore, there are multiple mechanisms that initiate HVD and further perpetuate the disease in otherwise normally functioning valve.

2.3. Current theories

As noted previously, degenerative HVD is believed to be caused by an over-activation of VICs through a process that depends on signaling associated with TGF-β1. This cytokine is secreted from cells in a latent form and stored in the ECM. Various ECM cues can result in the activation of TGF-β1, which then binds to and activates the TGF-β1 serine/threonine kinase receptors (TβRs). Following cytokine binding, TβRs modulate a wide variety of cellular processes by signaling through a family of transcription factors known as Smads (Howe 2003). In VICs, active TGF-β1 leads to myofibroblast activation *in vitro* (Walker, Masters et al. 2004), and the combination of active TGF-β1 and mechanical strain leads to a synergistic increase in hallmarks of HVD within porcine HV leaflets (Merryman, Lukoff et al. 2007). Confounding the problems associated with its mediation of cellular changes that lead to HVD, TGF-β1 signaling also leads to increased synthesis of the latent form of TGF-β1, which in turn may be activated by VICs (Merryman 2008). This feed-forward signaling mechanism may drive the progression of HVD.

2.4. Treatments

Currently, the only viable long-term treatment option for HVD is valve replacement surgery. Typically, the native diseased HV is either replaced by a decellularized HV of bovine or porcine origin or supplanted by a mechanical HV. While the techniques and resultant risks associated with HV replacement procedures have dramatically improved, surgery still remains a poor option for the two patient populations most affected by HVD: children and elderly.

In pediatric patients, the main concern is the integration of replacement HVs within tissues that are rapidly growing and changing. Compounding the problem in children, replacement HVs tend to wear quickly and typically need to be replaced within 20 years (Merryman, Engelmayr Jr et al. 2006). To circumvent these problems, many surgeons elect to perform the Ross procedure whereby the pulmonic HV is resected and placed in the aortic position. The replacement HV is then placed in the position formerly occupied by the pulmonic HV. The advantage of this procedure is that the pulmonic HV is able to incorporate within native tissue in the aortic position, and the replacement HV wears more slowly due to the lower

pressures associated with the pulmonic circulation. The major disadvantage of the Ross procedure is that it requires two very invasive surgical procedures (i.e., resection of both the aortic and pulmonic HVs), and often, the pulmonic HV is not able to adapt to the increased pressure associated with systemic circulation. For these reasons, the Ross procedure is rarely performed in elderly patients. Faced with the problems associated with HV replacement, physicians often delay surgical options until they are absolutely necessary; therefore, many patients would greatly benefit from an early treatment to delay or prevent the onset of HVD. Elderly patients face a difficult recovery following open-chest surgery. While these procedures may improve the cardiac function of these patients, their quality of life declines for a prolonged period of time (up to a year). Thus, elderly patients would benefit greatly from a pharmacological strategy against HVD.

2.5. Statins

The hopeful story in recent years has been that lipid-lowering drugs (*i.e.*, statins) might prevent HVD (Rajamannan, Subramaniam et al. 2002; Rajamannan, Edwards et al. 2003; Benton, Kern et al. 2009), similarly to their promise against atherosclerosis. In 2001, two retrospective studies indicated that statins may inhibit the progression of aortic valve stenosis (Aronow, Ahn et al. 2001; Novaro, Tiong et al. 2001), and it was suggested that large prospective studies be conducted. When these prospective studies were finally completed, the efficacy of statins proved disappointing. In 2005, a small double-blind, placebo controlled study showed no benefit of statins to reduce HVD (Cowell, Newby et al. 2005). Moreover, a recent report of a large clinical study demonstrated, rather conclusively, that statins do not reduce major cardiovascular outcomes, including aortic valve replacement, in patients with aortic valve stenosis (Rossebo, Pedersen et al. 2008). In light of these findings, the enthusiasm for statin therapy as a potential preventive treatment for HVD has been severely dampened. Thus, there is no pharmacological strategy currently available or being developed (to our knowledge) that has the potential to prevent or delay HVD progression.

3. Serotonergic drugs and heart valve disease

3.1. Carcinoid syndrome

Carcinoid syndrome occurs as a result of the formation of neuroendocrine tumors arising from oncogenic enterochromaffin cells. These cells synthesize and deliver 5-HT to the bloodstream for controlled systemic distribution; however, when tumor-forming enterochromaffin cells metastasize to the liver, 5-HT more readily enters systemic circulation resulting in an increase in plasma 5-HT levels and leading to the cardiac changes associated with carcinoid heart disease (CHD) (Druce, Rockall et al. 2009). CHD was first noted 80 years ago by the Dutch pathologist A.J. Scholte, who observed that a patient with a carcinoid tumor also had thickened tricuspid valve leaflets (Gustafsson, Hauso et al. 2008). Interestingly, CHD differs from most forms of HVD in that it mostly affects right-side HVs (*i.e.*, the pre-lung tricuspid and pulmonary valves). Elevated blood levels of 5-HT are carried to the right side of the heart through the inferior vena cava, where it is believed that interaction with 5-HT_{2B} receptors on cells in the tricuspid and pulmonic HVs leads to the extracellular matrix secretion and the thickening of the HV leaflets that characterize HVD. As the blood continues into pulmonary circulation, the 5-HT is inactivated by monoaminoxigenase in the lungs and, therefore, does not induce HV changes when the blood returns to the left side of the heart. For many years, the serotonergic receptor subtype responsible for CHD was not known due to the fact that serotonin has a similar affinity for many of its receptors, and several of the subtypes are expressed throughout the cardiovascular system. The current hypotheses identifying 5-HT_{2B} receptor as the major HV

target came about only as a result of inadvertent targeting of the 5-HT_{2B} receptors with Fen-Phen.

3.2. Fen-Phen

Among all the serotonergic drugs on the market, there may be none more recognizable to the general public than fenfluramine, one of the components of the 'Fen-Phen' anorexigen combination. This popular diet drug regimen consisting of *fen*fluramine and *phen*teramine was shown to be better tolerated than either alone in 1984 and the drug combination was subsequently and widely prescribed (Weintraub, Hasday et al. 1984). However, a study published in late 1997 identified both right-sided and left-sided HV defects in a number of patients who had been taking Fen-Phen for an average of 12 months (Connolly, Crary et al. 1997). Soon thereafter, the drugs (including other fenfluramine formulations) were voluntarily withdrawn from the market based upon recommendations by the FDA. In a retrospective study, the highest incidence of HVD development from Fen-Phen was found to be 25.2% among patients treated for an average of 20 months (Khan, Herzog et al. 1998).

Early histological analyses of diseased tissue from Fen-Phen patients indicated a pathophysiology similar to that observed in carcinoid patients and from tissues of subjects who had taken specific ergot-derived formulations. In this regard, in the mid-1960s, researchers and physicians noticed a strong connection between the ergot agents methysergide and ergotamine and the development of fibrotic pathologies including HVD (Graham, Suby et al. 1966; Graham 1967; Mason, Billingham et al. 1977). Some even went as far as to note that the "similarities in chemical structure of serotonin, methysergide, and ergotamine" may "suggest a common pathophysiologic mechanism for ergot alkaloid-associated valve disease and carcinoid valve disease" (Redfield, Nicholson et al. 1992). Much like with CHD, however, the exact molecular cause of the tissue changes that lead to HVD remained undetermined.

The Fen-Phen episodes sparked new interest in identifying the specific 5-HT receptor subtype involved in drug-induced HVD. Due to the known mitogenic roles of the 5-HT_2 subfamily, Fitzgerald et al. (Fitzgerald, Burn et al. 2000) examined the interaction of fenfluramine, norfenfluramine (the main metabolite of fenfluramine), ergotamine, and methysergide on human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. The ergot-derived compounds were found to possess high affinity for all three receptor subtypes. In contrast, fenfluramine rotamers demonstrated weak affinities for the 5-HT_2 receptors; however, the norfenfluramine rotamers exhibited relatively high affinity for both 5-HT_{2B} and 5-HT_{2C} receptors (Table 1) and slightly lower affinity for 5-HT_{2A} receptors. These results suggested that norfenfluramine, the main metabolite of fenfluramine, was chiefly responsible for 5-HT_2 receptor activation, and thus, the most likely candidate for causing Fen-Phen-mediated HVD. Furthermore, norfenfluramine was found to be two orders of magnitude more potent at 5-HT_{2B} and 5-HT_{2C} receptors compared with 5-HT_{2A} receptors (Table 2). Additionally, 5-HT_{2C} receptors, which are restricted to the central nervous system, were found to be expressed at exceedingly low levels within HV tissues (Fig. 5); whereas 5-HT_{2B} receptors are expressed in relatively high quantities within the HV leaflets. Simultaneously, Rothman et al. (2000) compared the *in vitro* pharmacology of norfenfluramine with that of known inducers of HVD (5-HT, methysergide), as well as with that of serotonergic drugs not associated with HVD (negative controls), and found that the HVD-associated compounds were, unlike the negative controls, potent 5-HT_{2B} receptor agonists (see below). Taken together with the expression of 5-HT_{2B} receptors in heart valve tissue (Fitzgerald, Burn et al. 2000) and the mitogenic effect of 5-HT_{2B} receptors in cardiac and other cell types (Nebigil, Choi et al. 2000; Nebigil, Launay et al. 2000), these results pointed to the 5-HT_{2B} receptor as the serotonergic target leading to HVD.

As noted above, the results of the Fitzgerald et al. study were supported in a seminal report published simultaneously by Rothman et al. (Rothman, Baumann et al. 2000) in which the affinities and potencies of 15 different molecules were tested at 11 different 5-HT receptor subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_6, and 5-HT_7. In this study, all but the 5-HT_{2B} receptor subtypes were systematically ruled out based on pharmacological similarity with HVD-associated molecules and pharmacological differences from negative control molecules that were not suspected mediators of HVD (*e.g.*, fluoxetine and its metabolite norfluoxetine). The 5-HT_2 receptor subtypes were the only receptors to display relatively high affinity (Table 1) and high potency (Table 2) for both the norfenfluramine rotamers and for the HVD-associated ergot-derived compounds (Tables 1 and 2, italicized). Similar to the Fitzgerald et al. study, the data indicate both high affinity (K_i) and potency (shown by phosphatidylinositol hydrolysis, K_{act}) at 5-HT_{2B} over 5-HT_{2A} for norfenfluramines and the known inducers of HVD. Taken together, these studies indicate that norfenfluramine, the main fenfluramine metabolite, is the likely culprit in fenfluramine-mediated HVD. It is important to note that in both the Fitzgerald et al. (2000) and Rothman et al. (2000) studies, phenteramine did not exhibit high affinity agonism of 5-HT_{2B} receptors (Table 1); correspondingly, formulations consisting of phenteramine alone (*i.e.*, in the absence of fenfluramine)—in use for decades prior to the Fen-Phen HVD outbreak—have not been associated with the disease (Rothman, Baumann et al. 2000).

3.3. Pergolide and Cabergoline

Following the Fen-Phen episode, a number of additional HVD-inducing drugs have been identified. First among these were the non-specific dopamine agonists pergolide and cabergoline, prescribed for Parkinson's disease (Antonini and Poewe 2007; Schade, Andersohn et al. 2007; Zanettini, Antonini et al. 2007). The stories of pergolide and cabergoline are similar to that of fenfluramine. The first preliminary account of potential pergolide-mediated HVD was reported in 2002 (Pritchett, Morrison et al. 2002). Dopamine agonists had become popular anti-Parkinsonian therapeutics, and many feared that these drugs would suffer the same fate as fenfluramine. Around the same period of time, many drugs were beginning to be profiled at 5-HT_{2B} receptors including anti-Parkinsonian drugs, amphetamine derivatives and other medications (Newman-Tancredi, Cussac et al. 2002; Setola, Hufeisen et al. 2003). Of the large number of drugs tested, pergolide, cabergoline, MDMA and its active N-demethylated metabolite 3,4-methylenedioxyamphetamine (MDA) were found to exhibit potent agonist activity at 5-HT_{2B} (Newman-Tancredi, Cussac et al. 2002; Setola, Hufeisen et al. 2003). It is interesting to note in this regard that Newman-Tancredi and colleagues were solely interested in differentiating activity of several Parkinsonian drugs at various serotonergic receptor subtypes and suggested that 5-HT_{2B} activity might be associated with some sort of therapeutic action of pergolide and cabergoline rather than mediating side-effects. On the other hand, Setola et al. correctly predicted that pergolide and other drugs which were 5-HT_{2B} agonists (including MDMA and MDA) would be associated with a high incidence of HVD. Accordingly, the incidence of HVD from pergolide and cabergoline has been found to be 23.4% and 28.6%, respectively (Zanettini, Antonini et al. 2007); no significant increase in HVD incidence has been associated with any of the other dopamine agonists. Further, three of the other drugs (lisuride, bromocripine, and terguride) were found to be potent agonists at 5-HT_{2A} receptors (Newman-Tancredi, Cussac et al. 2002), but have little to no activity at 5-HT_{2B} receptors (Huang, Setola et al. 2009). None of these drugs has been subsequently reported to be associated with HVD (Elangbam 2010). These findings lend further credence to the theory that the 5-HT_{2B} receptor is the molecular culprit for drug-mediated HVD. Tables 1 and 2 contain partial compilation of the results from these early studies and offer a representative view of the data that first indicated agonist activity at 5-HT_{2B} receptors leads to the development of HVD.

3.4. A search for others

Following the Fen-Phen and pergoline/cabergolide findings, many other drugs have been tested for agonist activity at 5-HT_{2B}. Specifically, the NIMH Psychoactive Drug Screening Program has sought to screen large numbers of FDA-approved drugs and drug-like compounds for agonist activity at 5-HT_{2B} receptors in order to identify drugs/chemotypes with HVD liability (Setola, Dukat et al. 2005; Roth 2007; Huang, Setola et al. 2009). The most recent findings in this effort identified 27 5-HT_{2B} receptor agonists among 2200 drugs and drug-like compounds (Table 3; (Huang, Setola et al. 2009)). Of these, 14 had previously been identified as 5-HT_{2B} receptor agonists, including 7 known HVD-associated drugs (Table 3). Six of the compounds that had not previously been identified as 5-HT_{2B} receptor agonists are currently approved medications (Table 3, italics), with five of these acting as potent agonists: guanfacine, oxymetazoline, quinidine, xylometazoline, and fenoldopam. Guanfacine and quinidine were noted to be of particular concern. Guanfacine is often prescribed as an antihypertensive agent, and quinidine is used to treat arrhythmia. As such, both tend to be prescribed for sustained periods and could potentially lead to valvular problems. The recent indication of guanfacine to treat attention deficit hyperactivity disorder in pediatric patients is of particular concern (Huang, Setola et al. 2009). Of less concern (perhaps) are xylometazoline, oxymetazoline, and fenoldopam, which are typically prescribed for relatively short time periods. However, all of these compounds should be studied closely to ensure that there is no link to HVD (Bhattacharyya, Schapira et al. 2009).

One of the identified agonists, ropinirole, was found to be much less potent than the other compounds (active only at 1 μM) and therefore may not be associated with inducing HVD. Indeed, ropinirole, which has been used for several years to treat restless legs syndrome, has been considered ‘safe’ vis-à-vis HVD. However, a very recent study documented a case of retroperitoneal fibrosis in a patient using ropinirole (Parissis, Papachristodoulou et al. 2010). Additionally, at least four other cases of HVD have been reported in patients using ropinirole (Parissis, Papachristodoulou et al. 2010). While these relatively few reports may not indicate a statistically increased risk of HVD with ropinirole use, these types of cases should be continuously monitored to ensure that no causal link exists.

3.5. Learning from previous mistakes

Though unfortunate in terms of human and economic loss, the fenfluramine case has led to some important insights. The first and most impactful of these is that screening drugs for 5-HT_{2B} receptor agonist activity is the best *in vitro* method for identifying potential drug-inducing HVD candidates (Rothman, Baumann et al. 2000; Setola, Hufeisen et al. 2003; Roth 2007; Elangbam 2010). The case of MDMA supports this recommendation. In 2003, we (VS and BLR), in collaboration with Richard Rothman’s group at NIDA, profiled the serotonergic amphetamine derivative MDMA to identify targets other than the monoamine plasmalemmal transporters. The screening campaign revealed that MDMA and MDA were both moderately potent 5-HT_{2B} receptor agonists, and that they stimulated the proliferation of primary human VIC cultures in a 5-HT_{2B} receptor-dependent manner (Setola, Hufeisen et al. 2003). These findings led us to predict that MDMA use may be a risk factor for HVD. In 2007, a clinical study reported an increased prevalence of HVD in MDMA users (Droogmans, Cosyns et al. 2007). Similarly, recent clinical findings have indicated that long-term usage of the fenfluramine-derivative benfluorex can lead to HVD (Noize, Sauer et al. 2006; Boutet, Frachon et al. 2009; Le Ven, Tribouilloy et al. 2010; Weill, Paita et al. 2010). Benfluorex is an anorexigen and hypolipidemic agent commonly prescribed to overweight diabetic patients in Asia, Europe and South America; however, the severity of the clinical case reports led the European Medicines Agency to withdraw benfluorex from the European market in December 2009 (Droogmans, Cosyns et al. 2010). Given that norfenfluramine is a known metabolite of benfluorex, the association of this drug with HVD

should not have come as a surprise (Droogmans, Cosyns et al. 2010). Thus, the aforementioned studies support the claim that *in vitro* screening of drugs at 5-HT_{2B} receptors can identify previously unknown drug-inducing HVD candidates.

Another lesson that can be learned from the role of 5-HT_{2B} receptors in HVD is that 5-HT_{2B} receptor antagonists may be potential prophylactics and/or treatments. Indeed, if the activation of 5-HT_{2B} receptors (on heart valve interstitial cells and/or other cells) contributes to HVD, it is likely that blockade of 5-HT_{2B} receptors may antagonize the onset and/or progression of the disease (Roth 2007). Furthermore, there are a number of 5-HT_{2B} receptor antagonists that are FDA-approved medications (e.g., antidepressants, antipsychotics, antihistamines) with established records of safety and tolerability (Rothman, Baumann et al. 2000; Setola, Hufeisen et al. 2003; Roth 2007; Huang, Setola et al. 2009). Along these lines, Droogmans and colleagues recently reported that the antihistamine cyproheptadine—which is also a potent 5-HT_{2B} receptor antagonist (Young, Khorana et al. 2005)—mitigated pergolide-induced HVD in rats (Droogmans, Roosens et al. 2009), as predicted by Roth (2007). Similar experiments using other 5-HT_{2B} receptor antagonists, and analyses of HVD prevalence among large numbers of patients taking medications with 5-HT_{2B} receptor antagonist activity (e.g., antihistamines, antidepressants, antipsychotics), will be informative in terms of HVD prevention/treatment strategies.

4. 5-HT_{2B} receptor as a novel treatment strategy

The development of a suitable therapeutic to prevent/retard HVD depends on the ability to target the root cause of the disease, which ultimately manifests itself as thickening and stiffening of HV leaflets, which diminishes the ability of the HV to maintain directionality in blood flow. At a cellular level, HV stiffening is believed to be caused by activation of VICs to a myofibroblast phenotype (Rabkin, Aikawa et al. 2001; Rabkin, Hoerstrup et al. 2002; Rabkin-Aikawa, Farber et al. 2004; Aikawa, Whittaker et al. 2006). Once activated, these cells increase ECM deposition, which directly leads to the decreased compliance of the leaflets observed in HVD. Additionally, increased mechanical strain has been shown to exacerbate VIC activation (Balachandran, Konduri et al. 2006; Merryman, Lukoff et al. 2007; Balachandran, Sucosky et al. 2009; Balachandran, Sucosky et al. 2010). In order to develop a strategy to prevent or treat HVD, a more thorough understanding of the cellular signaling and subsequent tissue-level changes involved in the progression of HVD is needed to elucidate the relevant molecular targets. Given the large number of currently-approved medications that exhibit antagonist/inverse agonist activity at 5-HT_{2B} receptors (Huang, Setola et al. 2009), we believe that the 5-HT_{2B} receptor is a tractable target to achieve these therapeutic goals.

Given its negative history, the idea of targeting 5-HT_{2B} for therapeutic gain may initially seem counter-intuitive; however, studies have begun to explore the potential benefits of controlling 5-HT_{2B} receptor signaling (Fabre, Marchal-Somme et al. 2008; Monassier, Laplante et al. 2008; Porvasnik, Germain et al. 2010). Just as agonists of the 5-HT_{2B} receptor have been observed to lead to HVD, many of these agonists have also been implicated in fibrotic responses and ECM alterations that lead to other pathologies such as ventricular hypertrophy (Jaffre, Callebert et al. 2004; Jaffre, Bonnin et al. 2009) and pulmonary arterial fibrosis and hypertension (Esteve, Launay et al. 2007). Correspondingly, genetic deletion of 5-HT_{2B} receptor expression in mice has been shown to lead to incomplete cardiac development characterized by ventricular dilation and a lack of tissue integrity (Nebigil, Choi et al. 2000; Nebigil, Hickel et al. 2001). Taken together, these results indicate that 5-HT_{2B} receptors play a crucial role in the maintenance of ECM homeostasis in cardiac tissues, and with a better understanding of the downstream effectors

of these receptors, the pathways may be able to be manipulated to therapeutically target cardiac fibrotic diseases (Hauso, Gustafsson et al. 2007).

As an illustration of this, studies in pulmonary fibrosis have shown that 5-HT_{2B} receptor antagonists can effectively reduce fibrotic lesions in a mouse model (Fabre, Marchal-Somme et al. 2008), and the selective 5-HT_{2B} receptor antagonist PRX-08066 has been shown to increase ventricular ejection fraction and reduce hypertrophy and vascular remodeling in a rat model of pulmonary arterial hypertension (Porvasnik, Germain et al. 2010). A 5-HT_{2B} receptor antagonist may be able to function similarly in preventing HVD by blocking the fibrotic response of VICs to other, non-serotonergic stimuli.

As mentioned previously, TGF-β1 is believed to be a key mediator of the cellular changes that lead to HVD. Unfortunately, the ubiquity of TGF-β1 signaling makes this molecule a poor therapeutic target. A more appropriate therapeutic goal would be to interrupt TGF-β1 signaling through a separate pathway that is more localized to HV tissues, and the 5-HT_{2B} receptor signaling pathway may be a promising candidate to achieve this goal. Evidence suggests that the signaling pathway from 5-HT_{2B} receptors may crosstalk with TGF-β1 signaling pathways (Jian, Xu et al. 2002), which may be mediated in part by the tyrosine kinase Src (Fig. 6). Src is known to play an important role in both 5-HT_{2B} and TGF-β1 receptor signaling pathways and has been shown to be involved in the 5-HT_{2B} receptor-dependent regulation of the platelet derived growth factor receptor (Nebigil, Launay et al. 2000; Xu, Jian et al. 2002; Mishra, Zhu et al. 2007; Pechkovsky, Scaffidi et al. 2008; Samarakoon, Higgins et al. 2008; Skhirtladze, Distler et al. 2008). Therefore, 5-HT_{2B} receptor blockade has the potential to function in two ways: 1) preventing proliferation and fibrotic ECM accumulation by VICs directly, and 2) interacting with TGF- β1 signaling pathways to prevent VIC myofibroblastic differentiation (Fig. 6).

Additionally, AT_1R antagonists have been observed to inhibit TGF-β1 signaling in Marfan's syndrome (Habashi, Judge et al. 2006; Cohn, van Erp et al. 2007; Brooke, Habashi et al. 2008), and as such, the AT_1R antagonist losartan has shown tremendous clinical promise in treating afflicted patients. Given that AT_1R and 5-HT_{2B} receptors may be functionally linked in other cell types (Jaffre, Bonnin et al. 2009), 5-HT_{2B} receptor antagonists may work in a similar manner to mitigate TGF-β1 signaling. Therefore, 5-HT_{2B} receptors may provide a localized pharmacological target to prevent the VIC-mediated fibrotic changes that characterize HVD. As noted above, many 5-HT_{2B} receptor antagonists are currently FDA-approved and used clinically to treat other diseases, with acceptable tolerance (including but not limited to: Amesergide, Amisulpride, Bromocryptine, Lisuride, Chlorpromazine, Clozapine, Cyproheptidine, Metergoline, Mianserin, Olanzapine, Aripiprazole, Risperidone, 9-OH-risperidone, Terguride, Yohimbine, Ziprasidone, Roxindole). Furthermore, it is interesting to note that lisuride has been shown to be an antagonist at 5-HT_{2B} receptors (Newman-Tancredi, Cussac et al. 2002; Elangbam 2010) and was prescribed for more than 30 years without a single known report of HVD (Hofmann, Penner et al. 2006). While the absence of documented cases does not necessarily lead to the conclusion that lisuride prevented HVD in these patients, it does seem reasonable to believe that given the large population of patients that received lisuride and its known serotonergic activity, even background levels (i.e., not significantly higher than the total population) would have been reported. In conclusion, we believe that these types of molecules should be tested for efficacy in preventing or treating HVD, as has been suggested (Roth 2007).

Acknowledgments

JDH was supported by an AHA Pre-doctoral Fellowship (10PRE4290020). BLR and VS were supported by the NIMH Psychoactive Drug Screening Program, U19MH82441, RO1MH61887. BLR was also supported by the

Michael Hooker Distinguished Professorship in Pharmacology. WDM was supported by the AHA (0835496N and 09GRNT2010125), Wallace H. Coulter Foundation (Early Career Award), and NIH (HL094707).

Abbreviations

5-HT	5-hydroxytryptomine
AT_1R	Angiotensin II type 1 receptor
CHD	Carcinoid heart disease
ECM	Extracellular matrix
GPCR	G protein-coupled receptor
GTP	Guanosine triphosphate
HV	Heart valve
HVD	Heart valve disease
MAPK	Mitogen activated protein kinase
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
TGF-β1	Transforming growth factor – β1
VIC	Valve interstitial cell

References

Abbas AI, Hedlund PB, et al. Amisulpride is a potent 5-HT7 antagonist: relevance for antidepressant actions in vivo. Psychopharmacology (Berl). 2009; 205(1):119–128. [PubMed: 19337725]

Abramowski D, Rigo M, et al. Localization of the 5-hydroxytryptamine2C receptor protein in human and rat brain using specific antisera. Neuropharmacology. 1995; 34(12):1635–1645. [PubMed: 8788961]

Agmon Y, Khandheria BK, et al. Aortic valve sclerosis and aortic atherosclerosis: different manifestations of the same disease? Insights from a population-based study. J Am Coll Cardiol. 2001; 38(3):827–834. [PubMed: 11527641]

Aikawa E, Whittaker P, et al. Human semilunar cardiac valve remodeling by activated cells from fetus to adult: implications for postnatal adaptation, pathology, and tissue engineering. Circulation. 2006; 113(10):1344–1352. [PubMed: 16534030]

Allen JA, Roth BL. Strategies to Discover Unexpected Targets for Drugs Active at G Protein-Coupled Receptors. Annu Rev Pharmacol Toxicol. 2010

Antonini A, Poewe W. Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. Lancet Neurol. 2007; 6(9):826–829. [PubMed: 17706566]

Armbruster BN, Roth BL. Mining the receptorome. J Biol Chem. 2005; 280(7):5129–5132. [PubMed: 15590622]

Aronow WS, Ahn C, et al. Association of coronary risk factors and use of statins with progression of mild valvular aortic stenosis in older persons. Am J Cardiol. 2001; 88(6):693–695. [PubMed: 11564402]

Balachandran K, Konduri S, et al. An ex vivo study of the biological properties of porcine aortic valves in response to circumferential cyclic stretch. Ann Biomed Eng. 2006; 34(11):1655–1665. [PubMed: 17031600]

Balachandran K, Sucosky P, et al. Elevated cyclic stretch alters matrix remodeling in aortic valve cusps: implications for degenerative aortic valve disease. Am J Physiol Heart Circ Physiol. 2009; 296(3):H756–764. [PubMed: 19151254]

Balachandran K, Sucosky P, et al. Elevated cyclic stretch induces aortic valve calcification in a bone morphogenic protein-dependent manner. Am J Pathol. 2010; 177(1):49–57. [PubMed: 20489151]

Banas SM, Doly S, et al. Deconstructing Antiobesity Compound Action: Requirement of Serotonin 5-HT(2B) Receptors for Dexfenfluramine Anorectic Effects. Neuropsychopharmacology. 2010

Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999; 38(8):1083–1152. [PubMed: 10462127]

Benton JA, Kern HB, et al. Statins block calcific nodule formation of valvular interstitial cells by inhibiting alpha-smooth muscle actin expression. Arterioscler Thromb Vasc Biol. 2009; 29(11): 1950–1957. [PubMed: 19679827]

Berger M, Gray JA, et al. The expanded biology of serotonin. Annu Rev Med. 2009; 60:355–366. [PubMed: 19630576]

Bhattacharyya S, Schapira AH, et al. Drug-induced fibrotic valvular heart disease. Lancet. 2009; 374(9689):577–585. [PubMed: 19683643]

Boutet K, Frachon I, et al. Fenfluramine-like cardiovascular side-effects of benfluorex. Eur Respir J. 2009; 33(3):684–688. [PubMed: 19251806]

Brooke BS, Habashi JP, et al. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med. 2008; 358(26):2787–2795. [PubMed: 18579813]

Chester AH, Taylor PM. Molecular and functional characteristics of heart-valve interstitial cells. Philos Trans R Soc Lond B Biol Sci. 2007; 362(1484):1437–1443. [PubMed: 17569642]

Choi DS, Maroteaux L. Immunohistochemical localisation of the serotonin 5-HT2B receptor in mouse gut, cardiovascular system, and brain. FEBS Lett. 1996; 391(1–2):45–51. [PubMed: 8706927]

Cohn RD, van Erp C, et al. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med. 2007; 13(2):204–210. [PubMed: 17237794]

Conn PJ, Sanders-Bush E. Selective 5HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacology. 1984; 23(8):993–996. [PubMed: 6483122]

Connolly HM, Crary JL, et al. Valvular heart disease associated with fenfluramine-phentermine. N Engl J Med. 1997; 337(9):581–588. [PubMed: 9271479]

Cowell SJ, Newby DE, et al. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. N Engl J Med. 2005; 352(23):2389–2397. [PubMed: 15944423]

Doly S, Bertran-Gonzalez J, et al. Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. PLoS One. 2009; 4(11):e7952. [PubMed: 19956756]

Doly S, Valjent E, et al. Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. J Neurosci. 2008; 28(11):2933–2940. [PubMed: 18337424]

Droogmans S, Cosyns B, et al. Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. Am J Cardiol. 2007; 100(9):1442–1445. [PubMed: 17950805]

Droogmans S, Cosyns B, et al. Benfluorex: the last chapter of drug-induced valvular heart disease? Eur J Echocardiogr. 2010

Droogmans S, Roosens B, et al. Dose dependency and reversibility of serotonin-induced valvular heart disease in rats. Cardiovasc Toxicol. 2009; 9(3):134–141. [PubMed: 19609730]

Druce M, Rockall A, et al. Fibrosis and carcinoid syndrome: from causation to future therapy. Nat Rev Endocrinol. 2009; 5(5):276–283. [PubMed: 19444261]

Roth, BL., editor. The Serotonin Receptors: From Molecular Pharmacology to Human Therapeutics. Humana Press; 2006.

Elangbam CS. Drug-induced valvulopathy: an update. Toxicol Pathol. 2010; 38(6):837–848. [PubMed: 20716786]

Elangbam CS, Job LE, et al. 5-hydroxytryptamine (5HT)-induced valvulopathy: compositional valvular alterations are associated with 5HT2B receptor and 5HT transporter transcript changes in Sprague-Dawley rats. Exp Toxicol Pathol. 2008; 60(4–5):253–262. [PubMed: 18511249]

Elangbam CS, Lightfoot RM, et al. 5-Hydroxytryptamine (5HT) receptors in the heart valves of cynomolgus monkeys and Sprague-Dawley rats. J Histochem Cytochem. 2005; 53(5):671–677. [PubMed: 15872060]

Elangbam CS, Wehe JG, et al. Evaluation of glycosaminoglycans content and 5-hydroxytryptamine 2B receptor in the heart valves of Sprague-Dawley rats with spontaneous mitral valvulopathy--a possible exacerbation by dl-amphetamine sulfate in Fischer 344 rats? Exp Toxicol Pathol. 2006; 58(2–3):89–99. [PubMed: 16996724]

Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature. 1952; 169(4306):800–801. [PubMed: 14941051]

Esteve JM, Launay JM, et al. Functions of serotonin in hypoxic pulmonary vascular remodeling. Cell Biochem Biophys. 2007; 47(1):33–44. [PubMed: 17406058]

Fabre A, Marchal-Somme J, et al. Modulation of bleomycin-induced lung fibrosis by serotonin receptor antagonists in mice. Eur Respir J. 2008; 32(2):426–436. [PubMed: 18321937]

Fitzgerald LW, Burn TC, et al. Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. Mol Pharmacol. 2000; 57(1):75–81. [PubMed: 10617681]

Graham JR. Cardiac and pulmonary fibrosis during methysergide therapy for headache. Am J Med Sci. 1967; 254(1):1–12. [PubMed: 6027684]

Graham JR, Suby HI, et al. Fibrotic disorders associated with methysergide therapy for headache. N Engl J Med. 1966; 274(7):359–368. [PubMed: 5903120]

Grashow JS, Yoganathan AP, et al. Biaixal stress-stretch behavior of the mitral valve anterior leaflet at physiologic strain rates. Ann Biomed Eng. 2006; 34(2):315–325. [PubMed: 16450193]

Gustafsson BI, Hauso O, et al. Carcinoid heart disease. Int J Cardiol. 2008

Gustafsson BI, Tommeras K, et al. Long-term serotonin administration induces heart valve disease in rats. Circulation. 2005; 111(12):1517–1522. [PubMed: 15781732]

Guyton, AC.; Hall, JE. Textbook of Medical Physiology. Philadelphia: W.B. Saunders Company; 1996.

Habashi JP, Judge DP, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006; 312(5770):117–121. [PubMed: 16601194]

Hauso O, Gustafsson BI, et al. Long-term serotonin effects in the rat are prevented by terguride. Regul Pept. 2007; 143(1–3):39–46. [PubMed: 17391782]

Hofmann C, Penner U, et al. Lisuride, a dopamine receptor agonist with 5-HT2B receptor antagonist properties: absence of cardiac valvulopathy adverse drug reaction reports supports the concept of a crucial role for 5-HT2B receptor agonism in cardiac valvular fibrosis. Clin Neuropharmacol. 2006; 29(2):80–86. [PubMed: 16614540]

Howe, PH. Transforming growth factor Beta. In: Thomson, AW.; Lotze, MT., editors. The Cytokine Handbook. London: Elsevier Science; 2003. p. 1119-1141.

Hsu CY, Persons PE, et al. Kinetic analysis of the inhibition of the epidermal growth factor receptor tyrosine kinase by Lavendustin-A and its analogue. J Biol Chem. 1991; 266(31):21105–21112. [PubMed: 1939153]

Huang XP, Setola V, et al. Parallel functional activity profiling reveals valvulopathogens are potent 5-hydroxytryptamine(2B) receptor agonists: implications for drug safety assessment. Mol Pharmacol. 2009; 76(4):710–722. [PubMed: 19570945]

Jaffre F, Bonnin P, et al. Serotonin and angiotensin receptors in cardiac fibroblasts coregulate adrenergic-dependent cardiac hypertrophy. Circ Res. 2009; 104(1):113–123. [PubMed: 19023134]

Jaffre F, Callebert J, et al. Involvement of the serotonin 5-HT2B receptor in cardiac hypertrophy linked to sympathetic stimulation: control of interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha cytokine production by ventricular fibroblasts. Circulation. 2004; 110(8):969–974. [PubMed: 15302781]

Jian B, Xu J, et al. Serotonin mechanisms in heart valve disease I: serotonin-induced up-regulation of transforming growth factor-beta1 via G-protein signal transduction in aortic valve interstitial cells. Am J Pathol. 2002; 161(6):2111–2121. [PubMed: 12466127]

Kaumann AJ, Levy FO. 5-hydroxytryptamine receptors in the human cardiovascular system. Pharmacol Ther. 2006; 111(3):674–706. [PubMed: 16960982]

Kellermann O, Loric S, et al. Sequential onset of three 5-HT receptors during the 5-hydroxytryptaminergic differentiation of the murine 1C11 cell line. Br J Pharmacol. 1996; 118(5): 1161–1170. [PubMed: 8818339]

Khan MA, Herzog CA, et al. The prevalence of cardiac valvular insufficiency assessed by transthoracic echocardiography in obese patients treated with appetite-suppressant drugs. N Engl J Med. 1998; 339(11):713–718. [PubMed: 9731086]

Kozikowski AP, Cho SJ, et al. HTS and rational drug design to generate a class of 5-HT(2C)-selective ligands for possible use in schizophrenia. ChemMedChem. 2010; 5(8):1221–1225. [PubMed: 20533502]

Kroeze WK, Kristiansen K, et al. Molecular biology of serotonin receptors structure and function at the molecular level. Curr Top Med Chem. 2002; 2(6):507–528. [PubMed: 12052191]

Kroeze WK, Sheffler DJ, et al. G-protein-coupled receptors at a glance. J Cell Sci. 2003; 116(Pt 24): 4867–4869. [PubMed: 14625380]

Kursar JD, Nelson DL, et al. Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. Mol Pharmacol. 1994; 46(2):227–234. [PubMed: 8078486]

Launay JM, Birraux G, et al. Ras involvement in signal transduction by the serotonin 5-HT2B receptor. J Biol Chem. 1996; 271(6):3141–3147. [PubMed: 8621713]

Le Ven F, Tribouilloy C, et al. Valvular heart disease associated with benfluorex therapy: results from the French multicentre registry. Eur J Echocardiogr. 2010

Li B, Zhang S, et al. Fluoxetine-mediated 5-HT2B receptor stimulation in astrocytes causes EGF receptor transactivation and ERK phosphorylation. Psychopharmacology (Berl). 2008; 201(3): 443–458. [PubMed: 18758753]

Liang YJ, Lai LP, et al. Mechanical stress enhances serotonin 2B receptor modulating brain natriuretic peptide through nuclear factor-kappaB in cardiomyocytes. Cardiovasc Res. 2006; 72(2):303–312. [PubMed: 16962085]

Lindner MD, Hodges DB Jr, et al. An assessment of the effects of serotonin 6 (5-HT6) receptor antagonists in rodent models of learning. J Pharmacol Exp Ther. 2003; 307(2):682–691. [PubMed: 12975483]

Lloyd-Jones D, Adams RJ, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation. 2010; 121(7):e46–e215. [PubMed: 20019324]

Manivet P, Mouillet-Richard S, et al. PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. J Biol Chem. 2000; 275(13):9324–9331. [PubMed: 10734074]

Manivet P, Schneider B, et al. The serotonin binding site of human and murine 5-HT2B receptors: molecular modeling and site-directed mutagenesis. J Biol Chem. 2002; 277(19):17170–17178. [PubMed: 11859080]

Maricq AV, Peterson AS, et al. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science. 1991; 254(5030):432–437. [PubMed: 1718042]

Mason JW, Billingham ME, et al. Methysergide-induced heart disease: a case of multivalvular and myocardial fibrosis. Circulation. 1977; 56(5):889–890. [PubMed: 912852]

Meltzer HY, Matsubara S, et al. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther. 1989; 251(1):238–246. [PubMed: 2571717]

Merryman WD. Insights into (the interstitium of) degenerative aortic valve disease. J Am Coll Cardiol. 2008; 51(14):1415. author reply 1416. [PubMed: 18387446]

Merryman WD, Engelmayr GC Jr, et al. Defining biomechanical endpoints for tissue engineered heart valve leaflets from native leaflet properties. Prog Pediat Cardiol. 2006; 21(2):153–160.

Merryman WD, Huang HY, et al. The effects of cellular contraction on aortic valve leaflet flexural stiffness. J Biomech. 2006; 39(1):88–96. [PubMed: 16271591]

Merryman WD, Liao J, et al. Differences in tissue-remodeling potential of aortic and pulmonary heart valve interstitial cells. Tissue Eng. 2007; 13(9):2281–2289. [PubMed: 17596117]

Merryman WD, Lukoff HD, et al. Synergistic effects of cyclic tension and transforming growth factor-beta1 on the aortic valve myofibroblast. Cardiovasc Pathol. 2007; 16(5):268–276. [PubMed: 17868877]

Merryman WD, Youn I, et al. Correlation between heart valve interstitial cell stiffness and transvalvular pressure: implications for collagen biosynthesis. Am J Physiol Heart Circ Physiol. 2006; 290(1):H224–231. [PubMed: 16126816]

Mishra R, Zhu L, et al. TGF-beta-regulated collagen type I accumulation: role of Src-based signals. Am J Physiol Cell Physiol. 2007; 292(4):C1361–1369. [PubMed: 17135298]

Mohler ER, Sheridan MJ, et al. Development and progression of aortic valve stenosis: atherosclerosis risk factors--a causal relationship? A clinical morphologic study. Clin Cardiol. 1991; 14(12):995–999. [PubMed: 1841025]

Monassier L, Laplante MA, et al. Serotonin 5-HT(2B) receptor blockade prevents reactive oxygen species-induced cardiac hypertrophy in mice. Hypertension. 2008; 52(2):301–307. [PubMed: 18591460]

Nebigil CG, Choi DS, et al. Serotonin 2B receptor is required for heart development. Proc Natl Acad Sci U S A. 2000; 97(17):9508–9513. [PubMed: 10944220]

Nebigil CG, Hickel P, et al. Ablation of serotonin 5-HT(2B) receptors in mice leads to abnormal cardiac structure and function. Circulation. 2001; 103(24):2973–2979. [PubMed: 11413089]

Nebigil CG, Jaffre F, et al. Overexpression of the serotonin 5-HT2B receptor in heart leads to abnormal mitochondrial function and cardiac hypertrophy. Circulation. 2003; 107(25):3223–3229. [PubMed: 12810613]

Nebigil CG, Launay JM, et al. 5-hydroxytryptamine 2B receptor regulates cell-cycle progression: cross-talk with tyrosine kinase pathways. Proc Natl Acad Sci U S A. 2000; 97(6):2591–2596. [PubMed: 10688905]

Newman-Tancredi A, Cussac D, et al. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. J Pharmacol Exp Ther. 2002; 303(2):815–822. [PubMed: 12388668]

Noize P, Sauer M, et al. Valvular heart disease in a patient taking benfluorex. Fundam Clin Pharmacol. 2006; 20(6):577–578. [PubMed: 17109650]

Novaro GM I, Tiong Y, et al. Effect of hydroxymethylglutaryl coenzyme a reductase inhibitors on the progression of calcific aortic stenosis. Circulation. 2001; 104(18):2205–2209. [PubMed: 11684632]

O'Brien KD. Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). Arterioscler Thromb Vasc Biol. 2006; 26(8):1721–1728. [PubMed: 16709942]

O'Brien KD, Reichenbach DD, et al. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of 'degenerative' valvular aortic stenosis. Arterioscler Thromb Vasc Biol. 1996; 16(4):523–532. [PubMed: 8624774]

O'Brien KD, Shavelle DM, et al. Association of angiotensin-converting enzyme with low-density lipoprotein in aortic valvular lesions and in human plasma. Circulation. 2002; 106(17):2224–2230. [PubMed: 12390952]

Olsson M, Dalsgaard CJ, et al. Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. J Am Coll Cardiol. 1994; 23(5):1162–1170. [PubMed: 8144784]

Olsson M, Rosenqvist M, et al. Expression of HLA-DR antigen and smooth muscle cell differentiation markers by valvular fibroblasts in degenerative aortic stenosis. J Am Coll Cardiol. 1994; 24(7):1664–1671. [PubMed: 7963113]

Otto, CM. Valvular Heart Disease. Philadelphia: Saunders; 2004.

Otto CM, Kuusisto J, et al. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. Circulation. 1994; 90(2):844–853. [PubMed: 7519131]

Parissis D, Papachristodoulou A, et al. Inflammatory aneurysm of the abdominal aorta in a patient treated with ropinirole. J Neurol. 2010; 257(9):1582–1584. [PubMed: 20407782]

Pazos A, Cortes R, et al. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. 1985; 346(2):231–249. [PubMed: 4052777]

Pechkovsky DV, Scaffidi AK, et al. Transforming growth factor beta1 induces alphavbeta3 integrin expression in human lung fibroblasts via a beta3 integrin-, c-Src-, and p38 MAPK-dependent pathway. J Biol Chem. 2008; 283(19):12898–12908. [PubMed: 18353785]

Porvasnik SL, Germain S, et al. PRX-08066, a novel 5-hydroxytryptamine receptor 2B antagonist, reduces monocrotaline-induced pulmonary arterial hypertension and right ventricular hypertrophy in rats. J Pharmacol Exp Ther. 2010; 334(2):364–372. [PubMed: 20430844]

Pritchett AM, Morrison JF, et al. Valvular heart disease in patients taking pergolide. Mayo Clin Proc. 2002; 77(12):1280–1286. [PubMed: 12479512]

Pytliak M, Vargova V, et al. Serotonin receptors - from molecular biology to clinical applications. Physiol Res. 2010

Rabkin-Aikawa E, Farber M, et al. Dynamic and reversible changes of interstitial cell phenotype during remodeling of cardiac valves. J Heart Valve Dis. 2004; 13(5):841–847. [PubMed: 15473488]

Rabkin E, Aikawa M, et al. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation. 2001; 104(21):2525–2532. [PubMed: 11714645]

Rabkin E, Hoerstrup SP, et al. Evolution of cell phenotype and extracellular matrix in tissue-engineered heart valves during in-vitro maturation and in-vivo remodeling. J Heart Valve Dis. 2002; 11(3):308–314. discussion 314. [PubMed: 12056720]

Rajamannan NM, Edwards WD, et al. Hypercholesterolemic aortic-valve disease. N Engl J Med. 2003; 349(7):717–718. [PubMed: 12917318]

Rajamannan NM, Subramaniam M, et al. Atorvastatin inhibits hypercholesterolemia-induced cellular proliferation and bone matrix production in the rabbit aortic valve. Circulation. 2002; 105(22): 2660–2665. [PubMed: 12045173]

Rapport MM, Green AA, et al. Serum vasoconstrictor, serotonin; isolation and characterization. J Biol Chem. 1948; 176(3):1243–1251. [PubMed: 18100415]

Raymond JR, Mukhin YV, et al. Multiplicity of mechanisms of serotonin receptor signal transduction. Pharmacol Ther. 2001; 92(2–3):179–212. [PubMed: 11916537]

Redfield MM, Nicholson WJ, et al. Valve disease associated with ergot alkaloid use: echocardiographic and pathologic correlations. Ann Intern Med. 1992; 117(1):50–52. [PubMed: 1596047]

Rosenkranz S. TGF-beta1 and angiotensin networking in cardiac remodeling. Cardiovasc Res. 2004; 63(3):423–432. [PubMed: 15276467]

Rossebo AB, Pedersen TR, et al. Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. N Engl J Med. 2008; 359(13):1343–1356. [PubMed: 18765433]

Roth BL. Drugs and valvular heart disease. N Engl J Med. 2007; 356(1):6–9. [PubMed: 17202450]

Roth BL. Neuropharmacology. 2010 in press.

Roth BL, Craigo SC, et al. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther. 1994; 268(3): 1403–1410. [PubMed: 7908055]

Roth BL, Nakaki T, et al. Aortic recognition sites for serotonin (5HT) are coupled to phospholipase C and modulate phosphatidylinositol turnover. Neuropharmacology. 1984; 23(10):1223–1225. [PubMed: 6521858]

Roth BL, Willins DL, et al. 5-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): where structure meets function. Pharmacol Ther. 1998; 79(3):231–257. [PubMed: 9776378]

Roth BL, Xia Z. Molecular and cellular mechanisms for the polarized sorting of serotonin receptors: relevance for genesis and treatment of psychosis. Crit Rev Neurobiol. 2004; 16(4):229–236. [PubMed: 15862107]

Rothman RB, Baumann MH, et al. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. Circulation. 2000; 102(23):2836–2841. [PubMed: 11104741]

Samarakoon R, Higgins SP, et al. TGF-beta1-induced plasminogen activator inhibitor-1 expression in vascular smooth muscle cells requires pp60(c-src)/EGFR(Y845) and Rho/ROCK signaling. J Mol Cell Cardiol. 2008; 44(3):527–538. [PubMed: 18255094]

Schade R, Andersohn F, et al. Dopamine agonists and the risk of cardiac-valve regurgitation. N Engl J Med. 2007; 356(1):29–38. [PubMed: 17202453]

Schoen, FJ.; Edwards, WD. Valvular heart disease: general principles and stenosis. In: Silver, MD.; Gotlieb, AI.; Schoen, FJ., editors. Cardiovascular Pathology. New York, NY: Churchill Livingstone; 2001. p. 402-442.

Setola V, Dukat M, et al. Molecular determinants for the interaction of the valvulopathic anorexigen norfenfluramine with the 5-HT2B receptor. Mol Pharmacol. 2005; 68(1):20–33. [PubMed: 15831837]

Setola V, Hufeisen SJ, et al. 3,4-methylenedioxymethamphetamine (MDMA, “E”. cstasy”) induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmacol. 2003; 63(6):1223–1229. [PubMed: 12761331]

Simmons CA, Grant GR, et al. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res. 2005; 96(7):792–799. [PubMed: 15761200]

Skhirtladze C, Distler O, et al. Src kinases in systemic sclerosis: central roles in fibroblast activation and in skin fibrosis. Arthritis Rheum. 2008; 58(5):1475–1484. [PubMed: 18438865]

Stewart BF, Siscovick D, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. J Am Coll Cardiol. 1997; 29(3):630–634. [PubMed: 9060903]

Tournois C, Mutel V, et al. Cross-talk between 5-hydroxytryptamine receptors in a serotonergic cell line. Involvement of arachidonic acid metabolism. J Biol Chem. 1998; 273(28):17498–17503. [PubMed: 9651340]

Ullmer C, Schmuck K, et al. Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett. 1995; 370(3):215–221. [PubMed: 7656980]

Vane JR. The relative activities of some tryptamine analogues on the isolated rat stomach strip preparation. Br J Pharmacol Chemother. 1959; 14(1):87–98. [PubMed: 13651584]

Walker GA, Masters KS, et al. Valvular myofibroblast activation by transforming growth factor-beta: implications for pathological extracellular matrix remodeling in heart valve disease. Circ Res. 2004; 95(3):253–260. [PubMed: 15217906]

Walton KW, Williamson N, et al. The pathogenesis of atherosclerosis of the mitral and aortic valves. J Pathol. 1970; 101(3):205–220. [PubMed: 4098425]

Weill A, Paita M, et al. Benfluorex and valvular heart disease: a cohort study of a million people with diabetes mellitus. Pharmacoepidemiol Drug Saf. 2010; 19(12):1256–1262. [PubMed: 20945504]

Weiner DM, Burstein ES, et al. 5-hydroxytryptamine2A receptor inverse agonists as antipsychotics. J Pharmacol Exp Ther. 2001; 299(1):268–276. [PubMed: 11561089]

Weintraub M, Hasday JD, et al. A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. Arch Intern Med. 1984; 144(6):1143–1148. [PubMed: 6375610]

Wipff PJ, Rifkin DB, et al. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. J Cell Biol. 2007; 179(6):1311–1323. [PubMed: 18086923]

Xu J, Jian B, et al. Serotonin mechanisms in heart valve disease II: the 5-HT2 receptor and its signaling pathway in aortic valve interstitial cells. Am J Pathol. 2002; 161(6):2209–2218. [PubMed: 12466135]

Yabanoglu S, Akkiki M, et al. Platelet derived serotonin drives the activation of rat cardiac fibroblasts by 5-HT2A receptors. J Mol Cell Cardiol. 2009; 46(4):518–525. [PubMed: 19167402]

Young R, Khorana N, et al. Pizotyline effectively attenuates the stimulus effects of N-methyl-3,4-methylenedioxyamphetamine (MDMA). Pharmacol Biochem Behav. 2005; 82(2):404–410. [PubMed: 16253319]

Zanettini R, Antonini A, et al. Valvular heart disease and the use of dopamine agonists for Parkinson’s disease. N Engl J Med. 2007; 356(1):39–46. [PubMed: 17202454]

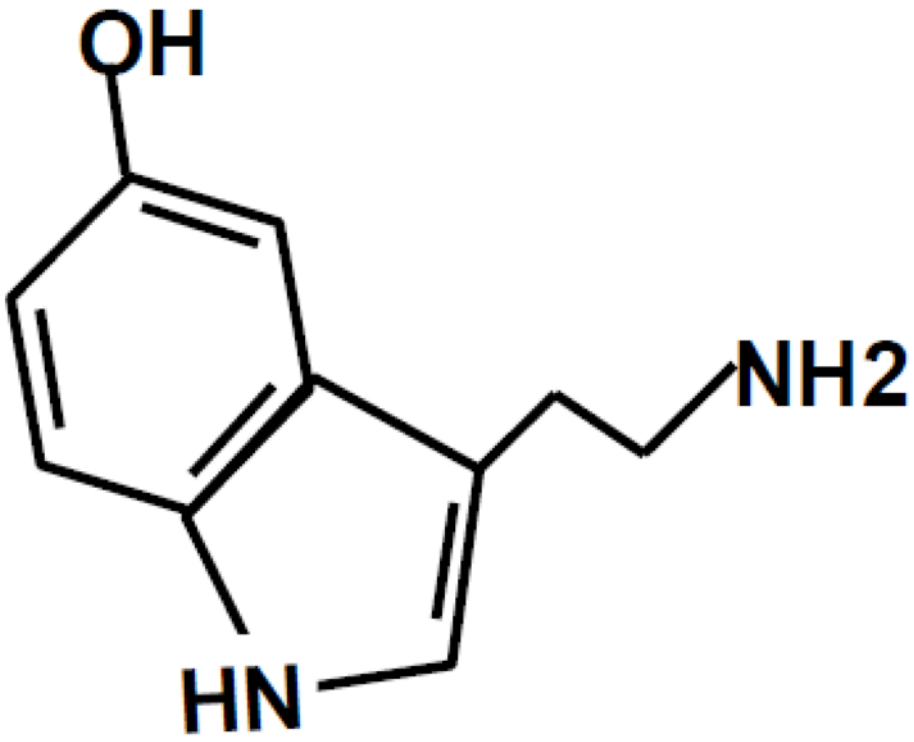

Fig. 1.
Molecular structure of serotonin.

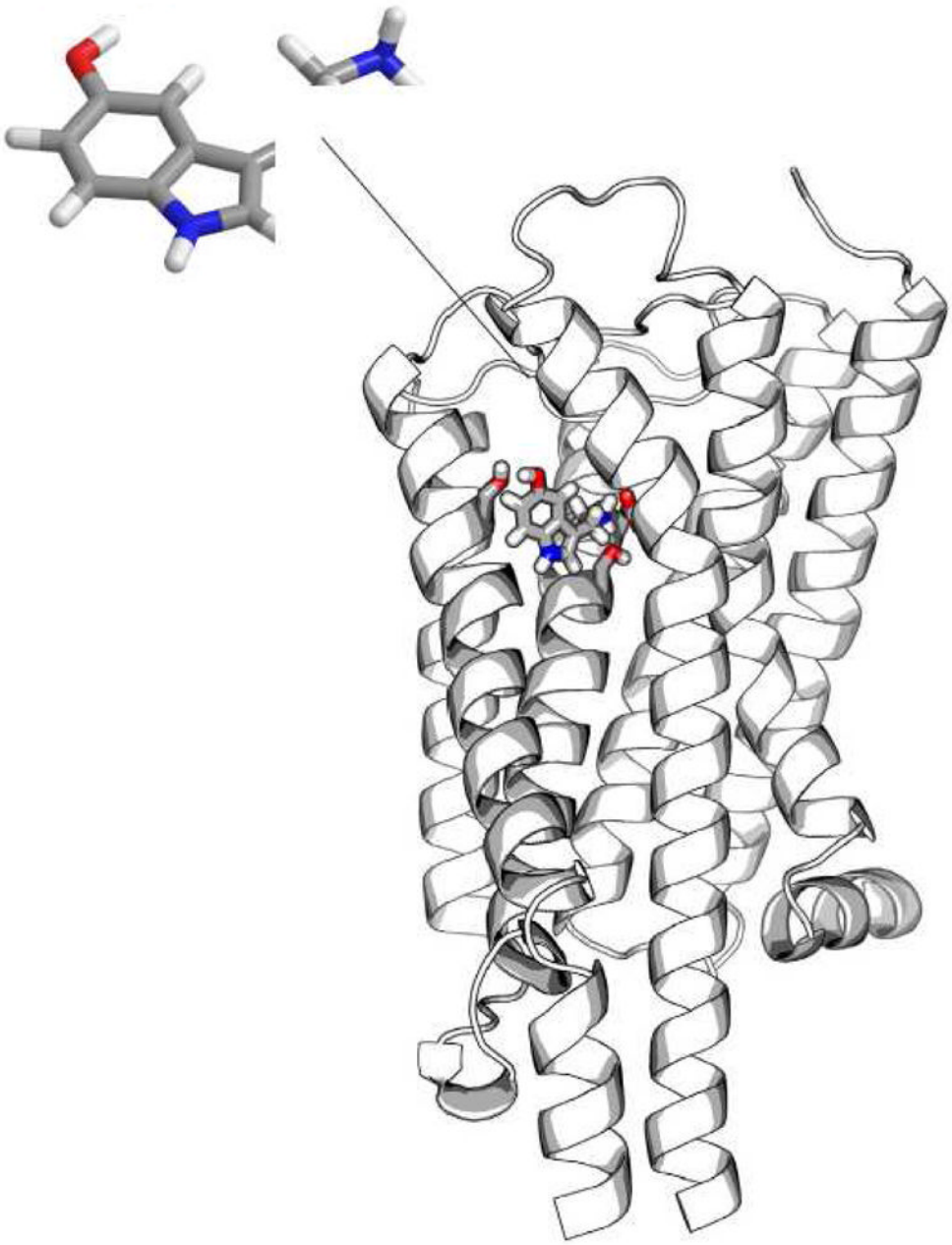

Fig. 2.
A three-dimensional homology model of the 5-HT2B receptor. The model is based on the crystal structure of squid rhodopsin, which has a high degree of homology with the 5-HT2B receptor. Shown are two TM3 residues and one TM6 residue known to interact with serotonin.

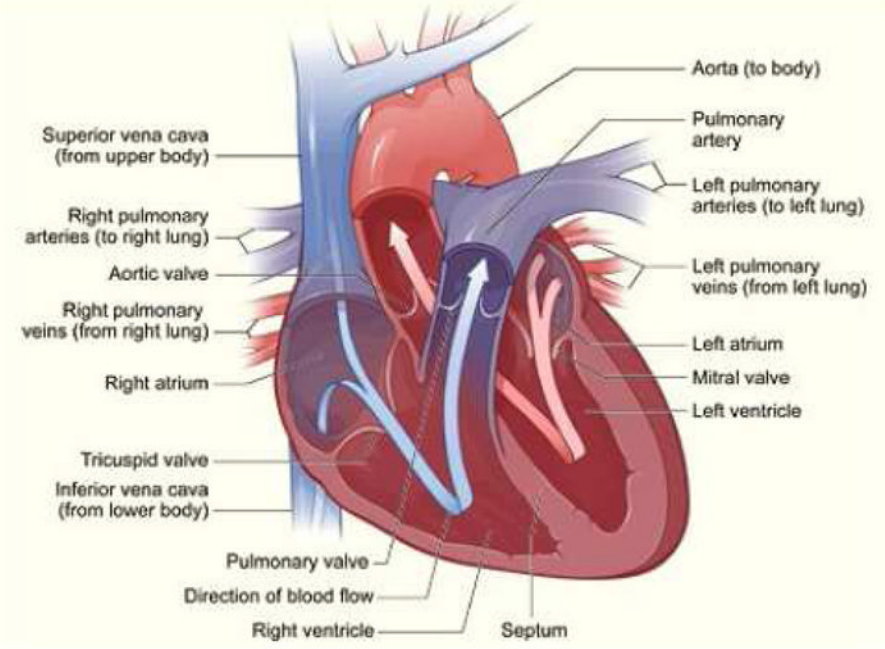

Fig. 3.

Schematic of blood flow through the four heart chambers. Blood from the body returns to the right atrium of the heart through the vena cava and proceeds to the right ventricle through the tricuspid valve. The ventricle pumps this deoxygenated blood through the pulmonary valve to the lungs. Oxygenated blood from the lungs returns to the left atrium through the pulmonary veins. The blood then proceeds through the mitral valve to the left ventricle; whereupon, it is pumped through the aortic valve for systemic distribution. Reprinted with permissions from the National Heart, Lung, and Blood Institute, a part of the National Institutes of Health and the U.S. Department of Health and Human Services.

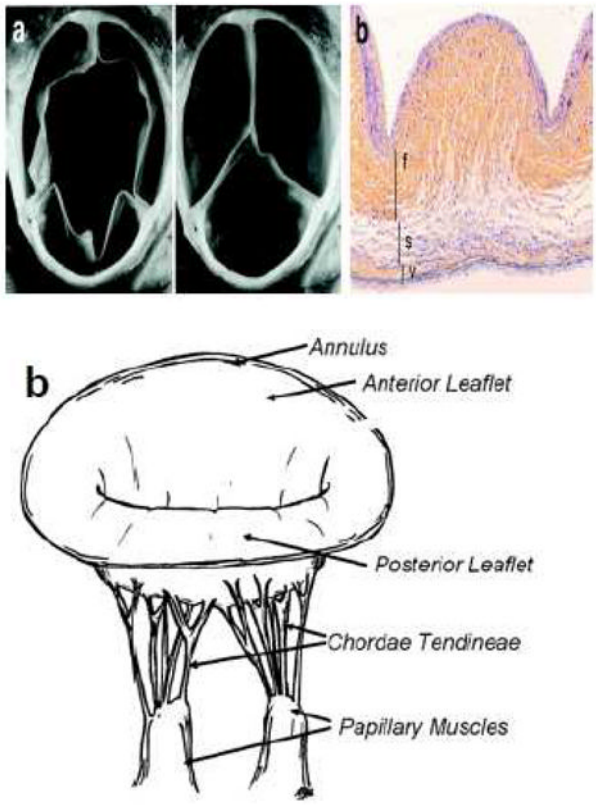

Fig. 4.

Structures of heart valves. (a) The three-leaflet aortic valve is shown in both its open (systolic) and closed (diastolic) states. The pulmonary valve is very similar in structure to the aortic valve. Reprinted with permissions from (Schoen and Edwards 2001). (b) The two leaflet mitral valve is anchored to the ventricular wall by chordae tendineae and papillary muscles to prevent prolapsed into the atrium during systole. The tricuspid valve is anchored in a similar manner as the mitral valve but contains three leaflets. Reprinted with permissions from (Grashow, Yoganathan et al. 2006).

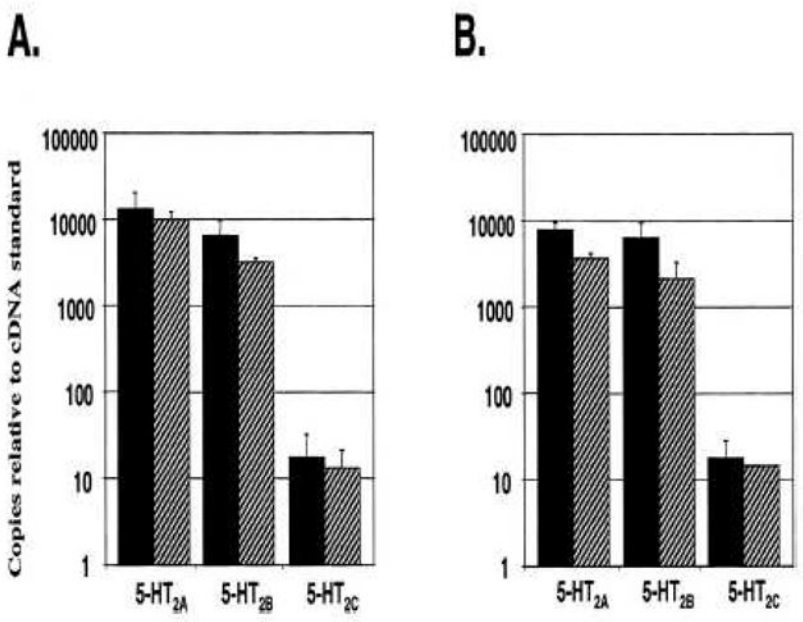

Fig. 5.

5-HT_2 receptor subtype expression in the aortic valve. 5-HT_{2A} and 5-HT_{2B} receptors are expressed in much greater numbers than 5-HT_{2C} as shown by random primed cDNA (solid bars) or oligo(dT) data (hatched bars). Reprinted with permissions from (Fitzgerald, Burn et al. 2000).

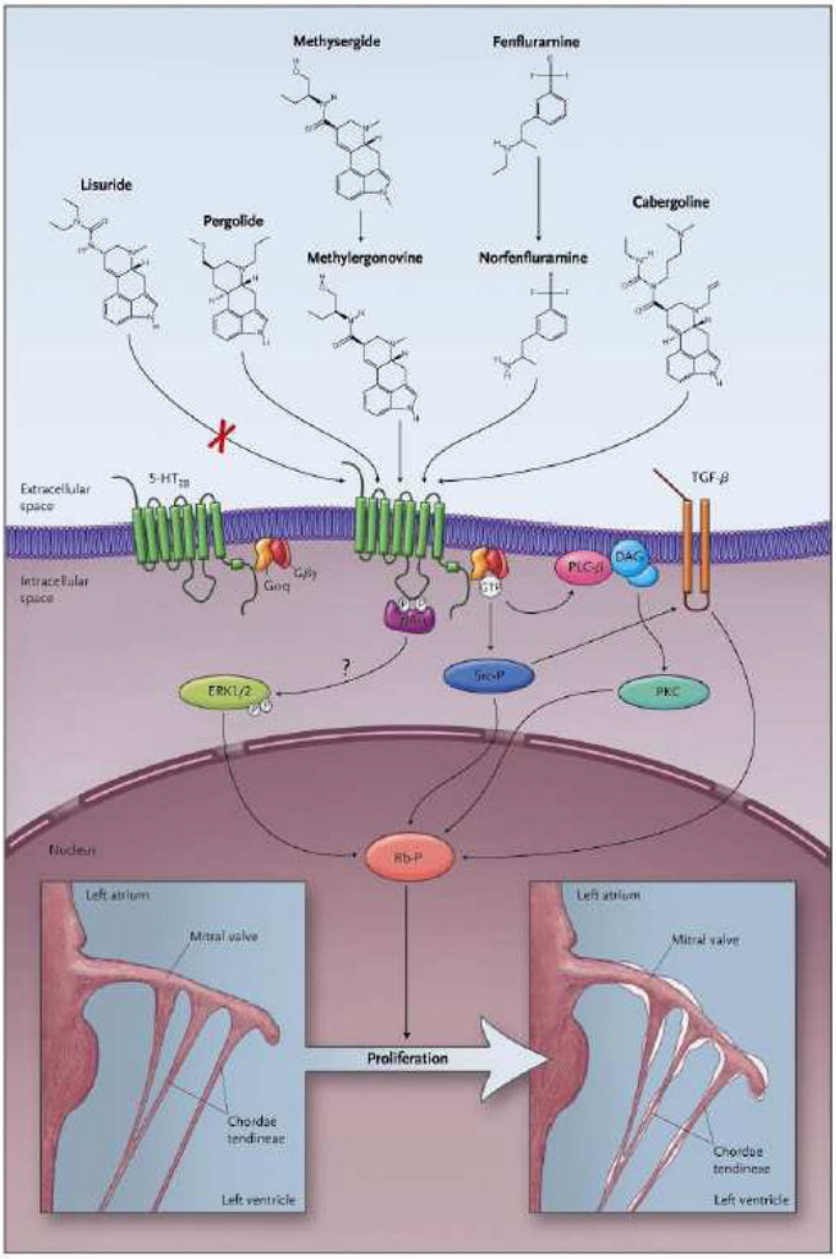

Fig. 6.

Potential pathways of 5-HT_{2B}. 5-HT_{2B} activation may lead to proliferation through pathways that depend on ERK1/2, Src, and PKC. Additionally, 5-HT_{2B} may crosstalk directly with TGF-β1 signaling via interaction with Src. A 5-HT_{2B} antagonist may function as an inverse agonist to inhibit these pathways and prevent the fibrotic response of VICs that leads to HVD. Reprinted with permissions from (Roth 2007).

Table 1

Comparison of Receptor Subtype Affinity (K_i in nM)

	Receptor Subtype		
Drug	**5-HT$_{2A}$**	**5-HT$_{2B}$**	**5-HT$_{2C}$**
5-HT[1]	614*	4.0	12.2
(+)-Fenfluramine[2]	2470	2080	3920
(−)-Fenfluramine[2]	1430	1620	680
(+)-Norfenfluramine[2]	187	56	27
(−)-Norfenfluramine[2]	267	99	65
Cabergoline[4]	7.8	2.6	190
Pergolide[3,4]	1.6	14	45
Ergotamine[1]	*9.0**	*3.0*	*12.0*
Methysergide[1]	*15.0**	*9.1*	*1.8*
Methylergonovine[1]	*12.6**	*0.49*	*12.4*
Phenteramine[1]	>10,000*	>10,000	>10,000

* from rat 5-HT$_{2A}$

[1] Rothman, R.B., et al., *Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications.* Circulation, 2000. **102**(23): p. 2836–41.

[2] Fitzgerald, L.W., et al., *Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine.* Mol Pharmacol, 2000. **57**(1): p. 75–81.

[3] Setola, V., et al., *3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro.* Mol Pharmacol, 2003. **63**(6): p. 1223–9.

[4] Newman-Tancredi, A., et al., *Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes.* J Pharmacol Exp Ther, 2002. **303**(2): 815–22.

Table 2

Comparison of Receptor Subtype Potency (K_{act} or EC_{50} for phosphoinositide hydrolysis in nM)

	Receptor Subtype		
Drug	**5-HT$_{2A}$**	**5-HT$_{2B}$**	**5-HT$_{2C}$**
5-HT[1]	66	2.4	0.6
(+)-Fenfluramine[1]	>10,000	379	362
(−)-Fenfluramine[1]	5,279	1,248	360
(+)-Norfenfluramine[2]	3,100	24	190
(−)-Norfenfluramine[2]	26,600	292	727
Cabergoline[3]	7.76	2.57	2.40
Pergolide[3]	1.62	6.03	4.47
Ergotamine[1]	*16*	*9.8*	*5*
Methysergide[1]	*3.5*	*150*	*2.9*
Methylergonovine[1]	*1.3*	*0.8*	*2.5*
Lisuride[3]	8.13	0*	7.76

*Lisuride exhibits antagonist signaling at the 5-HT$_{2B}$ receptor.[3]

[1]Rothman, R.B., et al., *Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications.* Circulation, 2000. **102**(23): p. 2836–41.

[2]Fitzgerald, L.W., et al., *Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine.* Mol Pharmacol, 2000. **57**(1): p. 75–81.

[3]Adapted from: Newman-Tancredi, A., et al., *Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes.* J Pharmacol Exp Ther, 2002. **303**(2): p. 815–22.

Table 3

Agonist efficacies and potencies at 5-HT_{2B} (E_{max} given as % of 5-HT, EC_{50} for calcium flux in nM)[1]

Drug	E_{max}	EC_{50}
5-HT	100.0	1.78
BW 723C86	92.9	1.41
DOI	88.2	1.45
Norfenfluramine	107.4	2.45
Cabergoline	98.5	398
Pergolide	88.5	74.1
Methylergonovine	49.5	21.4
Roprinirole	*73*	*2570*
Guanfacine	*93*	*123*
Oxymetazoline	*70.9*	*45.7*
Quinidine	*55.9*	*186*
Xylometazoline	*55.7*	*240*
Fenoldopam	*92.5*	*77.6*

[1] Adapted from: Huang, X.P., et al., *Parallel functional activity profiling reveals valvulopathogens are potent 5-hydroxytryptamine(2B) receptor agonists: implications for drug safety assessment.* Mol Pharmacol, 2009. **76**(4): p. 710–22.