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## HTR2B, 5-hydroxytryptamine (serotonin) receptor 2B

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Synonyms

5-hydroxytryptamine (serotonin) receptor 2B

5-HT<sub>2B</sub> receptor

5-HT(2B)

5-HT-2B

5-HT<sub>2B</sub>

Serotonin receptor 2B

5-hydroxytryptamine 2B receptor

### Historical Background

Because of its exquisite sensitivity to serotonin, the rat stomach fundus was used as a bioassay for serotonin before the development of more quantitative analytical assays for this biogenic amine (Vane 1957). Although the potency for the contractile effects of serotonin has been known for long time, the receptors mediating such a response have eluded definitive characterization for long time. Pharmacological studies attempting to characterize the contractile serotonergic receptor in the rat stomach fundus documented the similarity to the 5-HT<sub>2C</sub> receptor. In the absence of detectable 5-HT<sub>2C</sub> receptor mRNA in the rat stomach fundus, only molecular cloning allowed the identification of a new receptor in 1992 in rat and mouse (Foguet et al. 1992; Kursar et al. 1992; Loric et al. 1992; Wainscott et al. 1993) and in 1994 in humans (Choi et al. 1994; Kursar et al. 1994; Schmuck et al. 1994; Wainscott et al. 1996), now called 5-HT<sub>2B</sub> receptor. Subsequent pharmacological characterization of this receptor subtype in various species identified differences in its pharmacology, and confirmed the close identity of this pharmacology to that of 5-HT<sub>2C</sub> receptors. Its physiological and pathophysiological functions include many differentiation steps both in periphery and central nervous system that were not previously identified or attributed to other receptor subtypes.

### 1-Structure and properties of 5-HT<sub>2B</sub> receptors

#### 1.1-Selective agonists

- BW723C86: 1-methyl-2- [5-(2-thienylmethoxy)-1H-indole-3-yl] ethylamine hydrochloride has been reported to have 10-fold selectivity over the human 5-HT<sub>2C</sub> and 100-fold selectivity over the 5-HT<sub>2A</sub> receptors. Recommended use: ≤100 nM concentration or ≤3 mg/kg ip in rodents (Porter et al. 1999; Jerman et al. 2001; Knight et al. 2004; Cussac et al. 2008).

- Alpha-methyl serotonin is a full agonist with high affinity for the 5-HT<sub>2B</sub> receptor site (pEC<sub>50</sub>=8.4) and lower affinity for both 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub>.

- 5-Methoxytryptamine is also 25- and 400-fold selective over the 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor sites, respectively.

- Nor-dexfenfluramine (metabolite of dexfenfluramine), methylergonovine (metabolite of methysergide), and Ro 60-0175: 2(S)-1-(6-chloro-5-fluoro-1H-indol-1-yl)-2-propanamine fumarate are all preferential 5-HT<sub>2B</sub> agonists with about 10 fold selectivity over 5-HT<sub>2C</sub> receptor.

Pharmacological analysis has shown that the 5-HT<sub>2B</sub> receptor displays high affinity binding to its endogenous ligand serotonin (K<sub>d</sub> ~10 nM), a value significantly different from that for the 5-HT<sub>2A</sub> receptor. 5-HT<sub>2B</sub> receptors are the targets of many 5-HT<sub>2</sub> non-selective compounds, metabolites of therapeutic compounds and drugs of abuse. Agonists include MDA (3,4-methylene dioxamphetamine-MDA, metabolite of 3,4-Methylenedioxy methamphetamine-MDMA) (Setola et al. 2003), tryptamine, and lysergic acid diethylamide (LSD) all exhibit the highest potencies for 5-HT<sub>2B</sub>. 2,5-dimethoxy-4-iodoamphetamine (DOI) is a nearly full agonist at 5-HT<sub>2B</sub> receptors with similar affinity to 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors (Porter et al. 1999; Jerman et al. 2001; Knight et al. 2004; Cussac et al. 2008). Many substances from the class of "new" drugs known as "legal highs" were found to display notable affinity for 5-HT<sub>2B</sub> receptors, including 5-APB (K<sub>i</sub> = 14 nM) and 6-APB (K<sub>i</sub> = 3.7 nM), and 5-iodo-aminoindane (K<sub>i</sub> = 70 nM). Functional assays of 5-APB and 6-APB confirmed that these compounds acted as potent (i.e., nanomolar EC<sub>50</sub> values) full agonists at 5-HT<sub>2B</sub> receptors (Iversen et al. 2013; Rickli et al. 2015). Other compounds exhibited sub-micromolar affinities for the 5-HT<sub>2B</sub> receptor (mephedrone, naphyrone, 1-naphyrone and methylenedioxy-aminotetralin). 5-APB, commonly marketed as 'benzofury' a new psychoactive substance was shown to cause contraction of rat stomach fundus, which was reversed by the 5-HT<sub>2B</sub> receptor antagonist RS127445 (Dawson et al. 2014). This finding is potentially important because previous studies have shown that there was a correlation in a series of phenyliso-propylamines between hallucinogenic activity and affinity for the 5-HT<sub>2B</sub> receptor (Nelson et al.

1999). Activation of this receptor appears to play a key role in the behavioral stimulant and serotonin releasing effects of MDMA (Doly et al. 2008) and in the reinforcing effects of MDMA in mice (Doly et al. 2009).

## 1.2 Selective antagonists

- The first highly selective antagonist reported was LY266097: 1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9Hpyrido [3,4-b]indole hydrochloride with a pKi of 9.7 for the human cloned 5-HT<sub>2B</sub> receptor and a 100-fold greater selectivity over human 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> sites (Audia et al. 1996). Recommended use: 20 nM concentration or 0.5 mg/kg ip in rodents.

- SB204741: N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea has been reported as a selective 5-HT<sub>2B</sub> receptor antagonist with approximately 100-fold selectivity over the 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> sites but with quite low potency (Ki around 100 nM) (Bonhaus et al. 1995). Recommended use: 500 nM concentration or 10 mg/kg ip in rodents.

- The tetrahydro- $\beta$ -carboline, LY272015: 6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide is also a fairly selective and highly potent antagonist (Cohen et al. 1996). Recommended use: 50 nM concentration or 1 mg/kg ip in rodents.

- RS127445: 2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine was found to have sub-nanomolar affinity for the 5-HT<sub>2B</sub> receptor (pKi = 9.5) and 1,000 fold selectivity for this receptor as compared to numerous other receptor and ion channel binding sites and appears as the most selective, high affinity 5-HT<sub>2B</sub> receptor antagonist suitable now (Bonhaus et al. 1999). Recommended use: 20 nM concentration or 0.25 mg/kg ip in rodents.

- Recently, the methoxythioxanthene BF-1 was reported as a highly selective and potent 5-HT<sub>2B</sub> receptor antagonist lacking high affinities to 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, histamine H<sub>1</sub>, dopamine D<sub>1</sub> and D<sub>2</sub> as well as muscarinic M<sub>1</sub> and M<sub>2</sub> receptors (Schmitz et al. 2015).

- S33526: 6-chloro-2,3,4,9-tetrahydro-1H-b-carbolin-1-yl-phenyl-acetic acid ethyl ester, behaves as high affinity and relatively selective antagonist at 5-HT<sub>2B</sub> receptors (Cussac et al. 2002).

- SB215505: 6-chloro-5-methyl-N-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide behaves as high affinity and preferential inverse agonists at 5-HT<sub>2B</sub> receptors.

- SB206553: 5-methyl-N-(3-pyridyl)-1,2,3,5-tetrahydrobenzo[1,2-b:4,5-b']dipyrrole-1-carboxamide is a mixed 5-HT<sub>2C</sub>/5-HT<sub>2B</sub> receptor antagonist. It has been reported as a selective 5-HT<sub>2C/2B</sub> receptor inverse agonist with 50- to 100-fold lower affinity for the 5-HT<sub>2A</sub> and other sites.

Nonselective 5-HT<sub>2</sub> receptor antagonists such as ritanserin and metergoline antagonize 5-HT<sub>2B</sub> receptor-mediated effects. Also, the  $\alpha$ 2 adrenergic receptor antagonists yohimbine and rauwolscine are potent 5-HT<sub>2B</sub> antagonists and have a low affinity for the 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> receptor sites. Atypical antipsychotics have also fairly high affinity for 5-HT<sub>2B</sub> receptors including clozapine, asenapine, or cariprazine (Wainscott et al. 1996; Millan et al. 2003; Shahid et al. 2009; Kiss et al. 2010). Aripiprazole (OPC-14597) is a new generation atypical antipsychotic drug, which has high antagonist affinity (EC<sub>50</sub> = 11 nM) for the human 5-HT<sub>2B</sub> receptor (Shapiro et al. 2003).

## 1.3 Protein structure

The 5-HT<sub>2B</sub> receptor is a 7 TM receptor, with fairly long N-terminus of about 55 amino acids. A consensus site of N-glycosylation can be found that is missing in the rat 5-HT<sub>2B</sub> receptor, questioning the N-glycosylation of the N-terminus of this receptor. The receptor consists in 481 AA in human and 479 AA in rat or mouse, with 79% homology for human vs. rat and 82% homology for human vs. mouse. A new and unanticipated role of the 5-HT<sub>2B</sub> receptor N-terminus as a negative modulator, affecting both constitutive and agonist-stimulated activity of the receptor has been recently evidenced (Belmer et al. 2014).

The recently published 5-HT<sub>2B</sub> receptor bound to ergotamine crystal structure showed that it exhibits conformational characteristics of both the active and inactive states: an active-like state in the helix VII conformation of the 5-HT<sub>2B</sub> receptor, but only partial changes in helix VI, mirrored the strong  $\beta$ -arrestin bias of ergotamine at 5-HT<sub>2B</sub> receptors observed in pharmacological assays. The differential signaling patterns were also mirrored in the crystal structures, which showed features of a  $\beta$ -arrestin-biased activation state for the 5-HT<sub>2B</sub> receptor (Wacker et al. 2013; Wang et al. 2013). A likely structural explanation for the distinct conformational features and biased pharmacology of ergotamine for 5-HT<sub>2B</sub> receptors can be found in the region of the extracellular loop 2 (ECL2) junction with helix V, E212-R213-F214 forming an additional helical turn stabilized by a structured water molecule at the extracellular tip of helix V. The segment of ECL2 connecting helices III and V via the conserved disulfide bond is, therefore, shortened in the 5-HT<sub>2B</sub> receptor, and creates a conformational constraint on the position of the extracellular tip of helix V (Martí-Solano et al. 2014). However, this structured water molecule involved in ECL2 junction with helix V has been challenged since differential interactions of ergotamine with the top of helices V and VI could determine the rotational freedom of helix VI (Liu et al. 2013).

## 1.4 Allosteric modulators

No allosteric modulator has been definitely identified yet. Ergotamine has been shown to occupy two distinct sites in 5-HT<sub>2B</sub> receptors, the orthosteric site, where the indole nucleus of ergotamine resides, and the extended binding site,

where the tripeptide portion is engaged. The allosteric site in the muscarinic M2 receptor is the same extracellular region as the tripeptide portion of ergotamine. These similarities in both the M2 and 5-HT<sub>2B</sub> receptors suggest that the location of the extracellular allosteric site for Class A GPCRs is quite similar, and in fact, argue that ergotamine likely functions as a bitopic ligand; that is it occupies both the orthosteric and putative extracellular allosteric site in the 5-HT<sub>2B</sub> receptor. It is now thought that a sodium ion allosterically alters the binding pocket to dampen G-protein signaling, leaving  $\beta$ -arrestin recruitment intact. Structural consideration support that this sodium pocket is collapsed in the 5-HT<sub>2B</sub> receptor structure, (McCorvy and Roth 2015).

### 1.5 Heteromeric receptor associations

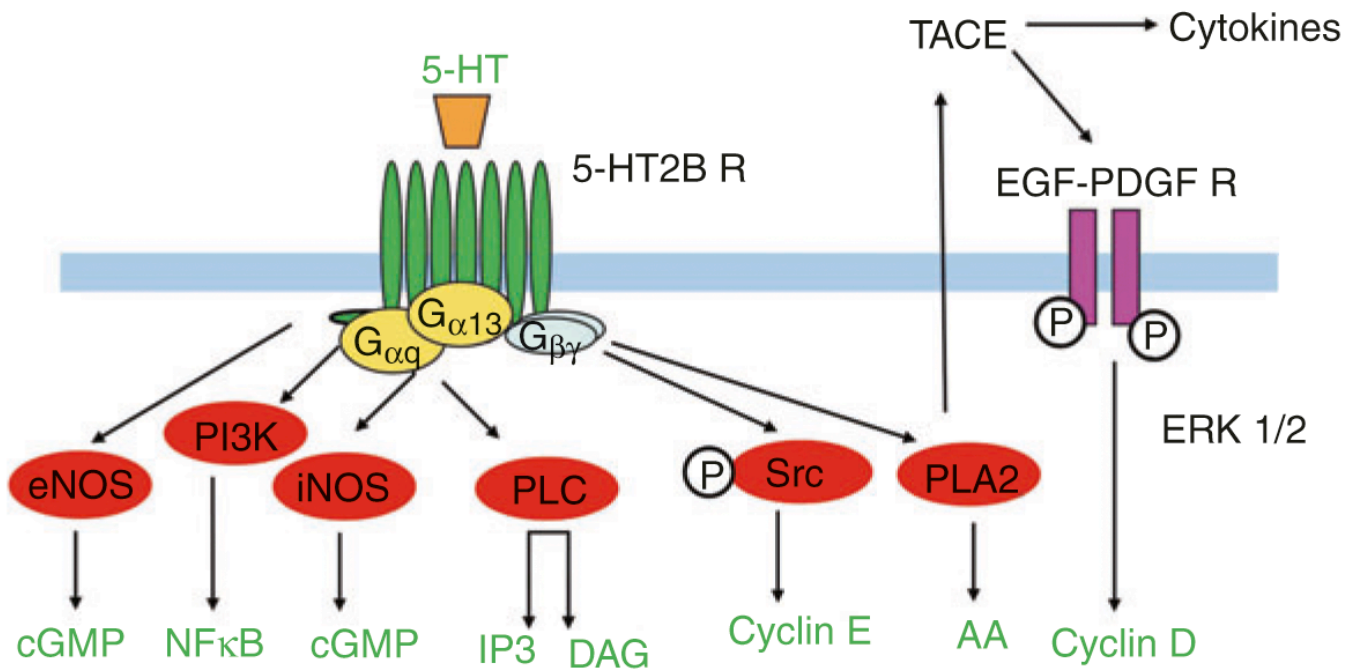
In cardiac fibroblasts, AT1 angiotensin receptors and 5-HT<sub>2B</sub> receptors, which have been reported to share common signaling pathways, which support a possible direct interaction between 5-HT<sub>2B</sub> and AT1 receptors. Using co-immunolocalization and a pull-down assay, the two receptors were shown to interact together, which suggested that these receptors could exist in heterodimeric complexes (Jaffre et al. 2009), but experimental confirmation is still lacking.

### 1.6 Transduction system(s)

#### In transfected cells

The 5-HT<sub>2B</sub> receptor, when stably transfected in mouse fibroblast L-cells, has been shown to activate GTPase activity and inositol 1,4,5- triphosphate production upon agonist stimulation, which could be blocked by antibodies against G $\alpha$ <sub>q/11</sub>, but not by pertussis or cholera toxins or by anti-G $\alpha$ i or anti-G $\alpha$ s antibodies. This GTPase activation was thus mediated by the G-protein G $\alpha$ <sub>q/11</sub>, but not by G $\alpha$ s or G $\alpha$ i. The GTPase activation was also blocked by anti- $\beta$ 1-4, or - $\gamma$ 2 subunit antibodies. Agonist stimulation of the 5-HT<sub>2B</sub> receptor caused a rapid and transient activation of the proto-oncogene product p21<sup>ras</sup> in response to serotonin, as measured by an increase in GTP bound-Ras (Launay et al. 1996). Furthermore, 5-HT<sub>2B</sub> receptor stimulation activated the mitogen-activated protein kinases (MAPKs) p42<sup>mapk</sup>/p44<sup>mapk</sup>, extracellular signal-regulated kinase 2 and 2 (ERK2/ERK1). In addition to a mitogenic action, a transforming activity of serotonin was mediated by the 5-HT<sub>2B</sub> receptor as it led to the formation of foci and to the formation of tumors from these foci in nude mice (Launay et al. 1996). Moreover, the 5-HT<sub>2B</sub> receptor-dependent cell cycle progression occurred through retinoblastoma protein hyperphosphorylation and the activation of both cyclin D1/cdk4 and cyclin E/cdk2 kinases. The induction of cyclin D1 expression, but not that of cyclin E, was under MAPK control, indicating an independent regulation of these two cyclins in 5-HT<sub>2B</sub> receptor mitogenesis. Platelet derived growth factor receptor (PDGFR) kinase activity was essential for 5-HT<sub>2B</sub>-triggered MAPK/cyclin D1, but not cyclin E, signaling pathways (Nebigil et al. 2000b). The 5-HT<sub>2B</sub> receptor activation also increases activity of the Src family kinases c-Src, Fyn and c-Yes. Strikingly, c-Src, but not Fyn or c-Yes, was the crucial molecule between the Gq protein-coupled 5-HT<sub>2B</sub> receptor and the cell cycle regulators. Inhibition of c-Src activity was sufficient to abolish the serotonin-induced: (i) PDGFR tyrosine kinase phosphorylation and MAPK activation; (ii) cyclin D1 and cyclin E expression levels; and (iii) thymidine incorporation. Thus, c-Src activation by the 5-HT<sub>2B</sub> receptor controlled cyclin E induction, and in concert with the receptor tyrosine kinase PDGFR, induced cyclin D1 expression *via* the MAPK/ERK pathway (Nebigil et al. 2000b). The 5-HT<sub>2B</sub> receptor is also coupled to the phospholipase A2 (PLA2)-mediated release of arachidonic acid (Tournois et al. 1998). In addition, stimulation of the 5-HT<sub>2B</sub> receptor triggered intracellular cGMP production through dual activation of constitutive nitric-oxide synthase (cNOS) and inducible NOS (iNOS). The group I PDZ motif at the carboxy terminus of the 5-HT<sub>2B</sub> receptor was shown to be required for recruitment of the cNOS transduction pathways, and iNOS stimulation was under control of the G $\alpha$ 13 pathways (Manivet et al. 2000). The 5-HT<sub>2B</sub> receptor shares the C-terminal E-X-V/I-S-X-V sequence with 5-HT<sub>2C</sub> receptors and also binds MUPP1-PDZ domains *in-vitro* (Becamel et al. 2001).

The signal transduction pathways are thus quite wide and include most of other known signal transduction pathways of 5-HT<sub>2A/2C</sub> receptors.



**Figure 1-** Summary of some of the identified transductions pathways that can be used by 5-HT<sub>2B</sub> receptors adapted from (Nebigil et al. 2000), (Manivet et al. 2000) and (Nebigil et al. 2003).

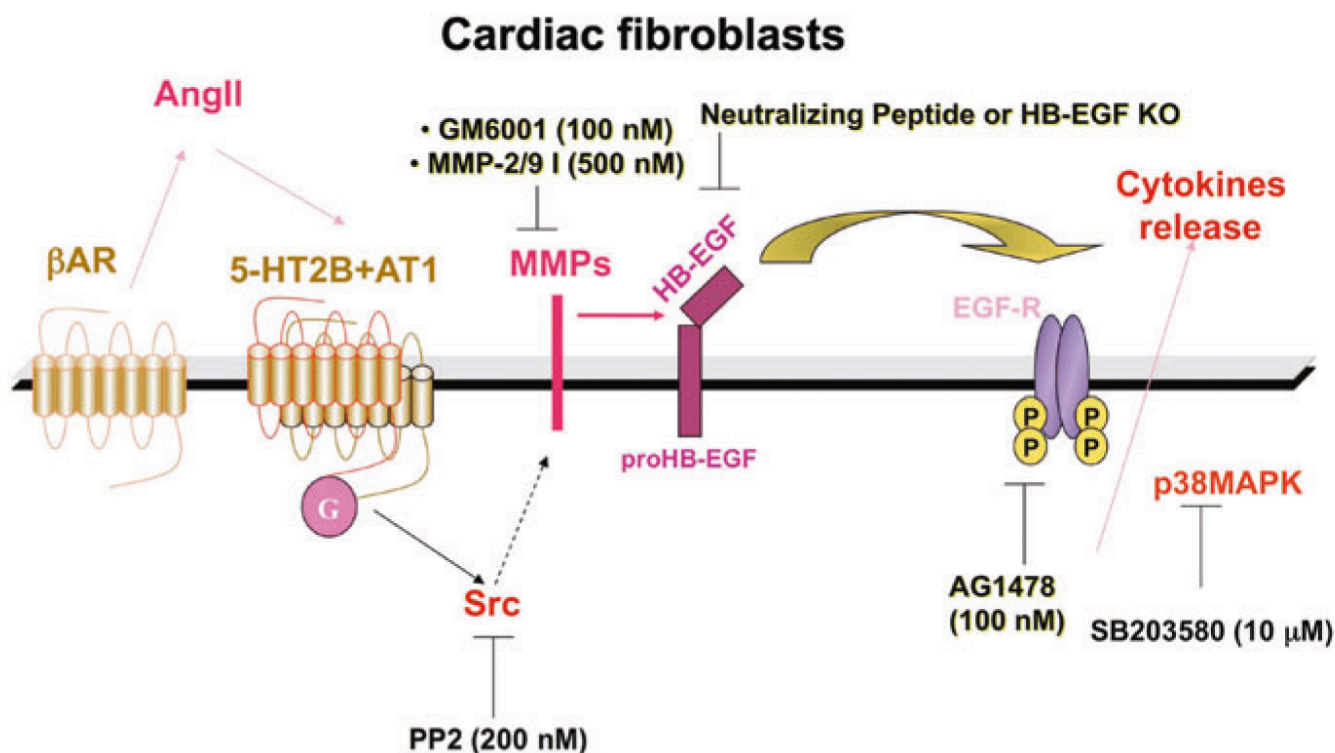
### In primary cultures

In primary culture of cardiac fibroblasts, angiotensin II (AngII) or serotonin-dependent cytokine release processes were critically dependent on HB-EGF and Src activation *via* endogenous AT<sub>1</sub> and 5-HT<sub>2B</sub> receptors (Jaffre et al. 2009). Matrix metalloproteinases (MMPs) were responsible for HB-EGF shedding and subsequent EGF-receptor transactivation that was induced by AngII or serotonin. Tumor necrosis factor- $\alpha$  converting enzyme (TACE, ADAM-17), was found to control HB-EGF shedding in fibroblasts and was directly regulated by 5-HT<sub>2B</sub> receptors (Pietri et al. 2005). All these findings support the idea that AT<sub>1</sub> and 5-HT<sub>2B</sub> receptors share common EGF receptor-dependent signaling pathways in adult cardiac fibroblasts. Blockade of one of the two receptors prevented cytokine release induced by the other receptor, supporting the idea of interactions between 5-HT<sub>2B</sub> and AT<sub>1</sub> receptors (Jaffre et al. 2009). Using co-immunolocalization and a pull-down assay, the two receptors were shown to interact in a common cell compartment. Reports have suggested that these receptors exist in heterodimeric complexes that may play a key role in receptor maturation and trafficking to the plasma membrane and/or signaling (Bulenger et al. 2005). Together, these findings indicate that heteromeric AT<sub>1</sub> and 5-HT<sub>2B</sub> receptors exist and result in common signaling complexes regulating hypertrophic factors in heart (Jaffre et al. 2009).

Stimulation of 5-HT<sub>2B</sub> receptors on hepatic stellate cells (HSC) was recently shown to activate expression of TGF $\beta$ 1 (a powerful suppressor of hepatocyte proliferation) *via* ERK/JunD signaling. Antagonists of 5-HT<sub>2B</sub> receptor have been shown to decrease mRNA levels of TGF $\beta$ 1, connective growth factor, plasminogen activator inhibitor-1, Smad-3 and JunD in lung and skin fibroblasts (Dees et al. 2011). Activation of the 5-HT<sub>2B</sub> receptor leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy of hepatocellular carcinomas (Soll et al. 2010).

The 5-HT<sub>2B</sub> receptor has been shown to protect newborn post-mitotic cardiomyocytes against serum deprivation-induced apoptosis as manifested by DNA fragmentation, nuclear chromatin condensation, and TUNEL labeling. Serotonin prevented cytochrome c release and caspase-9 and -3 activation after serum deprivation *via* cross talks between phosphatidylinositol-3 kinase (PI3K)/Akt and ERK1/2 signaling pathways. Serotonin binding to 5-HT<sub>2B</sub> receptor activated ERK kinases that inhibited Bax expression induced by serum deprivation. Serotonin *via* PI3K/Akt activated nuclear factor- $\kappa$ B (NF- $\kappa$ B) that was required for the regulation of the mitochondrial adenine nucleotide translocator (ANT-1) and mitochondrial permeability. These findings identified serotonin as a novel cardiomyocyte survival factor targeting mitochondria (Nebigil et al. 2003). Interestingly, NF- $\kappa$ B regulation by 5-HT<sub>2B</sub> receptors was confirmed in a large screen for genes regulating NF- $\kappa$ B and the MAPK pathways (Matsuda et al. 2003).

As in transfected cells, endogenously expressed 5-HT<sub>2B</sub> receptors can stimulate various transduction pathways according to the cell subtype including Src, MMPs, PLA2 activities.



**Figure 2-** Signal transduction mediated by 5-HT<sub>2B</sub> receptors in cardiac fibroblasts to control the release of hypertrophic cytokines by interacting with AT<sub>1</sub> receptors. Inhibitors used to determine this transduction pathway are shown, adapted from (Jaffre et al. 2009).

#### 1.7 Adult expression

##### Embryonic expression

The 5-HT<sub>2B</sub> receptor mRNA was detected in mouse embryos since 8.5 days post-coitum (dpc), whereas there were only low levels of 5-HT<sub>2A</sub> and no 5-HT<sub>2C</sub> receptor mRNA expression at this stage. Expression of this receptor was confirmed by pharmacological assays (Choi et al. 1997). Teratogenesis by retinoic acid or blockade of serotonergic signaling by 5-HT<sub>2</sub> receptor antagonists perturbs development, resulting in forebrain and olfactory placode anomalies, malformations of the face, eye and lens, as well as posterior neural tube and cardiac defects (Bhasin et al. 2004). These pathways may act as opposing signals for common targets in the mouse embryo. The importance of 5-HT<sub>2B</sub> receptors in cardiac development has been validated by genetic inactivation by homologous recombination of the 5-HT<sub>2B</sub> receptor gene, which leads to embryonic and neonatal death due to the following defects in the heart: *Htr<sub>2B</sub><sup>-/-</sup>* embryos exhibit a lack of trabeculae in the heart leading to mid-gestation lethality and newborn *Htr<sub>2B</sub><sup>-/-</sup>* mice exhibit cardiac dilation resulting from contractility deficits and structural deficits at the intercellular junctions between cardiomyocytes (Nebigil et al. 2000a). Report showed that serotonin acts downstream of lactogen signaling to stimulate  $\beta$ -cell proliferation. Blocking 5-HT<sub>2B</sub> signaling in pregnant mice also blocked  $\beta$ -cell expansion and caused glucose intolerance (Kim et al. 2010).

##### Peripheral expression

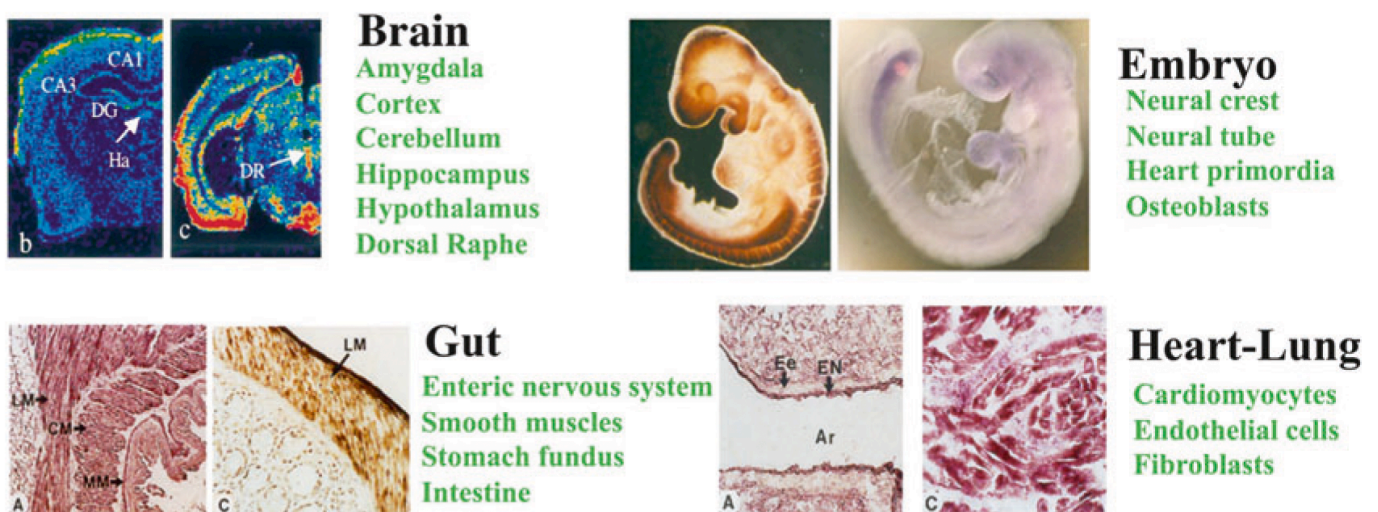
By Northern blot and immunohistochemistry, 5-HT<sub>2B</sub> receptor mRNA and protein expression was detected in the stomach fundus, intestine, liver, kidney, pancreas, spleen and lung, as well as in the myocardium of several species including rats, mice and humans (Kursar et al. 1992; Choi et al. 1994; Kursar et al. 1994; Choi and Maroteaux 1996). The presence of a functionally active 5-HT<sub>2B</sub> receptor subtype in human uterine smooth muscle cells was reported (Kelly and Sharif 2006). Reports using pharmacological or molecular studies indicated that the 5-HT<sub>2B</sub> receptor was also expressed in blood vessels, including smooth muscle cells (Ullmer et al. 1995) as well as on the endothelial cells of pig pulmonary arteries (Glusa and Pertz 2000), of human meningeal blood vessels (Schmuck et al. 1996), and of rat jugular vein (Ellis et al. 1995). The gene coding for the 5-HT<sub>2B</sub> receptor was found expressed in human and rodent anagen skin (but not telogen), in melanomas and Mel A melanocytes (Slominski et al. 2003; Slominski et al. 2004). In addition, 5-HT<sub>2B</sub> receptor expression has been detected in rat spleen, thymus, peripheral blood lymphocytes (Stefulj et al. 2000). A 5-HT<sub>2B</sub> receptor expression was detected in c-kit<sup>+</sup> bone-marrow cells (Launay et al. 2012). Finally, 5-HT<sub>2B</sub> receptor expression was also demonstrated in rat osteocytes, osteoblasts and a population of periosteal fibroblasts containing osteoblast precursor cells (Bliziotis et al. 2001; Westbroek et al. 2001).

## Central expression

In human brain, 5-HT<sub>2B</sub> receptor expression has been reported in the cerebral cortex, cerebellar nuclei and their projection areas, lateral septum, dorsal hypothalamus and medial amygdala (Kursar et al. 1992; Choi et al. 1994; Kursar et al. 1994; Choi and Maroteaux 1996). Expression of serotonin 5-HT<sub>2B</sub> receptor mRNA was also confirmed in several brain nuclei including the dorsal raphe nuclei by gene expression profiling in the mammalian brain, and by *in-situ* hybridization (Bonaventure et al. 2002). Human brain expression was confirmed by qPCR in frontal, temporal, parietal, and occipital lobe, olfactory region, cerebellum, diencephalon, hippocampus, thalamus, pituitary gland, pons, medulla oblongata and nucleus accumbens (NAcc) (Bevilacqua et al. 2010). The presence of microglial 5-HT<sub>2B</sub> receptors was confirmed by qPCR analysis of RNA isolated from primary cultured and acutely isolated adult microglia. Two photon microscopy on brain slices (Kolodziejczak et al. 2015) and patch-clamp experiments in cultured microglia (Krabbe et al. 2012) confirmed that modulation of microglial functions like phagocytosis and migration is fundamental for the central nervous system since microglia can influence the balance of synaptogenesis and neuronal death in pathology.

The 5-HT<sub>2B</sub> receptor has also been shown to participate in the release of rat growth hormone (GH) through a pituitary site since the 5-HT<sub>2B</sub> receptor agonist BW723C86 stimulated GH release that was blocked by the 5-HT<sub>2B</sub> receptor antagonist SB204741 (Papageorgiou and Deneff 2007). The expression of 5-HT<sub>2B</sub> receptor mRNA was reported in rat cultured astrocytes (Sanden et al. 2000; Osredkar and Krzan 2009). Expression of the 5-HT<sub>2B</sub> receptor was also found in spinal cord tissue (Helton et al. 1994; Holohean and Hackman 2004), in the rat organ of Corti lateral wall and spiral ganglion subfractions (Oh et al. 1999), and upregulated with age in the mice cochlea (Tadros et al. 2007). In human eye, *HTR<sub>2B</sub>* mRNA was found predominantly expressed in the retina, ciliary body, ciliary epithelium, choroid, conjunctiva, and iris. Optic nerve tissue of human donors exhibited the presence of *HTR<sub>2B</sub>* mRNA as human trabecular meshwork cells (Sharif and Senchyna 2006).

In addition to embryos, 5-HT<sub>2B</sub> receptor mRNA expression has been quite widely detected in peripheral organs, stomach, intestine, liver, kidney, pancreas, spleen and lung as well as in myocardium. Its expression has also been reported, although at fairly low level in the brain, in cerebral cortex, cerebellar nuclei and their projection areas lateral septum, dorsal hypothalamus and medial amygdala. Detection in humans or other vertebrate species is globally similar.



**Figure 3-** Major sites of expression of 5-HT<sub>2B</sub> receptors are shown. This includes brain as illustrated by *in situ* hybridization on rat brain sections, embryos as illustrated by *in situ* hybridization and immunohistochemistry on mouse embryos, immunohistochemistry in mouse gut and cardiopulmonary systems adapted from (Choi and Maroteaux 1996), (Choi et al. 1997) and (Bonaventure et al. 2002).

## 2-Pathophysiological functions in central nervous system

### 2.1 Response to antidepressants

Neurotransmission by serotonin is tightly regulated by autoreceptors that fine-tune serotonergic neurotransmission through negative feedback inhibition at the cell bodies (predominantly 5-HT<sub>1A</sub>) or at the axon terminals (predominantly 5-HT<sub>1B</sub>); however, different roles for 5-HT<sub>2B</sub> receptors have also been detected (McDevitt and Neumaier 2011). The therapeutic effects induced by serotonin-selective reuptake inhibitor (SSRI) antidepressants are initially triggered by blocking the SERT and rely on long-term adaptations of pre- and post-synaptic receptors. Long-term behavioral and neurogenic SSRI effects were abolished after either genetic or pharmacologic inactivation of 5-HT<sub>2B</sub> receptors (Diaz et al. 2012). Conversely, direct agonist stimulation of 5-HT<sub>2B</sub> receptors induced an SSRI-like response in behavioral and neurogenic assays. The 5-HT<sub>2B</sub> receptor was found expressed by raphe serotonergic

neurons by single cell PCR. The SSRI-induced increase in hippocampal extracellular serotonin concentration was strongly reduced in the absence of functional 5-HT<sub>2B</sub> receptors. Furthermore, a selective 5-HT<sub>2B</sub> agonist mimicked SSRI responses, supporting a positive regulation of serotonergic neurons by 5-HT<sub>2B</sub> receptors (Diaz et al. 2012). Furthermore, *Htr<sub>2B</sub><sup>-/-</sup>* mice display an antidepressant-like phenotype, which includes reduced latency to feed in the novelty suppressed feeding test, basal increase in hippocampal BDNF levels, no change in TrkB and p75 protein levels, and an increased preference for sucrose consumption compared to wild type mice. Nevertheless, these mice can develop depressive-like behaviors when socially isolated during four weeks. SSRI have been previously shown to be ineffective in non-stressed *Htr<sub>2B</sub><sup>-/-</sup>* mice and as well in chronically stressed *Htr<sub>2B</sub><sup>-/-</sup>* mice no behavioral or plastic effect was induced by SSRI antidepressants (Diaz et al. 2016). The 5-HT<sub>2B</sub> receptor appears, therefore, to positively modulate serotonergic activity and to be required for the therapeutic actions of SSRIs.

## 2.2 Response to anorexigens

The hypophagic response to the anorexigen and serotonin releaser, dexfenfluramine, observed in wild-type mice is also eliminated in *Htr<sub>2B</sub><sup>-/-</sup>* mice or in wild-type mice treated with RS127445. Using microdialysis, the dexfenfluramine-induced hypothalamic peak of serotonin release was found strongly reduced in *Htr<sub>2B</sub><sup>-/-</sup>* awake mice compared with wild type. Moreover, a strong serotonin release was observed upon dexfenfluramine stimulation of a synaptosomal preparation from wild type but not from *Htr<sub>2B</sub><sup>-/-</sup>* mice (Banas et al. 2011). A 5-HT<sub>2B</sub> receptor-dependent modulation of presynaptic effectors may explain this requirement for 5-HT<sub>2B</sub> receptors in releaser action.

## 2.3 Response to MDMA

The "club drug" 3,4-methylenedioxymethamphetamine (MDMA; also known as ecstasy) binds preferentially to and reverses the activity of the serotonin transporter, causing release of serotonin stores from nerve terminals. Subsequent activation of postsynaptic 5-HT receptors by released 5-HT has been shown to be critical for the unique psychostimulatory effects of MDMA. Acute pharmacological inhibition or genetic ablation of the 5-HT<sub>2B</sub> receptor in mice completely abolishes MDMA-induced hyperlocomotion and 5-HT release in NAcc and ventral tegmental area. Furthermore, the 5-HT<sub>2B</sub> receptor dependence of MDMA-stimulated release of endogenous 5-HT from superfused midbrain synaptosomes suggests that 5-HT<sub>2B</sub> receptors act, unlike any other 5-HT receptor, presynaptically to affect MDMA-stimulated 5-HT release. Thus, the 5-HT<sub>2B</sub> receptor is a novel regulatory component in the actions of MDMA (Doly et al. 2008). However, the role of serotonin/dopamine interactions in the behavioral effects of MDMA remains unclear. *Htr<sub>2B</sub><sup>-/-</sup>* mice do not exhibit behavioral sensitization or conditioned place preference following MDMA injections. In addition, MDMA-induced reinstatement of conditioned place preference after extinction and locomotor sensitization development are each abolished by a 5-HT<sub>2B</sub> receptor antagonist (RS127445) in wild type mice. Accordingly, MDMA-induced dopamine D1 receptor-dependent phosphorylation of extracellular regulated kinase in NAcc is abolished in mice lacking functional 5-HT<sub>2B</sub> receptors. These results underpin the importance of 5-HT<sub>2B</sub> receptors in the reinforcing properties of MDMA and illustrate the importance of serotonin/dopamine interactions on MDMA effects (Doly et al. 2009). Moreover, another selective 5-HT<sub>2B</sub> receptor antagonist LY 266097, which has no influence on basal accumbal and striatal DA outflow, reduces significantly accumbal DA outflow. A significant reduction of basal DA outflow in the NAcc was also observed after i.p. administration of the 5-HT<sub>2B</sub> receptor antagonist RS 127445. In contrast, the 5-HT<sub>2B</sub> receptor agonist BW 723C86 had no influence on basal DA outflow in either brain region. The increase in striatal and accumbal DA outflow induced by the 5-HT<sub>2B/2C</sub> receptor inverse agonist SB 206553 was unaltered by LY 266097 pretreatment. Conversely, LY 266097 significantly diminished the increase in DA outflow induced by haloperidol or amphetamine in the NAcc, but not in the striatum. Amphetamine-induced hyperlocomotion was also attenuated by LY 266097. Thus 5-HT<sub>2B</sub> receptors exert a facilitatory control on mesoaccumbens DA pathway activity, and may constitute a new target for improved treatment of DA-related neuropsychiatric disorders (Auclair et al. 2010).

These findings strongly suggest that activation of 5-HT<sub>2B</sub> receptors is a limiting step in the SERT dependent-increase in extracellular 5-HT that is required for SSRIs, MDMA, or anorexigen action.

## 2.4 Impulsivity and psychosis

Impulsivity and hyperactivity share common ground with numerous mental disorders, including schizophrenia. A population-specific serotonin<sub>2B</sub> (5-HT<sub>2B</sub>) receptor stop codon (i.e., *HTR<sub>2B</sub> Q20\**) was reported to segregate with severely impulsive individuals (Bevilacqua et al. 2010). Especially under conditions where control was impaired, the carriers of the stop codon being more vulnerable to alcohol were more impulsive if they drank. Similarly, *Htr<sub>2B</sub><sup>-/-</sup>* mice displayed more impulsive choice in delayed discounting tasks, sought novelty, and were more active after receiving a D1 dopamine receptor agonist (Bevilacqua et al. 2010). Interestingly, in the same cohort, early-onset schizophrenia was more prevalent in *HTR<sub>2B</sub> Q\*20* carriers. Recently, it was shown that domains related to the positive, negative and cognitive symptom-clusters of schizophrenia are affected in *Htr<sub>2B</sub><sup>-/-</sup>* mice, as shown by deficits in sensorimotor gating, in selective attention, in social interactions and in learning and memory processes. In addition, *Htr<sub>2B</sub><sup>-/-</sup>* mice presented with enhanced locomotor response to the psychostimulant dizocilpine and amphetamine, and with robust alterations in sleep architecture. Moreover, ablation of 5-HT<sub>2B</sub> receptors induced a region-selective decrease of dopamine and glutamate concentrations in the dorsal striatum. Importantly, selected schizophrenic-like phenotypes and endophenotypes were rescued by chronic haloperidol treatment (Pitychoutis et al. 2015). 5-HT<sub>2B</sub> receptor



deficiency confers a wide spectrum of antipsychotic-sensitive schizophrenic-like behavioral and psychopharmacological phenotypes in mice and provide first evidence for a role of 5-HT<sub>2B</sub> receptors in the neurobiology of psychotic disorders.

The phenotype of *Htr<sub>2B</sub><sup>-/-</sup>* mice results from a combination of both the direct absence of 5-HT<sub>2B</sub> receptor signaling and the neural adaptations triggered by the permanent lack of this receptor.

### 3-Pathophysiological peripheral functions

#### 3.1 Cardiopulmonary and vascular diseases

Inactivation of the 5-HT<sub>2B</sub> receptor gene by homologous recombination leads to partial embryonic lethality due to defects in the heart development. Neonates exhibit a second wave of partial lethality due to cardiac dilation resulting from contractility deficits and structural deficits at the intercellular junctions between cardiomyocytes. Echocardiography and electrocardiography studies in animals that past the first week and survive until adulthood, confirm the presence of left ventricular dilation and decreased systolic function (Nebigil et al. 2000a). Serotonin, via the 5-HT<sub>2B</sub> receptor, regulates differentiation and proliferation during development as well as cardiac structure and function in adults. In adults, 5-HT<sub>2B</sub> receptors were found to be overexpressed in heart from patients with congestive heart failure (Jaffre et al. 2009). Serotonin plasma levels are also increased in patients with heart failure and in animal studies with cardiac hypertrophy induced by aortic constriction. By mimicking sympathetic stimulation *in-vivo*, mice globally lacking serotonin 5-HT<sub>2B</sub> receptors did not develop isoproterenol-induced left ventricular hypertrophy (Jaffré et al. 2004). The exact cardiac cell type(s) expressing 5-HT<sub>2B</sub> receptors (cardiomyocytes versus non-cardiomyocytes) involved in this pathological heart hypertrophy was addressed *in-vivo*: mice expressing the 5-HT<sub>2B</sub> receptor solely in cardiomyocytes, like global 5-HT<sub>2B</sub> receptor-null mice, are resistant to isoproterenol-induced cardiac hypertrophy and dysfunction, as well as to isoproterenol-induced increases in plasma cytokine levels pointing to non-cardiomyocytes mediated effects (Jaffre et al. 2009). In primary culture of cardiac fibroblasts, angiotensin II and isoproterenol stimulated NOX activity that was prevented by a selective antagonist (SB215505). The 5-HT<sub>2B</sub> receptor blockade by SB215505 prevented the increase in cardiac superoxide generation and hypertrophy in two models of cardiac hypertrophy, i.e., angiotensin II and isoproterenol infusions in mice (Monassier et al. 2008). A functional interaction between AT1 and 5-HT<sub>2B</sub> receptors *via* a transinhibition mechanism that may involve heterodimeric receptor complexes was shown to trigger cytokine release in cardiac fibroblasts (Jaffre et al. 2009).

The 5-HT<sub>2B</sub> receptor has also been shown to be involved in cardiac hypertrophy by acting directly on cardiac myocytes. After two weeks of aortic banding surgery, mRNA and protein expression of 5-HT<sub>2B</sub> receptors increased significantly. The antagonist, SB215505, significantly reduced the increase in heart weight, heart wall thickness, left ventricular mass and the expression of the brain natriuretic peptide (BNP) but did not attenuate the up-regulation of 5-HT<sub>2B</sub> receptor protein expression in rats after aortic banding. Following *in-vitro* mechanical stretch of cardiomyocytes and incubation with serotonin, the level of 5-HT<sub>2B</sub> receptors and BNP protein increased time-dependently. When transfected with specific siRNA for 5-HT<sub>2B</sub> receptors in cardiomyocytes, the increase of NF-κB translocation and BNP protein induced by serotonin incubation plus mechanical stretch were both reversed (Liang et al. 2006). The involvement of 5-HT<sub>2B</sub> receptors was also reported in the generation of apoptotic events associated with cardiac remodeling during increased adrenergic stimulation (Bai et al. 2010).

These data revealed a dual role of 5-HT<sub>2B</sub> receptors on both cardiomyocytes and cardiac fibroblasts in regulating cardiac hypertrophy *in-vivo*.

Pulmonary arterial hypertension (PAH) is a progressive and often fatal disorder in humans that results from an increase in pulmonary blood pressure associated with abnormal vascular proliferation. Serotonin is associated with the pathogenesis of PAH (Chan and Loscalzo 2008). Therapeutic drugs with PAH as a side effect, like the amphetamine derivative and anorexigen dexfenfluramine, are potent serotonin releasers acting at SERT and/or agonists at 5-HT<sub>2B</sub> receptors (Weir et al. 2008). Serotonergic anorexigen-induced PAH is clinically indistinguishable from the heritable form of disease, associated with *BMPR2* mutations. Blockade of 5-HT<sub>2B</sub> receptors using independent approaches, either genetic (*Htr<sub>2B</sub><sup>-/-</sup>*) or pharmacologic inactivation (5-HT<sub>2B</sub> receptor antagonist RS127445), completely prevented the development of hypoxia-induced pulmonary hypertension in mice including the increase in pulmonary blood pressure and lung remodeling, the increase in vascular proliferation, elastase activity and transforming growth factor-β-1 (TGFβ1) levels (Launay et al. 2002). Using the monocrotaline-induced pulmonary hypertension model, more studies confirmed that other 5-HT<sub>2B</sub> receptor antagonists (terguride, PRX-08066, or C-122) significantly reduced pulmonary pressure, arterial wall thickening and lumen occlusion but maintained cardiac function (Porvasnik et al. 2010; Dumitrascu et al. 2011; Zopf et al. 2011). Pulmonary hypertension is associated with a substantial increase in 5-HT<sub>2B</sub> receptor expression in pulmonary arteries in rodents and humans (Launay et al. 2002; Dumitrascu et al. 2011). Activation of 5-HT<sub>2B</sub> receptors appears to be, therefore, a limiting step in the development of pulmonary hypertension.

In *Bmpr2R899X* mutant mice, which spontaneously develop pulmonary hypertension, the 5-HT<sub>2B</sub> receptor antagonist, SB204741 prevented the development of pulmonary hypertension, reduced recruitment of inflammatory cells to their lungs, and reduced muscularization of their blood vessels (West et al. 2016). Using bone-marrow transplantation, the restricted expression of 5-HT<sub>2B</sub> receptors to bone-marrow cells was shown as necessary and

sufficient for hypoxia- or monocrotaline-induced pulmonary hypertension to develop *via* an action at hematopoietic stem cell differentiation (Launay et al. 2012). Interestingly, *Bmpr2R899X* mutant bone-marrow cells were also shown to cause spontaneous pulmonary hypertension with remodeling and inflammation when transplanted into control mice, while control bone-marrow cells had a protective effect against the development of disease, when transplanted into mutant mice. Bone-marrow cells play thus a key role in genetic pulmonary hypertension pathogenesis (Yan et al. 2016).

These findings reveal the limiting role of serotonin via 5-HT<sub>2B</sub> receptors in PAH development and shift the contribution of serotonin to PAH to an extrapulmonary, hematopoietic event.

One of the most profound increases in vascular responsiveness in hypertension has been observed for serotonin. Mesenteric arteries from deoxycorticosterone- (DOCA) salt hypertensive predominantly contracted *via* 5-HT<sub>2B</sub> receptors. PCR analyses indicated an increase in mRNA for the 5-HT<sub>2B</sub> receptor in mesenteric arteries of DOCA-salt hypertensive arteries, supporting an increase in receptor number (Watts et al. 1996). The endothelium-denuded isolated superior mesenteric artery of DOCA-salt rats displayed a marked increase in maximum contraction to the 5-HT<sub>2B</sub> receptor agonist BW723C86 compared with that of arteries from sham rats, confirming that the 5-HT<sub>2B</sub> receptor plays a greater role in serotonin-induced contraction in arteries from DOCA-salt rats. In chronically instrumented rats, the 5-HT<sub>2B</sub>-receptor antagonist LY272015 significantly reduced mean blood pressure (Watts and Fink 1999). LY272015 produced a 4-fold rightward shift to serotonin in aorta from hypertensive rats made by exposure to the nitric-oxide synthase inhibitor N(omega)-nitro-L-arginine, and blockade by ketanserin did not occur at low concentrations of serotonin (Russell et al. 2002).

These findings revealed that the 5-HT<sub>2B</sub> receptor plays an important role in serotonin-induced contraction in arteries of hypertensive individuals.

### 3.2 Fibrosis

Serotonin was shown to increase proliferation and collagen synthesis by lung fibroblasts. Serotonin concentrations in lung homogenates increased significantly over the time course of bleomycin-induced fibrosis, with a maximum at day seven, together with the expression of serotonin receptors 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> (Königshoff et al. 2010). Blockade of 5-HT<sub>2B</sub> receptors by SB215505 reduced bleomycin-induced lung fibrosis, as demonstrated by reduced lung collagen content and reduced procollagen 1 and procollagen 3 mRNA expression. 5-HT<sub>2B</sub> receptor antagonists promoted an antifibrotic environment by decreasing the lung mRNA levels of TGFβ1, connective growth factor and plasminogen activator inhibitor-1 and JunD mRNA. Interestingly, the 5-HT<sub>2B</sub> receptor was strongly overexpressed by fibroblasts in the fibroblastic foci of human idiopathic pulmonary fibrosis samples (Fabre et al. 2008). Serotonin contribution to lung fibrosis is thus controlled by 5-HT<sub>2B</sub> receptors regulating TGFβ1 levels.

In the liver, fibrogenic hepatic stellate cells (HSC) are known to express also 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors that may regulate TGFβ1 and Smads (Li et al. 2006). After HSC activation, expression of 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors was found 100 and 50-fold that of quiescent cells, respectively. The 5-HT<sub>2B</sub> receptor expression level was strongly associated with fibrotic tissue in diseased liver. Treatment of HSCs with 5-HT<sub>2</sub> receptor antagonists suppressed proliferation and elevated their rate of apoptosis. Serotonin synergized with platelet-derived growth factor to stimulate increased HSC proliferation (Ruddell et al. 2006). Distinct from quiescent cells, activated HSCs exhibited [Ca<sup>2+</sup>]<sub>i</sub> transients following treatment with serotonin (Park et al. 2011). Stimulation of 5-HT<sub>2B</sub> receptors on HSC by serotonin was shown to activate expression of TGFβ1 *via* ERK/JunD signaling. Selective antagonism of 5-HT<sub>2B</sub> receptors enhanced hepatocyte growth in models of acute and chronic liver injury. Similar effects were observed in mice lacking 5-HT<sub>2B</sub> receptor or JunD and when HSCs have been selectively depleted (Ebrahimkhani et al. 2011). Therefore, 5-HT<sub>2B</sub> receptor appears to have a dual role on liver, promoting regeneration physiological conditions and fibrosis in pathological conditions.

Dermal fibrosis was independently shown to be reduced in *Htr2B*<sup>-/-</sup> mice using both inducible and genetic models of fibrosis. Pharmacologic inactivation of 5-HT<sub>2B</sub> receptor also effectively prevented the onset of experimental fibrosis and ameliorated established fibrosis by decreasing mRNA levels of TGFβ1, connective growth factor, plasminogen activator inhibitor-1 and Smad-3 (Dees et al. 2011). Serotonin contribution to skin fibrosis is thus controlled by 5-HT<sub>2B</sub> receptors via regulation of TGFβ1 levels.

Treatment of neonatal rat cardiac fibroblasts with serotonin increased the expression of smooth muscle α-actin, a marker of fibroblast differentiation into myofibroblasts and stimulated cardiac fibroblast migration. Incubation of cardiac fibroblasts with serotonin enhances secretion of TGFβ1 and expression of metalloproteinases (MMPs). Serotonin can thus directly act at neonatal cardiac fibroblasts by enhancing secretion of TGFβ1 and MMPs and promoting their migration and differentiation, which seem mediated through 5-HT<sub>2A</sub> receptors (Yabanoglu et al. 2009). Independently, serotonin- or AngII-stimulated cytokine release in adult cardiac fibroblasts was found to be sensitive to 5-HT<sub>2B</sub> receptor blockade, including secretion of TGFβ1. Treatments with epidermal growth factor receptor (EGFR, ErbB1/4)-selective inhibitors or with selective inhibitors of MMPs also abolished AngII- and serotonin-induced cytokine release. Finally, the use of *HB-EGF*<sup>-/-</sup> cardiac fibroblasts confirmed that EGFR stimulation is absolutely required for AngII- and serotonin-dependent cytokine release (Jaffre et al. 2009). Collectively, these

results revealed that convergent action of norepinephrine, AngII and serotonin *via* interactions between AT1 and 5-HT<sub>2B</sub> receptors coexpressed by non-cardiomyocytes are limiting key events in cardiac hypertrophy.

### 3.3 Valvular heart disease

Carcinoid heart disease occurs in over 65% of patients with the carcinoid syndrome and is characterized by fibrous thickening of cardiac valves, leading to heart failure for review (Roth 2007). Correlation of high plasma serotonin levels with valvular abnormalities detected by cardiac catheterization and echocardiography has been reported. Thus, serotonin overproduction has been proposed to be responsible for cardiac valvular disease in patients with carcinoid tumors (Robiolio et al. 1995). The similarity to lesions in carcinoid heart disease and in methysergide-associated valvular disease suggested direct stimulation of myofibroblast growth by serotonin agonism (Hendrikx et al. 1996). The occurrence of fenfluramine-associated valvular heart disease has raised concerns that other serotonergic medications might also increase the risk of developing valvular heart disease (Connolly et al. 1997). The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") and its N-demethylated metabolite 3,4-methylenedioxyamphetamine (MDA) each preferentially bind to and activate human recombinant 5-HT<sub>2B</sub> receptors and like fenfluramine and its N-deethylated metabolite norfenfluramine, elicit mitogenic responses in human valvular interstitial cells *via* activation of 5-HT<sub>2B</sub> receptors (Setola et al. 2003). Based on strikingly similar echocardiographic and histopathological features, it is now considered that ergot-derived dopamine agonists may cause a valvular heart disease nearly identical to that seen in patients with carcinoid syndrome (Horvath et al. 2004); population studies of patients with Parkinson's disease compared with non-parkinsonian controls have reported that pergolide and cabergoline have a similar risk of inducing fibrotic changes in cardiac valve leaflets. Pergolide and cabergoline have high affinity for the 5-HT<sub>2B</sub> serotonin receptors (Antonini and Poewe 2007; Roth 2007). A simultaneous mitral bioprosthesis hypertrophic scarring and native aortic valve stenosis was recently reported during benfluorex therapy in a 40-year-old woman. The bioprosthesis and aortic valves exhibited similar histopathological lesions. Thickening and plaque deposits made by smooth muscle  $\alpha$ -actin- and vimentin-positive cells in a glycosaminoglycan matrix were observed supporting that 5-HT<sub>2B</sub> receptors activated by norfenfluramine (also metabolite of benfluorex) trigger the development of drug-induced heart disease (Ayme-Dietrich et al. 2012). 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> receptor transcripts are easily detected in heart valves, while no 5-HT<sub>2C</sub> receptor transcript is detectable. Preferential stimulation of valvular 5-HT<sub>2B</sub> receptors (with or without accompanying 5-HT<sub>2A</sub> receptor activation) may contribute to valvular fibroplasia in humans (Fitzgerald et al. 2000). Mitral valve regurgitation has been associated with increased mRNA expression of valvular 5-HT<sub>2B</sub> receptors and SERT in pigs (Cremer et al. 2015b). Canine Myxomatous mitral valve disease was associated with higher expression of 5-HT<sub>2B</sub> receptors in mitral valve (Cremer et al. 2015a).

These findings suggest that 5-HT<sub>2B</sub> signaling links vascular damage and platelet activation to tissue remodeling and identify 5-HT<sub>2B</sub> as a novel therapeutic target to treat valvular heart diseases.

### 3.4 Pain

Serotonin released from mast cells or platelets in peripheral tissues is one important inflammatory mediators of pain. The involvement of serotonin in pain is complex because it could inhibit or facilitate nociceptive transmission, reflecting the presence of multiple serotonin receptor subtypes on peripheral and central nociceptors. An acute injection of serotonin or the 5-HT<sub>2</sub> agonist  $\alpha$ -methyl-serotonin in hindpaws of mice induces significant hyperalgesia to mechanical stimuli, which can be inhibited by the 5-HT<sub>2B/2C</sub> antagonist SB206553 that also blocks serotonin-induced transient [Ca<sup>2+</sup>] signaling in DRG neurons. Thus, 5-HT<sub>2B</sub> receptors are involved in mechanical hyperalgesia in mice (Lin et al. 2011). The Gq/11-PLCC $\beta$ /protein kinase C $\epsilon$  (PKC $\epsilon$ ) pathway mediated by 5-HT<sub>2B</sub> receptor is involved in 5-HT-induced mechanical hyperalgesia in mice. Administration of a transient receptor potential vanilloid 1 (TRPV1) antagonist inhibits the 5-HT-induced mechanical hyperalgesia. 5-HT injection enhanced 5-HT- and capsaicin-evoked calcium signals that are inhibited by a 5-HT<sub>2B/2C</sub> antagonist and PKC $\epsilon$  blocker. Thus, 5-HT<sub>2B</sub> mediates 5-HT-induced mechanical hyperalgesia by regulating TRPV1 function (Su et al. 2016).

Serotonin released from descending pain modulation pathways to the dorsal horn is also crucial to spinal nociception processing. Local peripheral ipsilateral but not contralateral injection of RS127445 significantly prevented 1% formalin-induced flinching behavior. Moreover, local peripheral ipsilateral but not contralateral injection of the 5-HT<sub>2</sub> receptor agonist DOI augmented 0.5% formalin-induced nociceptive behavior. The local pronociceptive effect of DOI was significantly prevented by the local injection of RS127445. Moreover, intrathecal injection of RS127445 also prevented 1% formalin-induced nociceptive behavior. In contrast, spinal injection of DOI significantly increased flinching behavior induced by 0.5% formalin. The spinal pronociceptive effect of DOI was prevented by the intrathecal injection of RS127445 (Cervantes-Durán et al. 2012). These results suggest that 5-HT<sub>2B</sub> receptors play a pronociceptive role in peripheral as well as spinal sites in the rat formalin test.

In rats subjected to spinal nerve ligation surgery, the preferential 5-HT<sub>2B</sub> agonist BW723C86 was reported to enhanced evoked potentials, while the 5-HT<sub>2B</sub> receptor-antagonist SB204741 depressed evoked potentials. Spinal hyperexcitation promoted by 5-HT<sub>2B</sub> receptors has been proposed as a pathogenic pathway contributing to pain (Aira

et al. 2010). Plasticity of spinal serotonergic neurotransmission selectively reduces spinal  $\mu$ -opioid receptor mechanisms via 5-HT<sub>2B</sub> receptors increased expression in dorsal horn neurons containing  $\mu$ -opioid receptor (Aira et al. 2012). The involvement of glutamate receptors in dorsal neuron hyperexcitation can also be promoted by 5-HT<sub>2B</sub> receptor after spinal nerve ligation. Augmentation of C-fiber-evoked potentials by spinal superfusion with 5-HT<sub>2B</sub> receptor agonist BW723C86 in nerve-ligated rats is impeded by co-administration of NMDA receptor antagonist. Evoked potentials are increased by NMDA receptor agonist in nerve-injured rats, irrespective of simultaneous 5-HT<sub>2B</sub> receptor blockade by SB204741. In uninjured rats, NMDA receptor agonist enhanced evoked potentials in the presence of BW723C86 but not if administered alone. Blockade of 5-HT<sub>2B</sub> receptors with selective antagonist SB204741 after spinal nerve ligation bilaterally decreases phosphorylation of NMDA receptor subunit NR1 (pNR1) in synaptic fraction. Delivery of SB204741 bilaterally attenuates thermal and mechanical allodynia occurring after spinal nerve ligation, at early time period, day 2 post injury. These findings suggest that transient activation of the PKC $\gamma$ /NMDAR pathway is critically involved in 5-HT<sub>2B</sub> receptor-mediated facilitation in the spinal nerve ligation model (Aira et al. 2013). The adaptor protein NADH dehydrogenase subunit 2 (ND2) is involved in NR1-phosphorylation and spinal hyperexcitability secondary to peripheral nerve injury. Spinal nerve ligation is followed by increased colocalization of ND2 with pNR1. C fiber-evoked dorsal horn field potentials are increased 60 min after spinal nerve ligation by superfusion with NMDA agonist. This increased postsynaptic up-regulation of ND2/pNR1 can be prevented by prior administration of selective 5-HT<sub>2B</sub> antagonist SB204741 (Aira et al. 2014). These results provide evidence that NMDAR-phosphorylation is instrumental in coupling 5-HT<sub>2B</sub> receptor-mediated input to NMDAR-expressing synapses in spinal hyperexcitation involved in pain.

Serotonin has also been involved in a rat model of neuropathic pain evoked by chronic constriction injury (CCI) of the sciatic nerve. The 5-HT<sub>2B</sub> receptor activation has been reported to both prevent and reduce CCI-induced allodynia at 3 weeks postinjury. Intrathecal administration of the 5-HT<sub>2B</sub> receptor agonist BW723C86 significantly attenuated established mechanical and cold allodynia; this effect was prevented by co-injection of RS127445. A single application of BW723C86 on the sciatic nerve concomitantly to CCI dose-dependently prevented mechanical allodynia and significantly reduced cold allodynia 17 days after CCI. This behavioral effect was accompanied with a marked decrease in macrophage infiltration into the sciatic nerve and, in the DRG, with an attenuated abnormal expression of several markers associated with local neuroinflammation and neuropathic pain. CCI resulted in a marked upregulation of 5-HT<sub>2B</sub> receptor expression in sciatic nerve and DRG. In the latter structure, it is biphasic, consisting of a transient early increase (23-fold), 2 days after surgery and before neuropathic pain emergence, followed by a steady (5-fold) increase, that remains constant until pain disappeared. In DRG and sciatic nerve, 5-HT<sub>2B</sub> receptors are immunolocalized on sensory neurons and infiltrating macrophages (Urtikova et al. 2012).

A role for serotonin in migraines has been supported by changes in circulating levels of serotonin and its metabolites during the phases of a migraine attack. A migraine headache is thought to be transmitted by the trigeminal nerve from the meninges and their associated blood vessels. Correlation of the receptor affinities with the potencies used in migraine prophylaxis showed significant correlations for the 5-HT<sub>2B</sub> receptor. Various human meningeal tissues express 5-HT<sub>2B</sub> mRNAs (Schmuck et al., 1996). The 5-HT<sub>2B</sub> receptor can activate the release of NO, and induce relaxation of the cerebral arteries and the jugular vein. 5-HT<sub>2B</sub> receptors located in endothelial cells of meningeal blood vessels may trigger migraine headache through the formation of NO, which results in the dilation of cerebral blood vessels and the concomitant activation of sensory trigeminovascular afferents, thus initiating the manifestation of head pain (Schmuck et al. 1996; Johnson et al. 2003). In addition, a recent genetic study identified 5-HT<sub>2B</sub> receptors as a susceptibility gene to migraine (Corominas et al. 2010). Endothelial 5-HT<sub>2B</sub> receptors may thus trigger dilation of meningeal blood vessels, which by activating sensory trigeminovascular afferents induces head pain.

5-HT<sub>2B</sub> receptors are also involved in signaling from the colon in rats in which there is visceral hypersensitivity. Oral administration of RS127445, significantly inhibits visceral hypersensitivity provoked by restraint stress without significant effect on the visceral nociceptive threshold of naïve rats (Ohashi-Doi et al. 2009). Moreover, when administered intracerebroventricularly RS127445 also decreases the number of pain behaviors during noxious colorectal distension. A selective 5-HT<sub>2B</sub> receptor antagonist has thus been proposed to have therapeutic potential for the treatment of gut disorders characterized by visceral hypersensitivity (O'Mahony et al. 2010). The 5-HT<sub>2B</sub> receptor appears thus to be involved in regulating sensory pathways only under hyperalgesic conditions, suggesting the possible utility of 5-HT<sub>2B</sub> receptor antagonism in reducing visceral hypersensitivity in patients with irritable bowel syndrome.

It thus appears that 5-HT<sub>2B</sub> receptor involvement in pain takes place at various sites and time periods, early events being more pronociceptive while at later this receptor contribution may be more antinociceptive, although this may vary according to the animal models.

### 3.5 Bones

The *Htr*<sub>2B</sub> mRNA expression, which was undetectable in anaplastic osteoblasts, appears in differentiated and matured osteoblasts (Bliziotis et al. 2001; Westbroek et al. 2001). The differentiation and maturation of osteoblasts might thus be regulated by the activation of the 5-HT<sub>2B</sub> receptor (Hirai et al. 2009). The *HTR*<sub>2A</sub> was found expressed only in osteoblasts, whereas *HTR*<sub>2B</sub> expression increased from precursor to mature osteoclasts (Hodge et al. 2013).

Of interest, *Htr<sub>2B</sub><sup>-/-</sup>* female mice displayed reduced bone density that was significant from the age of 4 months and intensifies by 12 and 18 months. This result confirmed that osteopenia was due to reduced bone formation because i) the alkaline phosphatase-positive colony-forming unit capacity of bone marrow precursors was markedly reduced in *Htr<sub>2B</sub><sup>-/-</sup>* mice from 4 to 12 months of age, ii) *ex-vivo* primary osteoblasts from *Htr<sub>2B</sub><sup>-/-</sup>* mice exhibited reduced proliferation and delayed differentiation, and iii) calcium incorporation was markedly reduced in osteoblasts after 5-HT<sub>2B</sub> receptor depletion (produced genetically or by pharmacological inactivation) (Collet et al. 2008). Using the osteoprogenitor cell line C1, blockade of 5-HT<sub>2B</sub> receptor intrinsic activity was reported to affect the efficiency of mineralization by decreasing calcium incorporation. Optimal bone matrix mineralization involves both NO and PLA2 signaling pathways and the 5-HT<sub>2B</sub> receptor promotes prostaglandin E2 production through cyclooxygenase (COX) activation. When C1 osteoblasts undergo conversion into osteocyte-like cells, COX activity was quenched. The 5-HT<sub>2B</sub> receptor contributed in an autocrine manner to osteogenic differentiation (Locker et al. 2006). A functional link between the 5-HT<sub>2B</sub> receptor and the activity of the tissue-non-specific alkaline phosphatase (TNAP) was established. Agonist stimulation of the receptor increased TNAP activity during the initial mineralization phase. Indeed, inhibition of 5-HT<sub>2B</sub> receptor intrinsic activity prevented TNAP activation. In contrast, agonist stimulation of the receptor further increased TNAP activity during the initial mineralization phase. Previous observations indicated that the 5-HT<sub>2B</sub> receptor coupled to PLA2 pathway and prostaglandin production at the beginning of mineral deposition. The 5-HT<sub>2B</sub> receptor also controlled leukotriene synthesis *via* PLA2 at the terminal stages of differentiation. These two 5-HT<sub>2B</sub> receptor-dependent eicosanoid productions delineate distinct time-windows of TNAP regulation during the osteogenic program. Finally, prostaglandins or leukotrienes were shown to relay the post-translational activation of TNAP *via* stimulation of the phosphatidylinositol-specific phospholipase C. In agreement with the above findings, primary calvarial osteoblasts from *Htr<sub>2B</sub><sup>-/-</sup>* mice were shown to exhibit defects in TNAP activity (Baudry et al. 2010). Brain serotonin was proposed to favor indirectly bone mass accrual following activation of 5-HT<sub>2C</sub> receptors on ventromedial hypothalamic neurons and 5-HT<sub>2B</sub> receptors on arcuate neurons (Yadav et al. 2009). Compared to control osteoblasts, the lack of 5-HT<sub>2B</sub> receptors was associated with a 10-fold over-production of prostacyclin (PGI<sub>2</sub>). Also, a specific prostacyclin synthase inhibitor (U51605) rescued totally osteoblast aggregation and matrix mineralization in *Htr<sub>2B</sub><sup>-/-</sup>* osteoblasts without having any effect on WT osteoblasts. Prostacyclin is the endogenous ligand of PPAR-β/δ, and its inhibition in *Htr<sub>2B</sub><sup>-/-</sup>* cells rescued totally the alkaline phosphatase and osteopontin mRNA levels, cell-cell adhesion, and matrix mineralization. The absence of 5-HT<sub>2B</sub> receptors leads to the overproduction of prostacyclin, inducing reduced osteoblast differentiation due to PPAR-β/δ - dependent target regulation and defective cell-cell adhesion and matrix mineralization (Chabbi-Achengli et al. 2013). The 5-HT<sub>2B</sub> receptor contributes thus in an autocrine manner to osteogenic differentiation.

### 3.6 Cancers

5-HT<sub>2B</sub> receptor expression was observed in spontaneous human carcinoid tumors, along with coupling to p21<sup>ras</sup> activation (Launay et al. 1996). The tumor proliferative activity of small intestinal neuroendocrine tumors (including cell growth and the development of desmoplasia) is associated with the particular microenvironment in the peritoneum and tumor cells support this necessary milieu through the secretion of profibrotic/ angiogenetic factors (Svejda et al. 2010).

Increased serotonin biosynthetic capacity accompanied by multiple changes in serotonin receptor expression and signaling favor malignant progression of human breast cancer cells. Among them, expression levels for 5-HT<sub>2B</sub> receptors was found increased in breast cancers and the *HTR<sub>2B</sub>* mRNA is also expressed in untransformed human mammary epithelium (Pai et al. 2009). *HTR<sub>2B</sub>* mRNA expression was found lower in basal tumors estrogen receptor (ER) negative, compared with luminal tumors, which are most commonly ER positive. *HTR<sub>2B</sub>* mRNA was elevated in carcinomas, increased with tumor stage, and concomitantly was higher in lymph node-positive tumors as compared to node-negative tumors. This observation was supported by a study, showing that c-Myc transformation induced an increase in *HTR<sub>2B</sub>* expression (Pai et al. 2009). In human breast cancer, correlation analysis revealed a significant correlation of *HTR<sub>2B</sub>* with estrogen receptor-α (ER-α) (Kopparapu et al. 2013).

Uveal (ocular) melanoma is an aggressive cancer that often forms undetectable micrometastases before diagnosis of the primary tumor. High increases in *HTR<sub>2B</sub>* mRNA transcript levels were found in all uveal melanomas with monosomy 3 compared with low expression in all tumors with disomy 3. As monosomy 3 is associated with metastatic disease, *HTR<sub>2B</sub>* expression has been proposed as a marker to identify patients with poor prognosis (Tschtscher et al. 2003). The 5-HT<sub>2B</sub> receptor signals through the heterotrimeric GTPase, *GNAQ*, which is mutated in half of uveal melanomas (Onken et al. 2010). The 5-HT<sub>2B</sub> receptor is among the genes, which show the highest overexpression in class 2 uveal melanoma (van Gils et al. 2008). A PCR-based 15-gene assay comprising 12 discriminating genes including *HTR<sub>2B</sub>* are now part of a prognostic assay, which provides an important addition to the armamentarium for managing patients with uveal melanoma (Onken et al. 2010). These genes, including *HTR<sub>2B</sub>*, provide candidates for distinguishing whether uveal melanomas contain liver metastases and thus aid in the diagnosis and prevention of uveal melanoma liver metastases, based on their different features (Zhang et al. 2014).

Prostate cancer is the most commonly diagnosed non-cutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain enigmatic. The 5-HT<sub>2B</sub> receptor was found to be upregulated in tumors relative to benign glands (Magee et al. 2001). Overexpression of receptors to

neuroendocrine cell products has been suggested to contribute to development of hormone-refractory prostate cancer. Immunostaining for the 5-HT<sub>2B</sub> receptor was observed in low-grade and high-grade tumors, prostatic intra-epithelial neoplastic and benign prostatic hyperplasia cells, and in vascular endothelial cells. Antagonists for the 5-HT<sub>2B</sub> receptor inhibited proliferation of prostate cancer cells in a dose-dependent manner (Dizeyi et al. 2005).

By contrast, gene expression profiles of adrenocortical tumors identified underexpression of *HTR<sub>2B</sub>* mRNA as a marker of malignant adrenocortical carcinoma (Fernandez-Ranvier et al. 2008). Analysis of biomarkers of malignancy of adrenocortical cancers in the meta-analysis has revealed that the combination of overexpressed anillin (*ANLN*) and underexpressed *HTR<sub>2B</sub>* mRNA appeared to be the best predictor of malignancy (Zsippai et al. 2011). Chronic adrenal stimulation by glucose-dependent insulinotropic peptide in macronodular adrenal hyperplasia was shown to lead to the significant induction of the *GPR54*, *HTR<sub>2B</sub>*, *GPR4*, and endothelial differentiation sphingolipid receptor *EDG8* (Lampron et al. 2006).

Serotonin has been reported to promote proliferation of serum-deprived hepatocellular carcinoma (HCC) cells. Among 64 genes for which mRNA expression levels differed between non-hepatitis B, non-hepatitis C compared to hepatitis C-type HCC, the most affected was found *HTR<sub>2B</sub>* (Iizuka et al. 2004). The function of serotonin as a survival factor of HCC cells was recently demonstrated: activation of the 5-HT<sub>2B</sub> receptor leads to sustained phosphorylation of two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy. Inhibiting the 5-HT<sub>2B</sub> receptor reduced cancer cell growth *in-vitro* and *in-vivo*. The presence of 5-HT<sub>2B</sub> receptors in HCC and the activation of autophagy-related mechanisms demonstrate novel insights of serotonin in cancer biology (Soll et al. 2010). The 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> receptors were found expressed, respectively, in 32% and 35% of the patients with hepatocellular cancer. Both receptors were associated with an increased proliferation index (Soll et al. 2012). The 5-HT<sub>2B</sub> receptor mediates serotonin-induced proliferation in the serum-deprived HCC Huh7 cells. Additionally, inhibition of 5-HT<sub>2B</sub> receptor in Huh7 cells using SB204741 significantly decreased the expression of FOXO3a (Liang et al. 2013). *In vitro* data suggest that 5-HT increased total  $\beta$ -catenin, active  $\beta$ -catenin and decreased phosphorylated  $\beta$ -catenin protein levels in serum deprived Huh-7 and HepG2 cells compared to control cells under serum free medium without 5-HT. Activation of Wnt/ $\beta$ -catenin signaling was evidenced by increased expression of  $\beta$ -catenin downstream target genes, Axin2, cyclin D1, dickkopf-1 (DKK1) and glutamine synthetase (GS) by qPCR in serum-deprived HCC cell lines treated with 5-HT. Additionally, 5-HT disrupted Axin1/ $\beta$ -catenin interaction, a critical step in  $\beta$ -catenin phosphorylation (Fatima et al. 2016).

In tumor-infiltrating macrophages, serotonin does not enhance colon cancer tumor cell proliferation but may act as a regulator of angiogenesis by reducing the expression of MMP-12, entailing lower levels of angiostatin—an endogenous inhibitor of angiogenesis (Nocito et al. 2008). Serotonin can stimulate the phosphorylation of ERK1/2 in bovine endothelial cells, and the 5-HT<sub>2B</sub> receptor was reported to play a role in the activation of eNOS in human endothelial cells. In SB204741-treated mice, the selective blockade of the 5-HT<sub>2B</sub> receptor resulted in the reduction of tumor angiogenesis and growth through the inhibition effect of ERK1/2 and eNOS (Asada et al. 2009). Therefore the possibility that 5-HT<sub>2B</sub> receptors participate in tumor angiogenesis is a likely possibility that remains to be evaluated in other tumors subtypes.

## Summary

Although expressed quite widely but at rather low levels, 5-HT<sub>2B</sub> receptors have been reported to play an important role at cardiac, pulmonary and central levels. Therapeutic interest concerning this receptor subtype has been delayed due to the lack of selective tools, radioligand, antibodies etc... However, selective antagonists at 5-HT<sub>2B</sub> receptors have gained much attention as new targets in therapeutics: 5-HT<sub>2B</sub> receptor antagonists are under active investigation for pulmonary hypertension and carcinoid tumors, since they produced encouraging results in animal models, and promising human trials are underway; 5-HT<sub>2B</sub> receptor may represent a new therapeutic target to reduce cardiac hypertrophy and hypertrophy-induced heart failure as well as valvulopathies. If the safety and efficacy of a 5-HT<sub>2B</sub> receptor antagonists can be confirmed, application of this kind of therapy to human study may be warranted. These receptors have also been recognized as off-targets because their agonist plays a significant role in the pathogenesis of valvulopathy and pulmonary hypertension.

## References

- Aira Z, Buesa I, García Del Caño G, Bilbao J, Doñate F, Zimmermann M, Azkue JJ Transient, 5-HT<sub>2B</sub> receptor-mediated facilitation in neuropathic pain: Up-regulation of PKC $\gamma$  and engagement of the NMDA receptor in dorsal horn neurons. *Pain*. 2013;154(9):1865-1877.
- Aira Z, Buesa I, García Del Caño G, Salgueiro M, Mendiabale N, Mingo J, . . . Azkue JJ Selective impairment of spinal mu-opioid receptor mechanism by plasticity of serotonergic facilitation mediated by 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors. *Pain*. 2012;153(7):1418-1425.

- Aira Z, Buesa I, Rada D, Gómez-Esteban JC, Azkue JJ Coupling of serotonergic input to NMDA receptor-phosphorylation following peripheral nerve injury via rapid, synaptic up-regulation of ND2. *Experimental Neurology*. 2014;255C:86-95.
- Aira Z, Buesa I, Salgueiro M, Bilbao J, Aguilera L, Zimmermann M, Azkue JJ Subtype-specific changes in 5-HT receptor-mediated modulation of C fibre-evoked spinal field potentials are triggered by peripheral nerve injury. *Neuroscience*. 2010;168(3):831-841.
- Antonini A, Poewe W Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. *Lancet Neurol*. 2007;6(9):826-829.
- Asada M, Ebihara S, Yamanda S, Niu K, Okazaki T, Sora I, Arai H Depletion of serotonin and selective inhibition of 2B receptor suppressed tumor angiogenesis by inhibiting endothelial nitric oxide synthase and extracellular signal-regulated kinase 1/2 phosphorylation. *Neoplasia*. 2009;11(4):408-417.
- Auclair AL, Cathala A, Sarrazin F, Depoortère R, Piazza PV, Newman-Tancredi A, Spampinato U The central serotonin 2B receptor: a new pharmacological target to modulate the mesoaccumbens dopaminergic pathway activity. *J Neurochem*. 2010;114(5):1323-1332.
- Audia JE, Evrard DA, Murdoch GR, Droste JJ, Nissen JS, Schenck KW, . . . Cohen ML Potent, selective tetrahydro-beta-carboline antagonists of the serotonin 2B (5-HT2B) contractile receptor in the rat stomach fundus. *J. Med. Chem*. 1996;39:2773-2780.
- Ayme-Dietrich E, Lawson R, Gasser B, Dallemand R, Bischoff N, Monassier L Mitral bioprosthesis hypertrophic scarring and native aortic valve fibrosis during benfluorex therapy. *Fundam Clin Pharmacol*. 2012;26(2):215-218.
- Bai C-F, Liu J-C, Zhao R, Cao W, Liu S-B, Zhang X-N, . . . Zhao M-G Role of 5-HT2B receptor in cardiomyocyte apoptosis of norepinephrine-induced cardiomyopathy model in rats. *Clin Exp Pharmacol Physiol*. 2010;37:e145-e151.
- Banas S, Doly S, Boutourlinsky K, Diaz S, Belmer A, Callebert J, . . . Maroteaux L Deconstructing antiobesity compound action: requirement of serotonin 5-HT2B receptors for dexfenfluramine anorectic effects. *Neuropsychopharmacology*. 2011;36:423-433.
- Baudry A, Bitard J, Mouillet-Richard S, Locker M, Poliard A, Launay J-M, Kellermann O The serotonergic 5-HT2B receptor controls tissue non-specific alkaline phosphatase activity in osteoblasts via eicosanoids and phosphatidylinositol-specific phospholipase C. *J Biol Chem*. 2010;285(34):26066-26073.
- Becamel C, Fige A, Poliak S, Dumuis A, Peles E, Bockaert J, . . . Ullmer C Interaction of Serotonin 5-HT2C receptors with PDZ10 of the multi PDZ protein MUPP1. *J Biol Chem*. 2001;276(16):12974-12982.
- Belmer A, Doly S, Setola V, Banas SM, Moutkine I, Boutourlinsky K, . . . Maroteaux L Role of the N-terminal Region in G-Protein Coupled Receptor Functions: Negative Modulation Revealed by 5-HT2B Receptor Polymorphisms. *Mol Pharmacol*. 2014;85(1):127-138.
- Bevilacqua L, Doly S, Kaprio J, Yuan Q, Tikkanen R, Paunio T, . . . Goldman D A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature*. 2010;468(8):1061-1066.
- Bhasin N, Kernick E, Luo X, Seidel HE, Weiss ER, Lauder JM Differential regulation of chondrogenic differentiation by the serotonin2B receptor and retinoic acid in the embryonic mouse hindlimb. *Dev Dyn*. 2004;230(2):201-209.
- Blizotes MM, Eshleman AJ, Zhang XW, Wiren KM Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. *Bone*. 2001;29(5):477-486.
- Bonaventure P, Guo H, Tian B, Liu X, Bittner A, Roland B, . . . Erlander MG Nuclei and subnuclei gene expression profiling in mammalian brain. *Brain Res*. 2002;943(1):38-47.
- Bonhaus DW, Bach C, DeSouza A, Salazar FHR, Matsuoka BD, Zuppan P, . . . Eglen RM The pharmacology and distribution of human 5-hydroxytryptamine 2B (5-HT2B) receptor gene products: comparison with 5-HT2A and 5-HT2C receptors. *Br. J. Pharmacol*. 1995;115:622-628.
- Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M, . . . Martin GR RS-127445: a selective, high affinity, orally bioavailable 5-HT2B receptor antagonist. *Br J Pharmacol*. 1999;127(5):1075-1082.
- Bulenger S, Marullo S, Bouvier M Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci*. 2005;26(3):131-137.
- Cervantes-Durán C, Vidal-Cantú GC, Barragán-Iglesias P, Pineda-Farias JB, Bravo-Hernández M, Murbartían J, Granados-Soto V Role of peripheral and spinal 5-HT(2B) receptors in formalin-induced nociception. *Pharmacol Biochem Behav*. 2012;102(1):30-35.
- Chabbi-Achengli Y, Launay J-M, Maroteaux L, De Vernejoul MC, Collet C Serotonin 2B Receptor (5-HT2B R) Signals through Prostacyclin and PPAR- $\beta/\delta$  in Osteoblasts. *PLoS ONE*. 2013;8(9):e75783.
- Chan SY, Loscalzo J Pathogenic mechanisms of pulmonary arterial hypertension. *J Mol Cell Cardiol*. 2008;44(1):14-30.
- Choi D-S, Birraux G, Launay J-M, Maroteaux L The human serotonin 5-HT2B receptor: pharmacological link between 5-HT2 and 5-HT1D receptors. *FEBS L*. 1994;352:393-399.
- Choi D-S, Maroteaux L Immunohistochemical localisation of the serotonin 5-HT2B receptor in mouse gut, cardiovascular system, and brain. *FEBS Lett*. 1996;391:45-51.

- Choi D-S, Ward S, Messaddeq N, Launay J-M, Maroteaux L 5-HT<sub>2B</sub> receptor-mediated serotonin morphogenetic functions in mouse cranial neural crest and myocardial cells. *Development*. 1997;124:1745-1755.
- Cohen ML, Schenck KW, Mabry TE, Nelson DL, Audia JE LY272015, a potent, selective and orally active 5-HT<sub>2B</sub> receptor antagonist. *J Ser Res*. 1996;3:131-144.
- Collet C, Schiltz C, Geoffroy V, Maroteaux L, Launay J-M, de Vernejoul M-C The serotonin 5-HT<sub>2B</sub> receptor controls bone mass via osteoblast recruitment and proliferation. *FASEB J*. 2008;22((2)):418-427.
- Connolly HM, Crary JL, McGoon MD, Hensrud DD, Edwards BS, Edwards WD, Schaff HV Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med*. 1997;337(9):581-588.
- Corominas R, Sobrido MJ, Ribasés M, Cuenca-León E, Blanco-Arias P, Narberhaus B, . . . Cormand B Association study of the serotonergic system in migraine in the Spanish population. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B(1):177-184.
- Cremer SE, Moesgaard SG, Rasmussen CE, Zois NE, Falk T, Reimann MJ, . . . Olsen LH Alpha-smooth muscle actin and serotonin receptors 2A and 2B in dogs with myxomatous mitral valve disease. *Research in Veterinary Science*. 2015a;100:197-206.
- Cremer SE, Zois NE, Moesgaard SG, Ravn N, Cirera S, Hønge JL, . . . Olsen LH Serotonin markers show altered transcription levels in an experimental pig model of mitral regurgitation. *Vet J*. 2015b;203(2):192-198.
- Cussac D, Boutet-Robinet E, Ailhaud MC, Newman-Tancredi A, Martel JC, Danty N, Raully-Lestienne I Agonist-directed trafficking of signalling at serotonin 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub>-VSV receptors mediated Gq/11 activation and calcium mobilisation in CHO cells. *Eur J Pharmacol*. 2008;594(1-3):32-38.
- Cussac D, Newman-Tancredi A, Quentric Y, Carpentier N, Poissonnet G, Parmentier JG, . . . Millan MJ Characterization of phospholipase C activity at h5-HT<sub>2C</sub> compared with h5-HT<sub>2B</sub> receptors: influence of novel ligands upon membrane-bound levels of [3H]phosphatidylinositols. *Naunyn Schmiedeberg's Arch Pharmacol*. 2002;365(3):242-252.
- Dawson P, Opacka-Juffry J, Moffatt JD, Daniju Y, Dutta N, Ramsey J, Davidson C The effects of benzofury (5-APB) on the dopamine transporter and 5-HT<sub>2</sub>-dependent vasoconstriction in the rat. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:57-63.
- Dees C, Akhmetshina A, Reich N, Jüngel A, Beyer C, Krönke G, . . . Distler JHW Platelet derived serotonin links vascular disease and tissue fibrosis. *J Exp Med*. 2011;208(5):961-972.
- Diaz SL, Doly S, Narboux-Nême N, Fernandez S, Mazot P, Banas S, . . . Maroteaux L 5-HT<sub>2B</sub> receptors are required for serotonin-selective antidepressant actions. *Mol Psychiatry*. 2012;17:154-163.
- Diaz SL, Narboux-Nême N, Boutourlinsky K, Doly S, Maroteaux L Mice lacking the serotonin 5-HT<sub>2B</sub> receptor as an animal model of resistance to selective serotonin reuptake inhibitors antidepressants. *Eur Neuropsychopharmacol*. 2016;26(2):265-279.
- Dizeyi N, Bjartell A, Hedlund P, Tasken KA, Gadaleanu V, Abrahamsson PA Expression of serotonin receptors 2B and 4 in human prostate cancer tissue and effects of their antagonists on prostate cancer cell lines. *Eur Urol*. 2005;47(6):895-900.
- Doly S, Bertran-Gonzalez J, Callebert J, Bruneau A, Banas SM, Belmer A, . . . Maroteaux L Role of serotonin via 5-HT<sub>2B</sub> receptors in the reinforcing effects of MDMA in mice *PLoS ONE*. 2009; 4(11):e7952.
- Doly S, Valjent E, Setola V, Callebert J, Herve D, Launay JM, Maroteaux L Serotonin 5-HT<sub>2B</sub> 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.
- Dumitrascu R, Kulcke C, Königshoff M, Kouri F, Yang X, Morrell N, . . . Pullamsetti SS Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. *Eur Respir J*. 2011;37(5):1104-1118.
- Ebrahimkhani M, Oakley F, Murphy L, Mann J, Moles A, Perugorria M, . . . Mann D Stimulating healthy tissue regeneration by targeting the 5-HT<sub>2B</sub> receptor in chronic liver disease. *Nature Medicine*. 2011;17(12):1668-1673.
- Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS Mediation by 5-hydroxytryptamine<sub>2B</sub> receptors of endothelium-dependent relaxation in rat jugular vein. *Br. J. Pharmacol*. 1995; 114((2)): 400-404.
- Fabre A, Marchal-Sommé J, Marchand-Adam S, Quesnel C, Borie R, Dehoux M, . . . Crestani B Modulation of bleomycin-induced lung fibrosis by serotonin receptor antagonists in mice. *Eur Respir J*. 2008;32(2):426-436.
- Fatima S, Shi X, Lin Z, Chen G-q, Pan X-h, Wu JC-Y, . . . Bian ZX 5-Hydroxytryptamine promotes hepatocellular carcinoma proliferation by influencing  $\beta$ -catenin. *Molecular Oncology*. 2016;10(2):195-212.
- Fernandez-Ranvier GG, Weng J, Yeh RF, Khanafshar E, Suh I, Barker C, . . . Kebebew E Identification of biomarkers of adrenocortical carcinoma using genomewide gene expression profiling. *Arch Surg*. 2008;143(9):841-846.
- Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, . . . Robertson DW Possible role of valvular serotonin 5-HT<sub>2B</sub> receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol*. 2000;57(1):75-81.
- Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, . . . Lübbert H Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J*. 1992;11(9):3481-3487.



- Glusa E, Pertz HH Further evidence that 5-HT-induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT<sub>2B</sub> receptors. *Br J Pharmacol.* 2000;130(3):692-698.
- Helton LA, Thor KB, Baez M 5-hydroxytryptamine<sub>2A</sub>, 5-hydroxytryptamine<sub>2B</sub>, and 5-hydroxytryptamine<sub>2C</sub> receptor mRNA expression in the spinal cord of rat, cat, monkey and human. *Neuroreport.* 1994;5(18):2617-2620.
- Hendrikx M, Van Dorpe J, Flameng W, Daenen W Aortic and mitral valve disease induced by ergotamine therapy for migraine: a case report and review of the literature. *J Heart Valve Dis.* 1996;5(2):235-237.
- Hirai T, Tokumo K, Tsuchiya D, Nishio H Expression of mRNA for 5-HT<sub>2</sub> receptors and proteins related to inactivation of 5-HT in mouse osteoblasts. *J Pharmacol Sci.* 2009;109(2):319-323.
- Hodge JM, Wang Y, Berk M, Collier FM, Fernandes TJ, Constable MJ, . . . Williams LJ Selective Serotonin Reuptake Inhibitors Inhibit Human Osteoclast and Osteoblast Formation and Function. *Biol Psychiatry.* 2013;74(1):32-39.
- Holohean AM, Hackman JC Mechanisms intrinsic to 5-HT<sub>2B</sub> receptor-induced potentiation of NMDA receptor responses in frog motoneurons. *Br J Pharmacol.* 2004;143(3):351-360.
- Horvath J, Fross RD, Kleiner-Fisman G, Lerch R, Stalder H, Liaudat S, . . . Lang AE Severe multivalvular heart disease: a new complication of the ergot derivative dopamine agonists. *Mov Disord.* 2004;19(6):656-662.
- Iizuka N, Oka M, Yamada-Okabe H, Hamada K, Nakayama H, Mori N, . . . Hamamoto Y Molecular signature in three types of hepatocellular carcinoma with different viral origin by oligonucleotide microarray. *Int J Oncol.* 2004;24(3):565-574.
- Iversen L, Gibbons S, Treble R, Setola V, Huang X-P, Roth BL Neurochemical profiles of some novel psychoactive substances. *Eur J Pharmacol.* 2013;700(1-3):147-151.
- Jaffre F, Bonnin P, Callebert J, Debbabi H, Setola V, Doly S, . . . Maroteaux L Serotonin and angiotensin receptors in cardiac fibroblasts coregulate adrenergic-dependent cardiac hypertrophy. *Circ Res.* 2009;104(1):113-123.
- Jaffré F, Callebert J, Sarre A, Etienne N, Nebigil CG, Launay JM, . . . Monassier L Involvement of the serotonin 5-HT<sub>2B</sub> receptor in cardiac hypertrophy linked to sympathetic stimulation: control of interleukin-6, interleukin-1 beta, and tumor necrosis factor-alpha cytokine production by ventricular fibroblasts. *Circulation.* 2004;110:969-974.
- Jerman JC, Brough SJ, Gager T, Wood M, Coldwell MC, Smart D, Middlemiss DN Pharmacological characterisation of human 5-HT<sub>2</sub> receptor subtypes. *Eur J Pharmacol.* 2001;414(1):23-30.
- Johnson KW, Nelson DL, Dieckman DK, Wainscott DB, Lucaites VL, Audia JE, . . . Phebus LA Neurogenic dural protein extravasation induced by meta-chlorophenylpiperazine (mCPP) involves nitric oxide and 5-HT<sub>2B</sub> receptor activation. *Cephalalgia.* 2003;23(2):117-123.
- Kelly CR, Sharif NA Pharmacological evidence for a functional serotonin-2B receptor in a human uterine smooth muscle cell line. *J Pharmacol Exp Ther.* 2006;317(3):1254-1261.
- Kim H, Toyofuku Y, Lynn FC, Chak E, Uchida T, Mizukami H, . . . German MS Serotonin regulates pancreatic beta cell mass during pregnancy. *Nat Med.* 2010;16(7):804-808.
- Kiss B, Horváth A, Némethy Z, Schmidt E, Laszlovszky I, Bugovics G, . . . Szombathelyi Z Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther.* 2010;333(1):328-340.
- Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, Bickerdike M Pharmacological characterisation of the agonist radioligand binding site of 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 2004;370(2):114-123.
- Kolodziejczak M, Bechade C, Gervasi N, Irinopoulou T, Banas SM, Cordier C, . . . Maroteaux L Serotonin Modulates Developmental Microglia via 5-HT<sub>2B</sub> Receptors: Potential Implication during Synaptic Refinement of Retinogeniculate Projections. *ACS Chem Neurosci.* 2015;6(7):1219-1230.
- Königshoff M, Dumitrascu R, Udalov S, Amarie OV, Reiter R, Grimminger F, . . . Eickelberg O Increased expression of 5-hydroxytryptamine<sub>2A/B</sub> receptors in idiopathic pulmonary fibrosis: a rationale for therapeutic intervention. *Thorax.* 2010;65(11):949-955.
- Kopparapu PK, Tinzl M, Anagnostaki L, Persson JL, Dizeyi N Expression and localization of serotonin receptors in human breast cancer. *Anticancer Res.* 2013;33(2):363-370.
- Krabbe G, Matyash V, Pannasch U, Mamer L, Boddeke HWGM, Kettenmann H Activation of serotonin receptors promotes microglial injury-induced motility but attenuates phagocytic activity. *Brain Behav Immun.* 2012;26(3):419-428.
- Kursar JD, Nelson DL, Wainscott D, Baez M Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine<sub>2B</sub> receptor. *Mol. Pharmacol.* 1994;46:227-234.
- Kursar JD, Nelson DL, Wainscott DB, Cohen ML, Baez M Molecular cloning, functional expression, and pharmacological characterisation of a novel serotonin (5-Hydroxytryptamine<sub>2F</sub>) from rat stomach fundus. *Mol. Pharmacol.* 1992;42:549-557.
- Lampron A, Bourdeau I, Hamet P, Tremblay J, Lacroix A Whole genome expression profiling of glucose-dependent insulinotropic peptide (GIP)- and adrenocorticotropin-dependent adrenal hyperplasias reveals novel targets for the study of GIP-dependent Cushing's syndrome. *J Clin Endocrinol Metab.* 2006;91(9):3611-3618.

- Launay J-M, Birraux G, Bondoux D, Callebert J, Choi D-S, Loric S, Maroteaux L Ras involvement in signal transduction by the serotonin 5-HT<sub>2B</sub> receptor. *J Biol Chem.* 1996;271:3141-3147.
- Launay J-M, Hervé P, Callebert J, Mallat Z, Collet C, Doly S, . . . Maroteaux L Serotonin 5-HT<sub>2B</sub> receptors are required for bone-marrow contribution to pulmonary arterial hypertension. *Blood.* 2012;119(7):1772-1780.
- Launay JM, Hervé P, Peoc'h K, Tournois C, Callebert J, Nebigil C, . . . Maroteaux L Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med.* 2002;8:1129-1135.
- Li T, Weng S-G, Leng X-S, Peng J-R, Wei Y-H, Mou D-C, Wang W-X Effects of 5-hydroxytryptamine and its antagonists on hepatic stellate cells. *Hepatobiliary Pancreat Dis Int.* 2006;5(1):96-100.
- Liang C, Chen W, Zhi X, Ma T, Xia X, Liu H, . . . Liang T Serotonin promotes the proliferation of serum-deprived hepatocellular carcinoma cells via upregulation of FOXO3a. *Mol Cancer.* 2013;12:14.
- Liang YJ, Lai LP, Wang BW, Juang SJ, Chang CM, Leu JG, Shyu KG Mechanical stress enhances serotonin 2B receptor modulating brain natriuretic peptide through nuclear factor-kappaB in cardiomyocytes. *Cardiovasc Res.* 2006;72(2):303-312.
- Lin S-Y, Chang W-J, Lin C-S, Huang C-Y, Wang H-F, Sun W-H Serotonin Receptor 5-HT<sub>2B</sub> Mediates Serotonin-Induced Mechanical Hyperalgesia. *J Neurosci.* 2011;31(4):1410-1418.
- Liu W, Wacker D, Gati C, Han GW, James D, Wang D, . . . Cherezov V Serial femtosecond crystallography of G protein-coupled receptors. *Science.* 2013;342(6165):1521-1524.
- Locker M, Bitard J, Collet C, Poliard A, Mutel V, Launay JM, Kellermann O Stepwise control of osteogenic differentiation by 5-HT<sub>2B</sub> receptor signaling: nitric oxide production and phospholipase A2 activation. *Cell Signal.* 2006;18(5):628-639.
- Loric S, Launay J-M, Colas J-F, Maroteaux L New mouse 5-HT<sub>2</sub>-like receptor: Expression in brain, heart, and intestine. *FEBS L.* 1992;312:203-207.
- Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, . . . Milbrandt J Expression profiling reveals hepsin overexpression in prostate cancer. *Cancer Res.* 2001;61(15):5692-5696.
- Manivet P, Mouillet-Richard S, Callebert J, Nebigil CG, Maroteaux L, Hosoda S, . . . Launay J-M PDZ-dependent activation of nitric-oxide synthases by the serotonin 2B receptor. *J Biol Chem.* 2000;275:9324-9331.
- Martí-Solano M, Sanz F, Pastor M, Selent J A dynamic view of molecular switch behavior at serotonin receptors: implications for functional selectivity. *PLoS ONE.* 2014;9(10):e109312.
- Matsuda A, Suzuki Y, Honda G, Muramatsu S, Matsuzaki O, Nagano Y, . . . Sugano S Large-scale identification and characterization of human genes that activate NF-kappaB and MAPK signaling pathways. *Oncogene.* 2003;22(21):3307-3318.
- McCorvy JD, Roth BL Structure and function of serotonin G protein-coupled receptors. *Pharmacol Ther.* 2015;150:129-142.
- McDevitt RA, Neumaier JF Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J Chem Neuroanat.* 2011;41(4):234-246.
- Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, . . . Cussac D The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine<sub>2C</sub> receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther.* 2003;306(3):954-964.
- Monassier L, Laplante MA, Jaffre F, Bousquet P, Maroteaux L, de Champlain J Serotonin 5-HT<sub>2B</sub> Receptor Blockade Prevents Reactive Oxygen Species-Induced Cardiac Hypertrophy in Mice. *Hypertension.* 2008;52:301-307.
- Nebigil CG, Choi D-S, Dierich A, Hickel P, Le Meur M, Messaddeq N, . . . Maroteaux L Serotonin 2B receptor is required for heart development. *Proc Natl Acad Sci U S A.* 2000a;97:9508-9513.
- Nebigil CG, Etienne N, Messaddeq N, Maroteaux L Serotonin is a novel survival factor of cardiomyocytes: mitochondria as a target of 5-HT<sub>2B</sub>-receptor signaling. *FASEB J.* 2003;17(10):1373-1375.
- Nebigil CG, Launay J-M, Hickel P, Tournois C, Maroteaux L 5-Hydroxytryptamine 2B receptor regulates cell-cycle progression: Cross talk with tyrosine kinase pathways. *Proc Natl Acad Sci U S A.* 2000b;97(6):2591-2596.
- Nelson DL, Lucaites VL, Wainscott DB, Glennon RA Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 1999;359(1):1-6.
- Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, . . . Clavien PA Serotonin regulates macrophage-mediated angiogenesis in a mouse model of colon cancer allografts. *Cancer Res.* 2008;68(13):5152-5158.
- O'Mahony SM, Bulmer DC, Coelho A-M, Fitzgerald P, Bongiovanni C, Lee K, . . . Cryan JF 5-HT<sub>2B</sub> receptors modulate visceral hypersensitivity in a stress-sensitive animal model of brain-gut axis dysfunction. *Neurogastroenterol Motil.* 2010;22(5):573-578.
- Oh CK, Drescher MJ, Hatfield JS, Drescher DG Selective expression of serotonin receptor transcripts in the mammalian cochlea and its subdivisions. *Brain Res Mol Brain Res.* 1999;70(1):135-140.
- Ohashi-Doi K, Himaki D, Nagao K, Kawai M, Gale JD, Furness JB, Kurebayashi Y A selective, high affinity 5-HT<sub>2B</sub> receptor antagonist inhibits visceral hypersensitivity in rats. *Neurogastroenterol Motil.* 2009;22(2):e69-e76.

- Onken MD, Worley LA, Tuscan MD, Harbour JW An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn.* 2010;12(4):461-468.
- Osredkar D, Krzan M Expression of serotonin receptor subtypes in rat brain and astrocyte cell cultures: an age-and tissue-dependent process. *Periodicum Biologorum.* 2009;111(1):129-135.
- Pai V, Marshall A, Hernandez L, Buckley A, Horseman N Altered serotonin physiology in human breast cancers favors paradoxical growth and cell survival. *Breast Cancer Res.* 2009;11(6):R81.
- Papageorgiou A, Deneff C Stimulation of growth hormone release by 5-hydroxytryptamine (5-HT) in cultured rat anterior pituitary cell aggregates: evidence for mediation by 5-HT<sub>2B</sub>, 5-HT<sub>7</sub>, 5-HT<sub>1B</sub>, and ketanserin-sensitive receptors. *Endocrinology.* 2007;148(9):4509-4522.
- Park K-S, Sin P-J, Lee DH, Cha S-K, Kim M-J, Kim N-H, . . . Kong ID Switching-on of serotonergic calcium signaling in activated hepatic stellate cells. *World J Gastroenterol.* 2011;17(2):164-173.
- Pietri M, Schneider B, Mouillet-Richard S, Ermonval M, Mutel V, Launay J-M, Kellermann O Reactive oxygen species-dependent TNF-alpha converting enzyme activation through stimulation of 5-HT<sub>2B</sub> and alpha<sub>1D</sub> autoreceptors in neuronal cells. *Faseb J.* 2005;19:1078-1087.
- Pitychoutis P, Belmer A, Moutkine I, Adrien J, Maroteaux L Mice lacking the serotonin Htr2B receptor gene present an antipsychotic-sensitive schizophrenic-like phenotype. *Neuropsychopharmacology.* 2015;40(12):2764-2773.
- Porter RH, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, . . . Sheardown MJ Functional characterization of agonists at recombinant human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors in CHO-K1 cells. *Br J Pharmacol.* 1999;128(1):13-20.
- Porvasnik SL, Germain S, Embury J, Gannon KS, Jacques V, Murray J, . . . Al-Mousily F 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.
- Rickli A, Kopf S, Hoener MC, Liechti ME Pharmacological profile of novel psychoactive benzofurans. *British Journal of Pharmacology.* 2015;172(13):3412-3425.
- Robiolio PA, Rigolin VH, Wilson JS, Harrison JK, Sanders LL, Bashore TM, Feldman JM Carcinoid heart disease. Correlation of high serotonin levels with valvular abnormalities detected by cardiac catheterization and echocardiography. *Circulation.* 1995;92(4):790-795.
- Roth BL Drugs and valvular heart disease. *N Engl J Med.* 2007;356(1):6-9.
- Ruddell RG, Oakley F, Hussain Z, Yeung I, Bryan-Lluka LJ, Ramm GA, Mann DA A role for serotonin (5-HT) in hepatic stellate cell function and liver fibrosis. *Am J Pathol.* 2006;169(3):861-876.
- Russell A, Baner A, Berlin H, Fink GD, Watts SW 5-Hydroxytryptamine<sub>2B</sub> Receptor Function Is Enhanced in the N(omega)-Nitro-L-arginine Hypertensive Rat. *J Pharmacol Exp Ther.* 2002;303(1):179-187.
- Sanden N, Thorlin T, Blomstrand F, Persson PA, Hansson E 5-Hydroxytryptamine<sub>2B</sub> receptors stimulate Ca<sup>2+</sup> increases in cultured astrocytes from three different brain regions. *Neurochem Int.* 2000;36(4-5):427-434.
- Schmitz B, Ullmer C, Segelcke D, Gwarek M, Zhu X-R, Lübbert H BF-1--a novel selective 5-HT<sub>2B</sub> receptor antagonist blocking neurogenic dural plasma protein extravasation in guinea pigs. *Eur J Pharmacol.* 2015;751:73-80.
- Schmuck K, Ullmer C, Engels P, Lübbert H Cloning and functional characterisation of the human 5-HT<sub>2B</sub> serotonin receptor. *FEBS L.* 1994;342:85-90.
- Schmuck K, Ullmer C, Kalkman H, Probst A, Lübbert H Activation of meningeal 5-HT<sub>2B</sub> receptors: an early step in the generation of migraine headache? *Eur J Neurosci.* 1996;8:959-967.
- Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon RA, Blough B, . . . Roth BL 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol.* 2003;63(6):1223-1229.
- Shahid M, Walker GB, Zorn SH, Wong EHF Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *J Psychopharm.* 2009;23(1):65-73.
- Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, . . . Mailman R Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology.* 2003;28(8):1400-1411.
- Sharif NA, Senchyna M Serotonin receptor subtype mRNA expression in human ocular tissues, determined by RT-PCR. *Mol Vis.* 2006;12:1040-1047.
- Slominski A, Pisarchik A, Wortsman J Expression of genes coding melatonin and serotonin receptors in rodent skin. *Biochim Biophys Acta.* 2004;1680(2):67-70.
- Slominski A, Pisarchik A, Zbytek B, Tobin DJ, Kauser S, Wortsman J Functional activity of serotonergic and melatonergic systems expressed in the skin. *J Cell Physiol.* 2003;196(1):144-153.
- Soll C, Jang JH, Riener M-O, Moritz W, Wild PJ, Graf R, Clavien P-A Serotonin promotes tumor growth in human hepatocellular cancer. *Hepatology.* 2010;51(4):1244-1254.
- Soll C, Riener M-O, Oberkofler CE, Hellerbrand C, Wild PJ, Deoliveira ML, Clavien P-A Expression of serotonin receptors in human hepatocellular cancer. *Clin Cancer Res.* 2012;18(21):5902-5910.

Stefulj J, Jernej B, Cicin-Sain L, Rinner I, Schauenstein K mRNA expression of serotonin receptors in cells of the immune tissues of the rat. *Brain Behav Immun.* 2000;14(3):219-224.

Su Y-S, Chiu Y-Y, Lin S-Y, Chen C-C, Sun W-H Serotonin Receptor 2B Mediates Mechanical Hyperalgesia by Regulating Transient Receptor Potential Vanilloid 1. *J Mol Neurosci.* 2016;59(1):113-125.

Svejda B, Kidd M, Giovinazzo F, Eltawil K, Gustafsson BI, Pfragner R, Modlin IM The 5-HT<sub>2B</sub> receptor plays a key regulatory role in both neuroendocrine tumor cell proliferation and the modulation of the fibroblast component of the neoplastic microenvironment. *Cancer.* 2010;116(12):2902-2912.

Tadros SF, D'Souza M, Zettel ML, Zhu X, Lynch-Erhardt M, Frisina RD Serotonin 2B receptor: upregulated with age and hearing loss in mouse auditory system. *Neurobiol Aging.* 2007;28(7):1112-1123.

Tournois C, Mutel V, Manivet P, Launay JM, Kellermann O Cross-talk between 5-hydroxytryptamine receptors in a serotonergic cell line. Involvement of arachidonic acid metabolism. *J Biol Chem.* 1998;273(28):17498-17503.

Tschentscher F, Husing J, Holter T, Kruse E, Dresen IG, Jockel KH, . . . Zeschning M Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res.* 2003;63(10):2578-2584.

Ullmer C, Schmuck K, Kalkman HO, Lübbert H Expression of serotonin receptor mRNA in blood vessels. *FEBS Lett.* 1995;370:215-221.

Urtikova N, Berson N, Van Steenwinckel J, Doly S, Truchetto J, Maroteaux L, . . . Conrath M Antinociceptive effect of peripheral serotonin 5-HT<sub>2B</sub> receptor activation on neuropathic pain. *Pain.* 2012;153(6):1320-1331.

van Gils W, Lodder EM, Mensink HW, Kiliç E, Naus NC, Brüggewirth HT, . . . de Klein A Gene expression profiling in uveal melanoma: two regions on 3p related to prognosis. *Investigative Ophthalmology & Visual Science.* 2008;49(10):4254-4262.

Vane JR A sensitive method for the assay of 5-hydroxytryptamine. *Br J Pharmacol Chemother.* 1957;12(3):344-349.

Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E, . . . Stevens RC Structural features for functional selectivity at serotonin receptors. *Science.* 2013;340(6132):615-619.

Wainscott DB, Cohen ML, Schenck KW, Audia JE, Nissen JS, Baez M, . . . Nelson DL Pharmacological characteristics of the newly cloned rat 5-Hydroxytryptamine 2F receptor. *Mol. Pharmacol.* 1993;43:419-426.

Wainscott DB, Lucaites VL, Kursar JD, Baez M, Nelson DL Pharmacologic characterization of the human 5-hydroxytryptamine<sub>2B</sub> receptor: evidence for species differences. *J Pharmacol Exp Ther.* 1996;276(2):720-727.

Wang C, Jiang Y, Ma J, Wu H, Wacker D, Katritch V, . . . Xu HE Structural basis for molecular recognition at serotonin receptors. *Science.* 2013;340(6132):610-614.

Watts SW, Baez M, Webb RC The 5-hydroxytryptamine<sub>2B</sub> receptor and 5-HT receptor signal transduction in mesenteric arteries from deoxycorticosterone acetate- salt hypertensive rats. *J Pharmacol Exp Ther.* 1996;277(2):1103-1113.

Watts SW, Fink GD 5-HT<sub>2B</sub>-receptor antagonist LY-272015 is antihypertensive in DOCA-salt- hypertensive rats. *Am J Physiol.* 1999;276(3 Pt 2):H944-952.

Weir EK, Obrezchikova M, Hong Z Fenfluramine: riddle or Rosetta stone? *Eur Respir J.* 2008;31(2):232-235.

West JD, Carrier EJ, Bloodworth NC, Schroer AK, Chen P, Ryzhova LM, . . . Merryman WD Serotonin 2B Receptor Antagonism Prevents Heritable Pulmonary Arterial Hypertension. *PloS one.* 2016;11(2):e0148657.

Westbroek I, van Der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ Expression of serotonin receptors in bone. *J Biol Chem.* 2001;276:28961-28968.

Yabanoglu S, Akkiki M, Seguelas MH, Mialet-Perez J, Parini A, Pizzinat N Platelet derived serotonin drives the activation of rat cardiac fibroblasts by 5-HT<sub>2A</sub> receptors. *J Mol Cell Cardiol.* 2009;46(4):518-525.

Yadav VK, Oury F, Suda N, Liu Z-W, Gao X-B, Confavreux C, . . . Karsenty G A Serotonin-Dependent Mechanism Explains the Leptin Regulation of Bone Mass, Appetite, and Energy Expenditure. *Cell.* 2009;138(5):976-989.

Yan L, Chen X, Talati M, Nunley BW, Gladson S, Blackwell T, . . . Hamid R Bone Marrow-derived Cells Contribute to Pathogenesis of Pulmonary Arterial Hypertension. *Am J Resp Crit Care Med.* 2016.

Zhang Y, Yang Y, Chen L, Zhang J Expression analysis of genes and pathways associated with liver metastases of the uveal melanoma. *BMC Med Genet.* 2014;15:29.

Zopf DA, Neves LAAd, Nikula KJ, Huang J, Senese PB, Gralinski MR C-122, a novel antagonist of serotonin receptor 5-HT<sub>2B</sub>, prevents monocrotaline-induced pulmonary arterial hypertension in rats. *Eur J Pharmacol.* 2011;670(1):195-203.

Zsippai A, Szabó DR, Szabó PM, Tömböl Z, Bendes MR, Nagy Z, . . . Igaz P mRNA and microRNA expression patterns in adrenocortical cancer. *Am J Cancer Res.* 2011;1(5):618-628.