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New therapeutic opportunities for 5-HT$_2$ receptor ligands

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ABSTRACT

Serotonergic dysfunction is mainly associated with neuropsychiatric and cardiovascular disorders but has also been linked with many other pathological conditions. Serotonin (5-hydroxytryptamine, 5-HT) mediates numerous physiological functions in the brain and the periphery by activating a variety of receptors. 5-HT receptors are divided into four classes, three of which belong to the G protein-coupled receptor family. This review provides an overview of the recent pharmacological developments involving the Gq-coupled 5-HT$_2$ receptor subfamily as well as the pathological implications of this receptor subfamily with regard to fibrosis, the central nervous system, cardiovascular disorders, and cancer. The final section highlights new therapeutic opportunities and emerging research revealing unexplored medical opportunities for this class of 5-HT receptors. The development of biased 5-HT$_2$ receptor ligands appears to be an interesting topic in various areas. In light of recent discoveries, the need for the development of new and safer drugs should take into account the risk of cardiovascular side effects such as pulmonary hypertension and heart valve disease.

Contents

1. Introduction

This review focuses on three serotonin (5-hydroxytryptamine, 5-HT) receptors belonging to the 5-HT$_2$ receptor subfamily: the 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ subtypes. Although the work by Julius et al. (1988) was the first to report the cloning of a full-length functional serotonin receptor from rat (the 5-HT$_{1c}$ receptor), this publication was shortly followed by considerable efforts from several groups that cloned other unidentified 5-HT receptors. The classical 5-HT$_2$ receptor described by Peroutka et al. (1981) was cloned from rats slightly later in 1988 (Pritchett et al., 1988) followed by human analogue (Branchek et al., 1990; Saltzman et al., 1991) and was renamed 5-HT$_{2A}$. The 5-HT$_{1c}$ receptor was renamed 5-HT$_{2C}$ because of its structural similarity to the other 5-HT$_2$ receptor, identical second messenger pathways, and similar pharmacological properties. Pharmacological studies attempting to characterize the contractile serotonergic receptor in the rat stomach fundus initially documented its similarity to the 5-HT$_{2C}$ receptor. Despite the absence of detectable 5-HT$_{2C}$ receptor mRNA in the rat

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stomach fundus, only homology cloning permitted the identification of a new receptor in 1992 in rat and mouse that was named 5-HT_{2B} (Foguet et al., 1992a, 1992b; 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).

The investigation of the contribution of these three 5-HT_2 receptors in mammalian physiology has led to a large number of reports in nearly all functions and organs. Some selective compounds stimulating or blocking these receptors provided an opportunity to explore various areas of human diseases. In this review, we will emphasize some important aspects of the cellular and molecular biology of these receptors and highlight some clinical situations in which these receptors appear as pathophysiological cornerstones.

2. 5-HT_2 receptors: structure, coupling, oligomerization, selective ligands, allosteric modulators, biased agonists

The closely related 5-HT_2 receptors are members of the rhodopsin family of G protein-coupled receptors (GPCRs) that activate multiple intracellular signalling networks. The classical signal transduction pathway for this subfamily is the Gq/11-coupled activation of phospholipase C (PLC), although these 5-HT_2 receptors can also activate phospholipase D and phospholipase A2 by interacting with additional pathways. These 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors are post-transcriptionally modified by alternative RNA splicing, a common mechanism for achieving protein diversity. RNA editing, on the other hand, is a less common process for generating molecular diversity. In fact, the 5-HT_{2C} receptor is one of the few GPCRs known to be edited. RNA editing of the 5-HT_{2C} receptor generates functionally distinct protein variants by altering the genetic code at the mRNA level.

2.1. Structure

5-HT_2 receptors are 7 transmembrane domain receptors with fairly long extracellular N-terminal loops ranging from 55 amino acids for the human 5-HT_{2B} and 5-HT_{2C} receptors to 75 amino acids for the human 5-HT_{2A} receptor and an intracellular C-terminus ranging from 85 amino acids for the human 5-HT_{2B} receptor to 75 amino acids for the human 5-HT_{2A} and 5-HT_{2C} receptors. A new and unanticipated role of the 5-HT_{2B} receptor N-terminus as a negative modulator affecting both constitutive and agonist-stimulated activity of the receptor has been shown (Belmer et al., 2014). The recently published crystal structure of the 5-HT_{2B} receptor bound to ergotamine showed that this receptor exhibits conformational characteristics in both the active and inactive states: an active-like state in the helix VII conformation of the 5-HT_{2B} receptor but only partial changes in helix VI. The differential signalling patterns were also mirrored in the crystal structures, which showed features of a β-arrestin-biased activation state for the 5-HT_{2B} 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_{2B} receptors can be found in the region of the extracellular loop 2 (ECL2) junction with helix V (E212-R213-F214), which forms 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 disulphide bond is shortened in the 5-HT_{2B} 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 the 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). No crystal structures have reported yet for the 5-HT_{2A} or 5-HT_{2C} receptors.

More work is needed to precisely understand the structure and function of these receptors as well as their specific properties.

2.2. Selective agonists

There is virtually no highly selective agonist for a particular 5-HT_2 receptor:

- 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_{2C} and 100-fold selectivity over the 5-HT_{2A} receptors (Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008). Lorcaserin [(1*R*)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1*H*-3 benzazepine] has approximately 10-fold higher affinity for 5-HT_{2C} receptor (Thomsen et al., 2008) over the 5-HT_{2A} and 5-HT_{2B} receptors.
- Nor-dexfenfluramine (a metabolite of dexfenfluramine), methylergonovine (a 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_{2B} agonists with approximately 10-fold selectivity over the 5-HT_{2C} receptor (Cussac et al., 2002).
- 2,5-dimethoxy-4-iodoamphetamine (DOI) is a non-selective nearly full agonist at 5-HT_2 receptors with similar affinity to the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004; Cussac et al., 2008).
- Alpha-methyl-5-HT is a non-selective nearly full agonist at 5-HT_2 receptors with a similar affinity towards the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (Porter et al., 1999; Jerman et al., 2001; Knight et al., 2004).

2.3. Selective antagonists

A few selective antagonists are available for the 5-HT_2 receptor subtypes:

- The first highly selective 5-HT_{2A} receptor antagonist reported was MDL100907 [(*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (Knight et al., 2004). Sarpogrelate [succinic acid mono-(1-dimethylaminomethyl-2-(2-[2-(3-methoxyphenyl) ethyl] phenoxy) ethyl) ester hydrochloride], SR46349B [4-((3Z)-3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl)phenol hemifumarate], and ketanserin [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]quinazoline-2,4(1*H*, 3*H*)-dione] are preferential 5-HT_{2A} receptor antagonists with a 10-fold higher affinity over the 5-HT_{2C} and/or 5-HT_{2B} sites.
- The first highly selective 5-HT_{2B} receptor antagonist reported was LY266097: 1-(2-chloro-3,4-dimethoxybenzyl)-6-methyl-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole hydrochloride, with a pKi of 9.7 for the human cloned 5-HT_{2B} receptor and a 100-fold greater selectivity over human 5-HT_{2C} and 5-HT_{2A} receptors (Audia et al., 1996). SB204741: *N*-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazolyl) urea has been reported as a selective 5-HT_{2B} receptor antagonist with approximately 100-fold selectivity over the 5-HT_{2C} and 5-HT_{2A} receptors but with a low potency (Ki approximately 100 nM) (Bonhaus et al., 1995). The tetrahydro-β-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). RS127445 [2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine] was found to have sub-nanomolar affinity for the 5-HT_{2B} receptor (pKi = 9.5) and 1000-fold selectivity for this receptor compared to numerous other receptor and ion channel binding sites and appears as the most selective, high-affinity 5-HT_{2B} receptor antagonist currently available (Bonhaus et al., 1999). SB215505 [6-chloro-5-methyl-*N*-(5-quinolinyl)-2,3-dihydro-1H-indole-1-carboxamide] behaves as a high-affinity and preferential inverse agonist at 5-HT_{2B} receptors (Reavill et al., 1999).
- SB242084 [6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl-carbamoyl]indoline] and RS-102221 [*N*-[5-[5-(2,5-dioxo-spiro[imidazolidine-4,4′-piperidin]-1′-yl)pentanoyl]-2,4-

dimethoxy-phenyl]-4-(trifluoromethyl)benzenesulfonamide] are selective 5-HT_{2C} antagonists (Bonhaus et al., 1997; Knight et al., 2004).

- 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_{2C}/5-HT_{2B} receptor antagonist. It has been reported as a selective 5-$HT_{2C/2B}$ receptor inverse agonist with 50- to 100-fold lower affinity for 5-HT_{2A} and other sites (Kennett et al., 1996; Knight et al., 2004).

Non-selective 5-HT_2 receptor antagonists such as ritanserin and mesulergine block 5-HT_2 receptor-mediated effects. Atypical antipsychotics including clozapine, asenapine, or cariprazine also have a fairly high affinity for all 5-HT_2 receptors (Wainscott et al., 1996; Millan et al., 2003; Shahid et al., 2009; Kiss et al., 2010). Aripiprazole (OPC-14597) is a novel atypical antipsychotic drug that has higher antagonist activity (EC_{50} = 11 nM) at the human 5-HT_{2B} receptor than at the 5-HT_{2A} or 5-HT_{2C} receptors (Shapiro et al., 2003). See Table 1 and the PDSP database, http://kidbdev.med.unc.edu/databases/pdsp.php.

2.4. Coupling

Intracellular signals are inherently challenging targets because they are often ubiquitous; indeed, drug vectorization comes from GPCRs not transduction pathways. Nonetheless, multi-target drugs acting at two key nodes in a signalling network offer one answer. Another approach to this concept is the manipulation of interactions between 5-HT receptors and their protein partners. One good example is the use of small peptides to decouple 5-HT_{2C} receptors from their PDZ partners, which mimics the desensitization elicited by antidepressants (Gavarini et al., 2006). Conversely, blocking the interactions between 5-HT_{2C} receptors and the phosphatase PTEN (phosphatase with tensin homology) reproduces the 5-HT_{2C} agonist-induced inhibition of the excitation of mesolimbic dopaminergic neurons by cannabinoids, thereby preventing their rewarding effects (Ji et al., 2006). Hence, interference with the association between 5-HT_{2C} receptors and PTEN might be an interesting way to counteract drug addiction.

β-Arrestins direct the agonist-induced internalization of 5-HT receptors; however, agonist-independent association with β-arrestins

Table 1
(Cussac et al., 2002; Rashid et al., 2003; Shapiro et al., 2003; Knight et al., 2004; Rosenzweig-Lipson et al., 2006; Siuciak et al., 2007; Cussac et al., 2008; Thomsen et al., 2008; Shahid et al., 2009; Banas et al., 2011).

		h5-HT_{2A} pKi	r5-HT_{2A} pKi	h5-HT_{2B} pKi	r5-HT_{2B} pKi	m5-HT_{2B} pKi	h5-HT_{2C} pKi	r5-HT_{2C} pki	m5-HT_{2C} pKi
BW723C86	Banas, 2011, h_{2C}INI, m_{2C}VNi			7.89 ± 0.01		8.04 ± 0.15	6.90 ± 0.01		6.78 ± 0.05
	Knight 2004 h_{2C}INI	7.2 ± 0.08		7.33 ± 0.03			7.11 ± 0.21		
	Cussac 2008 h_{2C}VSV	6.63 ± 0.06		7.85 ± 0.11			7.11 ± 0.01		
RO600175	Banas, 2011, h_{2C}INI, m_{2C}VNi			9.01 ± 0.13		8.64 ± 0.14	7.72 ± 0.22		7.35 ± 0.29
	Knight 2004 h_{2C}INI	7.44 ± 0.04		8.27 ± 0.06			8.22 ± 0.29		
	Cussac 2008 h_{2C}VSV	6.80 ± 0.08		8.66 ± 0.13			7.67 ± 0.07		
WAY161503	Banas, 2011, h_{2C}INI, m_{2C}VNi			7.28 ± 0.19		7.84 ± 0.12	7.46 ± 0.05		6.92 ± 0.11
	Rosenzweig 2006 h_{2C}?	7.74 ± 0.11		7.22 ± 0.03			8.48 ± 0.14		
CP809101	Siuciak $_{2C}$?	8.22 ± 0.15		7.19 ± 0.25			8.80 ± 0.11		
	Banas, 2011, h_{2C}INI, m_{2C}VNi			7.86 ± 0.18		8.41 ± 0.18	8.35 ± 0.02		7.72 ± 0.15
DOI	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.29 ± 0.18		7.87 ± 0.06	7.60 ± 0.02		7.41 ± 0.24
	Knight 2004 h_{2C}INI	9.02 ± 0.11		7.55 ± 0.05			8.08 ± 011		
	Cussac 2008 h_{2C}VSV	8.04 ± 0.05		7.78 ± 0.09			7.73 ± 0.04		
Norfenfluramine	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.02 ± 0.19		6.76 ± 0.23	7.09 ± 0.62		6.21 ± 0.07
	Knight 2004 h_{2C}INI	6.82 ± 0.29		7.00 ± 0.06			7.29 ± 0.04		
D-LSD	Knight 2004 h_{2C}INI	9.12 ± 0.06		9.01 ± 0.09			8.96 ± 0.06		
	Cussac 2008 h_{2C}VSV	9.49 ± 0.03		9.22 ± 0.02			8.52 ± 0.06		
Lorcaserine	Thomsen $_{2C}$?	6.95 ± 0.03	6.80 ± 0.08	6.76 ± 0.09	6.72 ± 0.01		7.82 ± 0.03	7.54 ± 0.12	
Clozapine	Banas, 2011, h_{2C}INI, m_{2C}VNi					7.97 ± 0.09			
	Knight 2004 h_{2C}INI	7.60 ± 0.08		7.99 ± 0.09			7.87 ± 0.05		
	Shahid 2009 $_{2C}$?	8.39 ± 0.03		8.79 ± 0.09			8.56 ± 0.06		
Aripiprazole	Banas, 2011, h_{2C}INI, m_{2C}VNi					7.21 ± 0.09			
	Schapiro 2003 h_{2C}INI	8.06 ± 0.10	7.66 ± 0.08	9.44 ± 0.16			7.12 ± 0.09	7.12 ± 0.04	
	Shahid 2009 $_{2C}$?	8.02 ± 0.16		9.59 ± 0.17				7.55 ± 0.14	
RS1022221	Banas, 2011, h_{2C}INI, m_{2C}VNi			6.47 ± 0.02		6.52 ± 0.08	8.01 ± 0.30		7.72 ± 0.22
	Knight 2004 h_{2C}INI	5.54 ± 0.03		5.95 ± 0.06			8.30 ± 0.05		
	Cussac 2002 h_{2C}VSV			6.63 ± 0.05			8.83 ± 0.04		
SB215505	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.12 ± 0.01		7.61 ± 0.21	7.40 ± 0.02		7.24 ± 0.26
	Knight 2004 h_{2C}INI								
	Cussac 2002 h_{2C}VSV			8.83 ± 0.09			7.95 ± 0.06		
SB206553	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.29 ± 0.04		7.06 ± 0.41	8.24 ± 0.01		8.21 ± 0.24
	Knight 2004 h_{2C}INI	5.64 ± 0.09		7.65 ± 0.07			7.79 ± 0.07		
	Cussac 2002 h_{2C}VSV			8.26 ± 0.17			8.50 ± 0.13		
SB242084	Banas, 2011, h_{2C}INI, m_{2C}VNi			6.36 ± 0.02		6.07 ± 0.01	8.19 ± 0.22		5.93 ± 0.27
	Knight 2004 h_{2C}INI	6.07 ± 0.18		6.84 ± 0.28			8.15 ± 0.10		
	Cussac 2002 h_{2C}VSV			7.34 ± 0.07			9.32 ± 0.06		
Mesulergine	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.39 ± 0.2		7.81 ± 0.15	9.01 ± 0.01		8.53 ± 0.21
	Knight 2004 h_{2C}INI	7.34 ± 0.03		8.46 ± 0.05			8.74 ± 0.03		
	Cussac 2002 h_{2C}VSV			8.71 ± 0.02			8.95 ± 0.06		
RS127445	Banas, 2011, h_{2C}INI, m_{2C}VNi			8.51 ± 0.07		8.22 ± 0.24	5.63 ± 0.05		5.33 ± 0.45
	Knight 2004 h_{2C}INI	6.03 ± 0.13		8.97 ± 0.09			6.33 ± 0.10		
MDL100907	Banas, 2011, h_{2C}INI, m_{2C}VNi			5.79 ± 0.60		5.03 ± 0.23	6.79 ± 0.51		6.64 ± 0.17
	Knight 2004 h_{2C}INI	8.73 ± 0.20		5.99 ± 0.06			7.52 ± 0.13		
SB204741	Knight 2004 h_{2C}INI	<5.00		6.90 ± 0.27			5.56 ± 0.07		
	Cussac 2002 h_{2C}VSV			7.29 ± 0.04			5.67 ± 0.11		
Sarpogrelate	Rashid 2003, $_{2C}$?	8.52 ± 0.12		6.57 ± 0.12			7.43 ± 0.03		
Ketanserin	Rashid 2003, $_{2C}$?	9.67 ± 0.12		6.55 ± 0.09			7.39 ± 0.11		

has been reported for non-edited (and partially edited) 5-HT_{2C} receptors (Marion et al., 2004). This interaction leads to constitutive internalization (Marion et al., 2004), an effect prevented by inverse agonists (Chanrion et al., 2008). Calmodulin binds to the proximal region of the 5-HT_{2C} receptor C-terminus upon receptor activation by 5-HT. Mutation of this motif inhibits both β-arrestin recruitment to the 5-HT_{2C} receptor and receptor-operated ERK1/2 signalling in HEK-293 cells, which is independent of G proteins and dependent on β-arrestins. Expression of the calmodulin mutant also prevents receptor-mediated ERK1/2 phosphorylation in cultured cortical neurons and choroid plexus epithelial cells that endogenously express 5-HT_{2C} receptors (Labasque et al., 2008). Intriguingly, although β-arrestins are implicated in the hallucinogenic effects mediated by 5-HT_{2A} receptors, their trafficking is independent (Bhatnagar et al., 2001). β-Arrestin 2 also directs agonist-induced internalization of 5-HT_{2B} receptors (Janoshazi et al., 2007). For instance, β-arrestins contribute to activation of ERK by the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors.

PDZ (postsynaptic-density-95/disc-large/zonulla-occludens-1) domain-containing proteins profoundly influence the internalization of the 5-$HT_{2A,2B,2C}$ receptors, and PDZ proteins are essential for targeting 5-HT_{2A} receptors to dendrites in cortical neurons (Xia et al., 2003b). PDZ partners are both receptor- and function-specific. Thus, the specific sets of PDZ proteins that interact with the 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors differ. Although PSD-95 (postsynaptic-density-95) prevents 5-HT_{2A} receptor internalization (Xia et al., 2003a), it favours the constitutive and agonist-dependent endocytosis of 5-HT_{2C} receptors (Gavarini et al., 2006). Conversely, MPP3 (membrane protein palmitoylated 3) stabilizes 5-HT_{2C} receptors at the plasma membrane (Gavarini et al., 2006). The 5-HT_{2B} receptor shares the C-terminal E-X-V/I-S-X-V sequence with 5-HT_{2C} receptors and also binds MUPP1-PDZ domains in vitro (Becamel et al., 2001). MUPP1 was shown to interact with the -SSV sequence on the C-terminus of the 5-HT_{2C} receptor. Moreover, 5-HT_{2A} and 5-HT_{2B} receptors sharing the C-terminal -E-X-V/I-S-X-V sequence with 5-HT_{2C} receptors also bind MUPP1-PDZ domains in vitro (Becamel et al., 2001). The PDZ motif on the C-terminus of the 5-HT_{2B} receptor was also found to be necessary for the recruitment of the constitutive NO synthase (cNOS-NOS3)(Manivet et al., 2000). In addition, stimulation of the 5-HT_{2B} receptor triggered intracellular cGMP production through dual activation of NOS3 and inducible NOS (iNOS–NOS2). The group I PDZ motif at the carboxy terminus of the 5-HT_{2B} receptor was shown to be required for the recruitment of the NOS3 transduction pathways, and NOS2 stimulation was controlled by the Gα13 pathways (Manivet et al., 2000).

The human polyomavirus JCV causes the fatal demyelinating disease progressive multifocal leukoencephalopathy in immunocompromised patients. Elphick et al. (2004) found that the 5-HT_{2A} serotonergic receptor could act as the cellular receptor on human glial cells for JCV. 5-HT_{2A} receptor antagonists inhibited JCV infection, and monoclonal antibodies targeting 5-HT_{2A} receptors blocked the infection of glial cells by JCV but not by SV40. Transfection of 5-HT_{2A} receptor-negative HeLa cells with a 5-HT_{2A} receptor restored virus infection, and this infection was blocked by an antibody targeting the 5-HT_{2A} receptor. A tagged 5-HT_{2A} receptor colocalized with labelled JCV in an endosomal compartment following internalization. Later, it was observed that endothelial cells not expressing the 5-HT_{2A} receptor could be infected. Following this observation, it was reported that virus entry into HEK293A cells was specifically observed when any of the 5-HT_2 receptors were expressed. Recent data confirmed that virus internalization into HEK293A cells was significantly and specifically allowed by 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} serotonin receptors in a way somewhat similar to CCR5 chemokine receptor, which acts as a co-receptor for HIV-1 viral entry. This work shows that the 5-HT_2 subfamily of serotonin receptors contributes to JCPyV infection by facilitating viral entry (Assetta et al., 2013).

A better understanding of the interactions between viruses and the 5-HT_2 receptors may lead to new antiviral opportunities.

2.5. Oligomerization

Oligomeric associations of GPCRs often comprise signalling units, and 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors may all form homo- or heterodimers (Herrick-Davis et al., 2004; Brea et al., 2009; Jaffre et al., 2009). In contrast to some classes of GPCRs, agonist binding does not greatly influence the formation of 5-HT-receptor dimers, indicating a constitutive assembly before membrane insertion. For example, 5-HT_{2C} receptors generate dimers in the endoplasmic reticulum and Golgi of living cells (Herrick-Davis et al., 2005). 5-HT_{2C} receptor dimers possess an interface between transmembrane helices IV and V, and dimer proximity is increased and decreased by agonists and inverse agonists, respectively (Mancia et al., 2008). Furthermore, analysis of functionally compensating, coexpressed mutant 5-HT_{2C} receptors linked to Gq (fusion proteins) indicates that the dimer is asymmetric versus Gq, with both subunits binding to 5-HT and exerting distinct roles during signalling (Herrick-Davis et al., 2005; Mancia et al., 2008).

The 5-HT_{2A} and metabotropic glutamate 2 (mGlu2) receptors assemble into heterodimers via a transmembrane-IV and transmembrane-V linking domain (Gonzalez-Maeso et al., 2008). Intriguingly, mGlu2 receptor agonists blunt heterodimer coupling to Gi, providing one substrate for their anti-hallucinogenic properties (Gonzalez-Maeso et al., 2008; Fribourg et al., 2011). Direct evidence of mGlu2/5-HT_{2A} heterodimers in the human brain and a reduced density of these heterodimers in patients with schizophrenia has been acquired (Gonzalez-Maeso et al., 2008). Nonetheless, this study remains one of the few demonstrations of heterodimers in tissues. Of particular interest are ligands specific for 5-HT_{2A}/mGlu2 complexes or other putative heterodimers possessing distinctive binding and coupling profiles (Gonzalez-Maeso et al., 2008). Evidence of functional crosstalk between the 5-HT_{2A} and D_2 receptors was reported in HEK293 cells. D2 receptor activation increases the hallucinogenic agonist affinity for the 5-HT_{2A} receptor and decreases inositol phosphate production. Co-immunoprecipitation studies show that these two receptors can physically interact in HEK293 cells and raise the possibility that a receptor heterocomplex mediates the observed crosstalk. In vivo, 5-HT_{2A} receptor expression is necessary for the full effects of a D_2 antagonist on MK-801-induced locomotor activity (Albizu et al., 2011). Behavioural studies performed in mice lacking 5-HT_{2A} receptors also revealed a remarkable 5-HT_{2A} receptor-dependent dissociation in the beneficial antinociceptive effects of cannabinoid CB1 receptors agonist delta-9-tetrahydrocannabinol (THC) and its detrimental amnesic properties. Biochemical studies have shown that CB1 and 5-HT_{2A} receptors form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment. Remarkably, costimulation of both receptors by agonists reduces cell signalling; antagonist binding to one receptor blocks signalling of the interacting receptor; and heteromer formation leads to a switch in G protein coupling of the 5-HT_{2A} receptor from Gq to Gi proteins (Vinals et al., 2015).

In cardiac fibroblasts, the angiotensin receptor AT_1 and 5-HT_{2B} receptors have been reported to share common signalling pathways, which support a possible direct interaction between 5-HT_{2B} and AT_1 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 in vivo experimental confirmation is still lacking. Ghrelin, an orexigenic peptide present in the stomach, has gastroprokinetic properties. In vivo studies have shown that the ghrelin receptor (GHS-R1a) antagonist [D-Lys(3)]-GHRP-6 reduces food intake and delays gastric emptying in rodents, but these effects are dissimilar with the observed phenotype of ghrelin knockout mice. [D-Lys(3)]-GHRP-6 induced a pronounced contraction of the stomach strips, which was blocked by the 5-HT_2 receptor antagonists methysergide and yohimbine resulting in smooth muscle contractions and suggesting the possibility of direct interactions with 5-HT_{2B} receptors (Depoortere et al., 2006).

A possibility for GHS-R1a/5-HT_{2C} dimer-induced attenuation of calcium signalling was also observed. Flow cytometry fluorescence resonance energy transfer (fcFRET) assays confirmed the direct interaction between the GHS-R1a receptor and 5-HT_{2C} receptor. Colocalized expression of the 5-HT_{2C} and GHS-R1a receptors in cultured primary hypothalamic and hippocampal rat neurons further supports the biological relevance of this physiological interaction. When 5-HT_{2C} receptor signalling is blocked, ghrelin's orexigenic effect is potentiated in vivo. In contrast, the 5-HT_{2C} receptor-selective agonist lorcaserin attenuates ghrelin-induced food intake (Schellekens et al., 2015). Physical associations of the melatonin MT2 and 5-HT_{2C} receptors as functional heteromers were also found by co-immunoprecipitation, bioluminescence resonance energy transfer, and pharmacological techniques both in transfected cells and in cells from human cortex and hippocampus. MT2/5-HT_{2C} heteromers amplify the 5-HT-mediated Gq/phospholipase C response and trigger melatonin-induced unidirectional transactivation of the 5-HT_{2C} protomer in MT2/5-HT_{2C} heteromers. Pharmacological studies reveal distinct functional properties for agomelatine, which indicates “biased signalling” (Kamal et al., 2015).

Future studies must focus on the putative in situ heterodimerization of native 5-HT receptors and on their pharmacological profiles with the goal of identifying novel targets for therapeutic intervention.

2.6. Allosteric modulators

Positive allosteric modulators (PAMs) represent alternative approaches to orthosteric agonists (i.e., compounds that interact with the native ligand-binding site). PAMs can increase the affinity and/or efficacy of the orthosteric agonist for its target receptor by acting at a site other than the native ligand-binding site (allostery). Importantly, so-called pure GPCR PAMs, which lack intrinsic agonist activity within a specific signalling pathway, have been described. These compounds modulate the basal tone of the endogenous ligand in a manner that conserves the spatial and temporal elements of native neurotransmission (Christopoulos & Kenakin, 2002). Indeed, multiple PAMs have been identified for GPCRs and may circumvent the challenges of orthosteric agonists. First, PAMs could amplify endogenous signalling through the 5-HT_2 receptors, likely resulting in a more physiologically relevant enhancement of function compared to a direct orthosteric agonist. Second, because of generally higher sequence diversity in allosteric sites relative to the conserved orthosteric domain, PAMs could potentially achieve higher receptor selectivity than orthosteric agonists. Indeed, some 5-HT_{2C} receptor PAMs have been reported, although the pharmacological profiles of these compounds have not yet been widely reported (Ding et al., 2012). Ergotamine has been shown to occupy two distinct sites in 5-HT_{2B} receptors: the orthosteric site, where the indole nucleus of ergotamine resides, and an extended binding site, where the tripeptide portion is engaged. The allosteric site in the muscarinic M2 receptor is the same extracellular region as that interacting with the tripeptide portion of ergotamine. These similarities in both the M2 and 5-HT_{2B} 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, ergotamine occupies both the orthosteric and putative extracellular allosteric site in the 5-HT_{2B} receptor. It is now thought that a sodium ion allosterically alters the binding pocket to dampen G protein signalling, leaving β-arrestin recruitment intact. Recent structural considerations support that this sodium pocket is collapsed in the 5-HT_{2B} receptor structure (McCorvy & Roth, 2015).

The identification of specific PAMs of the 5-HT_2 receptors may conceivably lead to improved therapeutics.

2.7. Biased agonists

Another area for the development of agonists targeting 5-HT_2 receptors might emerge from so-called biased agonist compounds that share a functional selectivity for specific intracellular signalling pathways (Kenakin et al., 2012). 5-HT_2 receptors couple to multiple intracellular pathways including PLC and PLA2, and pharmacological evidence using recombinant cell-based systems suggest that non-selective 5-HT_2 agonists such as mCPP and quipazine may differentially activate these signalling pathways downstream from the 5-HT_{2C} receptor (Berg et al., 1998). Lysergic acid diethylamide and ergotamine displayed bias for β-arrestin signalling at 5-HT_{2B} receptors; other ergolines such as dihydroergotamine, methylergonovine, pergolide, and cabergoline also exerted similar effects. Ergotamine and dihydroergotamine, both of which contain a large tripeptide moiety substitution at the amide scaffold, displayed more extreme signalling bias at the 5-HT_{2B} receptor compared to lysergic acid diethylamide (Wacker et al., 2013). An additional approach is the identification of compounds with 5-HT_{2C} receptor agonist activity combined with antagonist activity at the 5-HT_{2A} receptor. Experimental evidence supports a potential synergy between these two pharmacological properties, raising the theoretical possibility that a single drug possessing both characteristics may be a superior therapeutic compared to either characteristic alone (Booth et al., 2009; Pockros et al., 2012; Cunningham et al., 2013; Canal et al., 2014).

Currently, it is unknown whether this example of functional selectivity could be translated into any therapeutic gain, although this notion does open up an interesting opportunity for future drug discovery.

3. Pathologies and 5-HT_2 receptors

3.1. Fibrosis and inflammation

Healing is the process of the restoration of health in an unbalanced, diseased, or damaged organism. Fibrogenesis is a critical process in wound repair that generates scar tissue and helps protect an injured organ until the damaged or lost cells are regenerated. When an injury naturally resolves, the fibrogenic response is usually limited, and the temporary scar tissue replaced by healthy functional cells (Mann & Oakley, 2013). States of chronic tissue infection or damage, ageing, tumours, feeding habits and/or exposure to drugs that generate microenvironments in which the inflammatory and fibrogenic phases of wound healing fail to resolve can induce a state of overactive wound healing and loss of the normal regenerative process (Kapetanaki et al., 2013; Mann & Oakley, 2013). This latter state can lead to the development of progressive fibrotic disease, in which normal tissue is gradually replaced by scar tissue. This type of fibrotic disease progression can occur as a consequence of uncontrolled repair processes in many organs in response to a wide variety of chronic insults. Unless the underlying injury process is effectively managed, the spread of fibrotic matrix ultimately impairs the architecture and function of the organ. In fact, fibrosis is the final common pathological outcome of many chronic and inflammatory diseases that subsequently leads to permanent scarring, organ malfunction, and ultimately death, as exemplified by end-stage liver disease, chronic kidney disease, idiopathic pulmonary fibrosis, and heart failure. Fibrosis is also a major pathological feature of many chronic autoimmune diseases such as scleroderma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, glomerulonephritis, and myelofibrosis. Pathologic fibrosis can either affect a single organ or become systemic when it affects numerous organs, and its incidence increases with low exercise or a high-fat high sugar diet as well as with age and thus with the ageing of the population. However, the causative agents triggering the development of fibrosis are often unknown. The penetrance varies among gender; the female:male ratio is 3:1 for systemic sclerosis and lung fibrosis and nearly the opposite for kidney, liver, and heart fibrosis.

Over the last decade, several investigations revealed the fact that the 5-HT system is activated during the early phases of wound repair and exerts a major influence on fibrogenesis. Modulating the activities of specific 5-HT receptors that trigger the activation of fibrogenic signal transduction seems to be a promising approach to control pathological

fibrosis. Mechanistic links between fibrosis and 5-HT were first reported in the 1960s for a condition known as carcinoid syndrome, which is caused by tumours arising from neuroendocrine enterochromaffin cells that synthesize 5-HT in the gut (i.e., carcinoid tumours that secrete vast quantities of 5-HT). While there is still much to learn about the method by which 5-HT and its different receptors converge in various target cell types to regulate tissue fibrotic repair, initial evidence reported the implication of the 5-HT_2 receptor subtypes in different pathological fibrotic tissues, including skin (Dees et al., 2011), lung (Launay et al., 2002), heart (Jaffre et al., 2009; Pavone et al., 2012), valves (Ayme-Dietrich et al., 2012), and liver (Ebrahimkhani et al., 2011). In addition to fibroblasts, these same receptors have been identified in haematopoietic stem cells (Amireault et al., 2011; Launay et al., 2012) and immune cells (de Las Casas-Engel et al., 2013), which also contribute to fibrosis.

However, there is still a complete lack of therapeutic compounds that target a specific 5-HT receptor and are selective for treating fibrotic diseases.

3.1.1. Lung fibrosis

In mouse lung homogenates, 5-HT concentrations significantly increase over the progression of bleomycin-induced fibrosis, with maximum values observed at day seven; these increases occur in conjunction with increased expression of the 5-HT_{2A} and 5-HT_{2B} receptors (Königshoff et al., 2010). Pharmacological blockade of either the 5-HT_{2A} or 5-HT_{2B} receptors reduces bleomycin-induced lung fibrosis, as demonstrated by reduced lung collagen content and reduced procollagen 1 and procollagen 3 mRNA expression levels. Serotonin antagonists promote an antifibrotic environment by decreasing the lung mRNA levels of TGF-β1, connective tissue growth factor and plasminogen activator inhibitor-1 as well as JunD mRNA. Interestingly, the 5-HT_{2B} receptor is strongly overexpressed in fibroblasts originating from the fibroblastic foci in either human idiopathic pulmonary fibrosis samples or bleomycin-induced pulmonary fibrosis samples in rodents (Fabre et al., 2008).

3.1.2. Liver fibrosis

In the liver, fibrogenic hepatic stellate cells (HSC), which are negative regulators of hepatocyte regeneration, are known to express 5-HT_{2A} and 5-HT_{2B} receptors that regulate TGF-β1 and the downstream signalling Smads (Li et al., 2006). HSCs are key cellular components of hepatic wound healing and fibrosis. After HSC activation, expression of 5-HT_{2A} and 5-HT_{2B} receptors becomes 100- and 50-fold that of quiescent cells, respectively. Treatment of HSCs with 5-HT_2 receptor antagonists suppresses proliferation and increases their rate of apoptosis. Serotonin synergizes with platelet-derived growth factor to stimulate increased HSC proliferation (Ruddell et al., 2006). Distinct from quiescent cells, activated HSCs exhibit transient [Ca2 +]i increases following treatment with 5-HT that are blocked by 5-HT_2 receptor antagonists (Park et al., 2011). Stimulation of 5-HT_{2B} receptors on HSCs by 5-HT was recently shown to activate the expression of TGF-β1 (a powerful suppressor of hepatocyte proliferation) via ERK/JunD signalling. Selective antagonism of 5-HT_{2B} receptors enhanced hepatocyte growth in models of acute and chronic liver injury. Similar effects are observed in mice lacking either 5-HT_{2B} or JunD as well as when HSCs are selectively depleted. Antagonism of 5-HT_{2B} attenuates CCl4-induced liver fibrogenesis and improves liver function in disease models in which fibrosis is pre-established and progressive (Ebrahimkhani et al., 2011).

3.1.3. Skin fibrosis

In skin experiencing systemic sclerosis (SSc), expression of 5-HT_{2B} receptors was strongly increased in the fibrotic tissue of patients, and almost all fibroblasts stained positive for 5-HT_{2B} receptors. Dermal fibrosis is reduced in $Htr_{2B}^{-/-}$ mice using both inducible and genetic models of fibrosis. Pharmacologic inactivation of the 5-HT_{2B} receptor also effectively prevents the onset of experimental fibrosis and ameliorates established fibrosis by decreasing the mRNA levels of TGF-β1, connective tissue growth factor, plasminogen activator inhibitor-1 and Smad-3 (Dees et al., 2011). Moreover, inhibition of platelet activation prevents fibrosis in different rodent models (including bleomycin-induced) of skin fibrosis. Consistently, mice deficient for TPH1, which is the rate-limiting enzyme for 5-HT production outside the central nervous system, show reduced experimental skin fibrosis (Dees et al., 2011).

3.1.4. Heart fibrosis

In rats, treatment of neonatal cardiac fibroblasts with 5-HT increases the expression of smooth muscle α-actin (a marker of fibroblast differentiation into myofibroblasts), stimulates their migration, and enhances the secretion of TGF-β1 and expression of MMPs, all of which seem to be mediated through 5-HT_{2A} receptors (Yabanoglu et al., 2009). Independently, either 5-HT- or AngII-stimulated cytokine release, including the secretion of IL-6, IL-1β, TNF-α, and TGF-β1 in adult cardiac fibroblasts is sensitive to 5-HT_{2B} receptor blockade. Treatments with epidermal growth factor receptor (EGFR, ErbB1/4)-selective inhibitors or selective inhibitors of MMPs also abolish AngII- and 5-HT-induced cytokine release. Finally, the use of HB-$EGF^{-/-}$ cardiac fibroblasts confirms that EGFR transactivation is absolutely required for AngII- and 5-HT-dependent cytokine release. Collectively, these results reveal that the convergent actions of AngII and 5-HT via interactions between the AT1 and 5-HT_{2B} receptors coexpressed in non-cardiomyocytes are the key limiting events in cardiac fibroblast activation (Jaffre et al., 2009).

Embryonic morphogenesis of cardiac valves and fibrotic events is a critical event linked to endothelial-mesenchymal transformation (EMT). Inducers of EMT during valvulogenesis include VEGF, TGF-β1, and Wnt/β-catenin, all of which are regulated in a spatiotemporal manner. Serotonin can initiate TGF-β signalling, which in turn has been strongly implicated in fibrosis. Recent evidence has suggested that degenerative valvular disease may be mediated by developmental pathways including bone morphogenic protein (BMP), Wnt and Notch signalling, nitric oxide, and angiotensin II (Orton et al., 2012). Wnt2 acts as an angiogenic factor for endothelium in vitro and in vivo, and its target genes undergo complex regulation by the tissue microenvironment (Klein et al., 2009). Gene profiling identified 5-HT_{2B} as a down-regulated target gene of Wnt2 signalling in HUVECs. The existence of valve interstitial cells derived at different times and from different origins (i.e., the embryonic epicardium and endocardial cushions and adult bone marrow) raises the interesting possibility that these populations of fibroblasts are functionally different and thus differ in their susceptibility to and/or participation in fibrotic pathological processes (Visconti et al., 2006).

If correct, deciphering the contribution of 5-HT_2 receptors in these events should advance our understanding of fibrosis and lead to new antifibrotic compounds.

3.2. Central nervous system
(depression, psychosis, addiction, feeding, impulsivity)

3.2.1. Feeding and anorexigens

Maintenance of energy balance requires regulation of the amount and timing of food intake. Eating disorders are an important health problem in developed countries (Leibowitz & Alexander, 1998; Vickers & Dourish, 2004); see (http://www.cdc.gov/obesity/data/facts.html). It has been well established that in the central nervous system, 5-HT is one of the major neurotransmitters that control numerous physiological processes affecting food intake. The most extensive characterization of the serotonergic influences on energy balance pathways relates to the modulation of the arcuate nucleus POMCs and NPY/AgRP neuronal populations. Previous studies have established that released 5-HT (i) hyperpolarizes and inhibits AgRP/NPY neurons and decreases the inhibitory drive onto POMC cells by activation of 5-HT_{1B} receptors and (ii) activates POMC/CART neurons via stimulation of

5-HT_{2C} receptors (Heisler et al., 2006), which leads to reciprocal increases in α-MSH release and decreases in AgRP release at melanocortin 4 receptors in the target regions. Subsequent increases in 5-HT neurotransmission have also been shown to regulate the hypothalamic–pituitary–adrenal (HPA) axis upstream of corticotropin-releasing hormone among others (Heisler et al., 2007). Many studies have suggested that 5-HT_{1B} receptor activation inhibits neurons that promote hunger, while 5-HT_{2C} receptors activate neurons in the hypothalamic nuclei that promote satiety (Heisler et al., 2006; Nonogaki et al., 2007; Lam et al., 2008). However, activation of these receptors is not sufficient to fully explain the modulatory effects of 5-HT in feeding behaviour. Other 5-HT receptors such as 5-HT_4 or 5-HT_6 have also been suggested to participate in the control of energy intake (Vickers & Dourish, 2004; Conductier et al., 2005; Jean et al., 2007).

Dexfenfluramine (*d*-fenfluramine, DF) is an amphetamine congener that has been utilized therapeutically as a highly efficient anorectic molecule for the treatment of obesity (Garfield & Heisler, 2009). Previously, DF was used in the treatment of obesity and had potential for the treatment of bulimia. However, clinical use of DF has been associated with several unacceptable side effects including primary pulmonary hypertension and valvular heart disease (Fitzgerald et al., 2000; Rothman et al., 2000; Launay et al., 2002), and this anorexigen was withdrawn from the market in 1997. In the early 1980s, Mennini and colleagues performed pioneering work initially describing the effect of DF and derivatives on the release of 5-HT into nerve terminals by targeting the serotonin transporter (SERT) (Garattini et al., 1986). Administration of DF suppresses food intake in both animals and humans. Animal studies have reported either a complete or partial blockade of DF-induced hypophagia by the 5-HT_2 antagonist ritanserin (Goodall et al., 1993; Neill & Cooper, 1989), the 5-$HT_{2B/2C}$ antagonist SB-200646 (Bourson et al., 1996) and the 5-HT_{2C} antagonist SB242084 (Clifton et al., 2000). Thus, the anorectic effect of DF has been proposed to be mediated by activation of 5-HT_{2C} receptors (Vickers et al., 1999, 2001), while 5-HT_{2B} receptors have been shown to participate in the DF-induced pulmonary hypertension (Launay et al., 2002) and valvulopathy (Setola et al., 2005). Since the hypophagic effect of DF persisted in 5-HT_{2C} receptor knockout ($Htr_{2C}^{-/-}$) mice (Vickers et al., 1999), other 5-HT receptor subtypes must be involved in DF-induced hypophagia.

The 5-HT_{2B} receptor has also been proposed to play a role in the regulation of food intake (De Vry & Schreiber, 2000). An early study showed an orexigenic effect of the preferential 5-HT_{2B} receptor agonist BW723C86 (Kennett et al., 1997). Furthermore, it has been reported that 5-HT regulates appetite possibly via 5-HT_{2B} receptors expressed on hypothalamic neurons (Yadav et al., 2009). In particular, POMC-specific $Htr_{2B}^{-/-}$ mice show mild hypophagia and a reduction in fat pad mass. Like all 5-HT_2 receptors, the 5-HT_{2B} receptor is Gq-coupled and therefore excitatory, while POMC neurons have a well-established anorexigenic function. The mechanisms through which 5-HT_{2B} receptors on POMC neurons may produce orexigenic effects have not been established. Reports that 5-HT_{2B} receptors on POMC neurons mediate orexigenic effects are especially puzzling since a series of studies recently reported that expression of the 5-HT_{2C} receptor on POMC neurons has a critical anorexigenic function (Xu et al., 2008).

Whether and how multiple types of 5-HT receptors might be functioning with overlapping purposes in the same or different populations of POMC neurons are open questions that require additional investigation.

Interestingly, the hypophagic response to the anorexigen and 5-HT releaser DF as observed in wild-type (WT) mice was eliminated in $Htr_{2B}^{-/-}$ mice and in WT mice treated with the highly selective 5-HT_{2B} receptor antagonist RS127445. Using microdialysis, the DF-induced hypothalamic peak of 5-HT release was found to be strongly reduced in conscious $Htr_{2B}^{-/-}$ mice compared with WT mice. Moreover, the pronounced 5-HT release observed upon DF stimulation of a synaptosomal preparation from WT mice was not observed in the synaptosomes from $Htr_{2B}^{-/-}$ mice (Banas et al., 2011). 5-HT_{2B} receptor-dependent phosphorylation of SERT (Launay et al., 2006) may explain the requirement of 5-HT_{2B} receptors in the release action. These findings strongly support that activation of 5-HT_{2B} receptors is a limiting step in the SERT-dependent release effect of DF, whereas other 5-HT receptors may act downstream with respect to feeding behaviour. The results using the 5-HT_{2C} receptor agonist WAY-161503 (Rosenzweig-Lipson et al., 2006) in $Htr_{2B}^{-/-}$ mice confirm the participation of 5-HT_{2C} receptors in feeding behaviour (Banas et al., 2011) as observed in humans during the recent clinical trial for the 5-HT_{2C} receptor-selective agonist lorcaserin (Smith et al., 2010; Thomsen et al., 2008). Moreover, it has been reported that 5-HT_{2C} receptor-expressing POMC neurons are required to control energy and glucose homeostasis (Berglund et al., 2013). Nevertheless, postsynaptic receptors including the 5-HT_{1B}, 5-HT_{2C}, and possibly 5-HT_{2B} receptors seem to be indirectly activated by DF-induced SERT-dependent and 5-HT_{2B} receptor-dependent 5-HT release. Central 5-HT neurons have recently been reported to play a major role in regulating glucose and lipid homeostasis through the recruitment and metabolic activation of brown and beige adipocytes (McGlashon et al., 2015).

The interplay between the central and peripheral 5-HT regulation of feeding behaviour and energy homeostasis has not yet been elucidated.

3.2.2. Raphe neurons and depression

In the raphe nuclei, neurotransmission by 5-HT is tightly regulated by autoreceptors that fine-tune serotonergic neurotransmission through negative feedback inhibition at either the cell bodies (predominantly 5-HT_{1A}) or the axon terminals (predominantly 5-HT_{1B}); however, different roles for 5-HT_{2B} receptors have also been identified (McDevitt & 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 postsynaptic receptors.

The 5-HT_2 receptor agonist DOI decreases the firing rate of 5-HT neurons in the dorsal raphe (DR) nucleus of anaesthetised WT mice. This inhibitory response persists in $Htr_{2C}^{-/-}$ mice but is completely blunted in $Htr_{2A}^{-/-}$ knockout mice. Moreover, the reduction of DR 5-HT neuronal activity in WT mice by DOI can be attenuated by the loss of norepinephrine (NE) neurons. In WT mice, pharmacological inactivation of 5-HT_{2A} receptors by the selective antagonist MDL100907 reverses the escitalopram-induced decrease in DR 5-HT neuronal activity. In microdialysis experiments, a single injection of escitalopram increases cortical extracellular 5-HT but not NE levels in awake WT mice. Although the addition of MDL100907 does not potentiate 5-HT neurotransmission, it allows escitalopram to increase cortical NE outflow and consequently elicit an increase in swim time in the forced swimming test. Blockade of the 5-HT_{2A} receptor may strengthen the antidepressant-like effect of escitalopram by facilitating the enhancement of NE transmission in the brain (Quesseveur et al., 2013).

These results provide support for the use of atypical antipsychotics (which target 5-HT_2 receptors), with SSRIs as a relevant antidepressant augmentation strategy.

Both short-term and long-term behavioural and neurogenic SSRI effects were abolished after either genetic or pharmacologic inactivation of 5-HT_{2B} receptors (Diaz et al., 2012). Conversely, direct agonist stimulation by the preferential 5-HT_{2B} receptor agonist BW723C86 induced an SSRI-like response in acute behavioural and chronic neurogenic assays. The 5-HT_{2B} receptor is expressed in raphe serotonergic neurons as shown by single cell PCR. The SSRI-induced increase in the hippocampal extracellular 5-HT concentration was strongly reduced in the absence of functional 5-HT_{2B} receptors. These results support the positive regulation of serotonergic neurons by 5-HT_{2B} receptors (Diaz et al., 2012).

Based on this observation, the 5-HT_{2B} receptor appears to positively modulate serotonergic activity and to be required for the therapeutic actions of SSRIs, although direct 5-HT_{2B} agonists cannot be used as

therapeutic option unless a biased agonist without the adverse cardiopulmonary effects could be developed.

Evidence from various sources indicates alterations in the function of the 5-HT_{2C} receptor with regard to anxiety, depression, suicide, and other stress-related disorders upon treatment with antidepressant drugs. Although the notion of 5-HT_{2C} receptor desensitization following antidepressant treatment is well established in the literature, this concept is mainly based on in vitro assays and/or behavioural assays (hypolocomotion, hyperthermia) that have poor relevance to anxiodepressive disorders. Various serotonergic projections to distinct 5-HT_{2C} receptor populations exert complex modulations. Targeting the 5-HT_{2C} receptor in specific brain areas rather than activating or blocking the receptors in the whole brain would be the most rational therapeutic strategy (Martin et al., 2014). Nevertheless, agomelatine, which is a potent melatonin receptor agonist, is also an effective antidepressant and a potent 5-$HT_{2B/2C}$ receptor antagonist (Millan et al., 2003). Administration of melatonin twice daily increases the number of spontaneously active dopamine (DA) neurons but does not affect the firing of NE neurons. Long-term administration of either melatonin or the 5-HT_{2C} receptor antagonist SB242084 alone has no effect on the firing rate and burst parameters of dorsal raphe 5-HT and ventral tegmental area DA neurons. The combination of both drugs, however, only enhances the number of spontaneously active DA neurons while leaving the firing rate of 5-HT neurons unchanged. The addition of the selective 5-HT_{2B} receptor antagonist LY266097, which by itself is devoid of any effect, to this combinatory regimen increases the number of bursts per minute and the percentage of spikes occurring in bursts of DA neurons (Chenu et al., 2014). In conclusion, the combination of melatonin receptor activation and 5-HT_{2C} receptor blockade results in a disinhibition of DA neurons; when 5-HT_{2B} receptors are also blocked, the firing and the bursting activity of DA neurons are both enhanced, thus reproducing the antidepressant effect of agomelatine and supporting the effect of these receptors on DA neurons.

3.2.3. Drugs of abuse

Dopaminergic projections to the striatum inhibit the medium spiny neurons (MSN) in the striatopallidal (indirect) pathway and excite MSNs in the striatonigral (direct) pathway. There are dense 5-HT projections to the striatum from the dorsal raphe nucleus, and it is known that increased 5-HT in the striatum facilitates DA release from terminals. The direct pathway excites various cortical nuclei, and some of these nuclei send inhibitory projections to the DRN (dorsal raphe nuclei). Among drugs of abuse, cocaine blocks all 3 monoamine transporters at similar concentrations, amphetamine and methamphetamine are most potent at the norepinephrine transporter (NET) but 5- to 9-fold less potent at the dopamine transporter (DAT) and 200- to 500-fold less potent at SERT; the compound 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') has a higher affinity for SERT than either DAT or NET (Han & Gu, 2006).

3.2.3.1. MDMA and its metabolite MDA. The amphetamine derivative MDMA is a psychostimulant drug that is widely used recreationally among young people in Europe and North America. MDMA is metabolized into N-demethylated metabolite 3,4-methylenedioxyamphetamine (MDA). The serotonergic system appears crucial for the reinforcing properties of MDMA. MDMA binds preferentially to and reverses the activity of the SERT, which also causes a release of 5-HT 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.

Current evidence indicates that 5-HT_{2A} receptors modulate mesolimbic DA activity and several behavioural responses related to the addictive properties of psychostimulants. A study evaluating the role of 5-HT_{2A} receptors in MDMA-induced reinforcement, hyperlocomotion, and the reinstatement of MDMA-seeking behaviour investigated the basal and MDMA-stimulated extracellular levels of DA in the nucleus accumbens (NAcc) and of 5-HT and NE in the prefrontal cortex. Self-administration of MDMA is blunted in $Htr_{2A}^{-/-}$ mice compared to their WT littermates. Horizontal locomotion is increased by MDMA administration to a higher extent in $Htr_{2A}^{-/-}$ than in WT mice. DA outflow in the NAcc is lower in $Htr_{2A}^{-/-}$ compared to WT mice under basal conditions and after MDMA challenge. In WT mice, priming does not reinstate MDMA-seeking behaviour, while cue-induced reinstatement is prominent. This cue-induced reinstatement is blocked by the administration of the 5-HT_{2A} receptor-selective antagonist SR46349B (eplivanserin). 5-HT_{2A} receptors are crucial for MDMA-induced reinforcement and cue-induced reinstatement of MDMA-seeking behaviour. These effects are probably due to the modulation of mesolimbic dopaminergic activity (Orejarena et al., 2011). An increase in the functionality of cortical 5-HT_{2A} receptors was observed in mice pretreated with MDMA compared with mice pretreated with saline, but this activation was significantly greater in pretreated mice in the locomotor environment. In contrast, the functional activity of striatal D2 receptors was significantly decreased only in mice pretreated with MDMA. These results reveal neuroadaptations in cortical 5-HT_{2A} and striatal D2 receptors after MDMA-induced behavioural sensitization in mice (Varela et al., 2011). Using in vivo microdialysis and locomotor activity monitoring, repeated injections of MDMA induce long-term sensitization of noradrenergic and serotonergic neurons, which correlates with behavioural sensitization. The development of this phenomenon, which lasts for at least 1 month after withdrawal, requires repeated stimulation of the α_{1B}-adrenergic and 5-HT_{2A} receptors. Moreover, behavioural and neuroendocrine assays indicate that hyper-reactivity of noradrenergic and serotonergic networks is associated with persistent desensitization of the somatodendritic α_{2A}-adrenergic, 5-HT_{2A}, and 5-HT_{1A} receptors. Repeated MDMA exposure causes strong neural and behavioural adaptations, and inhibitory feedback mediated by α_{2A}-adrenergic and 5-HT_{1A} autoreceptors plays an important role in the physiopathology of this addictive behaviour (Lanteri et al., 2014).

The central 5-HT_{2B} receptor has been only recently considered as an interesting pharmacological target to treat drug addiction. Acute pharmacological inhibition or genetic ablation of the 5-HT_{2B} receptor in mice completely abolishes MDMA-induced hyperlocomotion and 5-HT release in the NAcc and ventral tegmental area. Furthermore, the 5-HT_{2B} receptor dependence of MDMA-stimulated release of endogenous 5-HT from superfused midbrain synaptosomes suggests that 5-HT_{2B} receptors act to favour MDMA-stimulated 5-HT release. Thus, the 5-HT_{2B} receptor is a regulatory component in the actions of MDMA (Doly et al., 2008). $Htr_{2B}^{-/-}$ mice did not exhibit behavioural sensitization or conditioned place preference following MDMA injections. In addition, both MDMA-induced reinstatement of conditioned place preference after extinction and locomotor sensitization development are abolished by treatment with RS127445 in WT mice. Accordingly, MDMA-induced dopamine D1 receptor-dependent phosphorylation of extracellular regulated kinase in the NAcc is abolished in mice lacking functional 5-HT_{2B} receptors. These results underpin the importance of 5-HT_{2B} receptors in the reinforcing properties of MDMA (Doly et al., 2009).

The selective 5-HT_{2C} receptor antagonist RS102221 can suppress MDMA-induced hyperlocomotion. These findings provide evidence that inactivation of 5-HT_{2C} receptors may reduce the motor response to MDMA (Conductier et al., 2005). A role has been ascribed to the inhibitory effects of 5-HT_{2C} receptor activation on the physiology and behaviour mediated by the mesolimbic dopaminergic pathway, particularly in the terminal region of the NAcc. The influence of this receptor subtype on functions mediated by the nigrostriatal dopaminergic pathway is less clear.

Therefore, the contribution of 5-HT_2 receptors to the psychostimulant effects of MDMA is complex, with the 5-HT_{2A} and 5-HT_{2B} receptors responsible for enhanced effects but the 5-HT_{2C} receptor lowering the effects of MDMA.

3.2.3.2. Amphetamine. Although the locomotor response to d-amphetamine is mediated by an increased release of DA and NE, blockade of either the α_{1B}-adrenergic or 5-HT_{2A} receptors almost completely inhibits the d-amphetamine-induced locomotor response in mice. In agreement with these results, mice lacking α_{1B}-adrenergic receptors hardly respond to d-amphetamine. Paradoxically, mice lacking 5-HT_{2A} receptors ($Htr_{2A}^{-/-}$) exhibit a twofold higher locomotor response to d-amphetamine than their WT littermates. Repeated amphetamine injections still increase the locomotor response of $Htr_{2A}^{-/-}$ mice to d-amphetamine at a level similar to that of sensitized WT mice. A 1 mg/kg dose of prazosin, an α_{1B}-adrenergic antagonist, completely blocks d-amphetamine-induced locomotor response in $Htr_{2A}^{-/-}$ naïve animals, but a dose of 3 mg/kg is necessary in sensitized $Htr_{2A}^{-/-}$ mice. Because naïve $Htr_{2A}^{-/-}$ mice exhibit an increased cortical noradrenergic response to d-amphetamine, these data confirmed that repeated d-amphetamine modifies noradrenergic transmission in $Htr_{2A}^{-/-}$ mice. Stimulation of 5-HT_{2A} receptors would inhibit noradrenergic neurons. The dramatic decrease in 5-HT_{2A} receptor antagonist efficiency in sensitized WT mice indicates that disruption of the regulatory role of 5-HT_{2A} receptors on noradrenergic transmission occurs during sensitization and thus represents a putative physiological basis of the behavioural sensitization to d-amphetamine (Salomon et al., 2007).

Acute exposure to the selective 5-HT_{2B} receptor antagonist LY266097 significantly diminished the increase in DA outflow induced by d-amphetamine in the ventral striatum/NAcc but not in the dorsal striatum (DSt) (Auclair et al., 2010). The locomotor response of $Htr_{2B}^{-/-}$ mice to d-amphetamine was found to be significantly enhanced compared with $Htr_{2B}^{+/+}$ mice (Pitychoutis et al., 2015), supporting the notion of this receptor participating in the control of DA/NE pathways. $Htr_{2C}^{-/-}$ mice showed marked alterations in the activity and functional output of the DA pathway. $Htr_{2C}^{-/-}$ mice also displayed increased activity of substantia nigra pars compacta (SNc) DA neurons, elevated baseline extracellular DA concentrations in the DSt, alterations in grooming behaviour, and enhanced sensitivity to the stereotypic behavioural effects of d-amphetamine and the DAT blocker GBR 12909. These psychostimulant responses occurred in the absence of phenotypic differences in the drug-induced extracellular DA concentration, suggesting a phenotypic alteration in behavioural responses to released DA. This possibility was further suggested by enhanced behavioural responses of $Htr_{2C}^{-/-}$ mice to the D1 receptor agonist SKF 81297; similar responses were also observed in $Htr_{2B}^{-/-}$ mice (Bevilacqua et al., 2010). Differences in DSt D1 or D2 receptor expression were not found, nor were there any differences in the medium spiny neuron firing patterns or intrinsic membrane properties following DA stimulation (Abdallah et al., 2009).

Therefore, 5-$HT_{2B/2C}$ receptors regulate nigrostriatal dopaminergic activity and function both on SNc dopaminergic neurons and on a locus downstream of the DSt, whereas 5-HT_{2A} receptors act on NE neurons.

3.2.3.3. Cocaine. Several studies indicate that acute treatment with 5-HT_{2A} receptor antagonists attenuates the reinstatement of cocaine-maintained behaviour but not cocaine self-administration in rodents. Investigation of the effects of the selective 5-HT_{2A} receptor antagonist MDL100907 on intravenous cocaine self-administration as well as drug and cue-primed reinstatement was performed in rhesus macaques. The role of 5-HT_{2A} receptors in cocaine-induced DA overflow in the NAcc and the caudate nucleus/DSt was evaluated using in vivo microdialysis. MDL100907 significantly attenuates drug- and cue-induced reinstatement but had no significant effects on cocaine self-administration across a range of maintenance doses. Importantly, MDL100907 attenuates cocaine-induced DA overflow in the DSt but not in the NAcc (Murnane et al., 2013).

This work revealed that 5-HT_{2A} receptors differentially contribute to the abuse-related effects of cocaine and cocaine-induced nigrostriatal and mesolimbic DA overflow.

The peripheral administration of the selective 5-HT_{2B} receptor antagonists RS127445 or LY266097 significantly reduced basal DA outflow in the NAcc shell but had no effect on cocaine-induced DA outflow in this brain region. Additionally, RS127445 failed to modify both basal and cocaine-induced DA outflow in the NAcc core and the DSt. This interaction may occur downstream of DA neurons and could involve activity at the level of DSt and/or NAcc DA transmission in keeping with the importance of these brain regions in the behavioural responses to cocaine. Regulatory control may therefore be exerted by the 5-HT_{2B} receptor on ascending DA pathways (Devroye et al., 2015).

Thus, 5-HT_{2B} receptors may also exert facilitatory control on mesoaccumbens DA pathway activity in addition to serotonergic neurons.

$Htr_{2C}^{-/-}$ mice completely devoid of 5-HT_{2C} receptors display enhanced exploration of a novel environment and increased sensitivity to the stimulatory locomotor effects of cocaine. In an operant intravenous self-administration model under a progressive ratio schedule of reinforcement, $Htr_{2C}^{-/-}$ mice display elevated numbers of lever pressing for cocaine injections, indicating that the drug is more reinforcing in these mice. Moreover, $Htr_{2C}^{-/-}$ mice exhibit enhanced cocaine-induced elevations of DA levels in the NAcc, a brain region implicated in the stimulant and rewarding properties of cocaine. In contrast, phenotypic differences in DSt DA levels were not observed after cocaine treatment. These findings strongly implicate 5-HT_{2C} receptors in the serotonergic suppression of DA-mediated behavioural responses to cocaine (Rocha et al., 2002). Depleting forebrain 5-HT levels induces compulsive cocaine-seeking behaviour in rats with a limited history of cocaine use; this can be reversed by systemic treatment with a 5-HT_{2C} receptor agonist and mimicked by systemic treatment with a 5-HT_{2C} receptor antagonist in intact animals (Pelloux et al., 2012).

These results indicate the causal involvement of reduced serotoninergic transmission in the emergence of compulsive drug seeking after a long history of cocaine use that is dependent on 5-HT_{2C} receptors.

3.2.4. Impulsivity

A feature of multiple neuropsychiatric disorders is impulsivity. Recent studies have implicated 5-HT systems (notably the 5-HT_2 receptor) in the medial prefrontal cortex (mPFC) in mediating individual differences in motor impulsivity. High and low impulsive rats were identified in a 1-choice serial reaction time task (1-CSRTT). In western blots, protein levels of the 5-HT_{2A} and 5-HT_{2C} receptors predicted the intensity of motor impulsivity, and their ratio in the mPFC was positively correlated with levels of premature responses in individual outbred rats. High phenotypic motor impulsivity is associated with diminished co-immunoprecipitation of mPFC synaptosomal 5-HT_{2A} and 5-HT_{2C} receptors. The shRNA knockdown of the 5-HT_{2C} receptor in the mPFC results in increased motor impulsivity and triggers a functional disruption of the local 5-HT_{2A}:5-HT_{2C} receptor ratio as evidenced by a compensatory upregulation of 5-HT_{2A} receptor protein expression and a leftward shift in the potency of M100907 to suppress impulsive behaviour. There seems to be a direct relationship between the 5-HT_{2A} and 5-HT_{2C} receptors in the mPFC, and their imbalance may be a functionally relevant mechanism in motor impulsivity (Anastasio et al., 2015).

A functional polymorphism in the human *HTR_{2B}* gene that introduces a stop codon after 20 amino acids (Q20*) was found to enhance impulsive behaviour (Bevilacqua et al., 2010). Especially under conditions where control was impaired, the carriers of this stop codon were more vulnerable to alcohol and were more impulsive if they drank. Similarly, $Htr_{2B}^{-/-}$ mice displayed more impulsive choices in delayed discounting tasks, sought novelty and were more active after receiving a D1 dopamine receptor agonist (Bevilacqua et al., 2010). The phenotype of $Htr_{2B}^{-/-}$ mice results from a combination of both the direct absence of 5-HT_{2B} receptor signalling and the neural adaptations triggered by the permanent lack of signalling through this receptor during development.

In operant-based behavioural paradigms, $Htr_{2C}^{-/-}$ mice display deficits in executive functions. $Htr_{2C}^{-/-}$ mice are impaired in the acquisition of a visuospatial attention task as assessed in the 5-CSRTT. In this task, $Htr_{2C}^{-/-}$ mice exhibit marked impairment of attentional processes with normal response inhibition. Based on the results from in vivo microdialysis, the elevated extracellular DA concentrations in the NAcc of $Htr_{2C}^{-/-}$ mice during task performance indicate that 5-HT_{2C} receptors impact DA homeostasis during a visuospatial attention task. The disinhibition of mesolimbic DA pathways may contribute to the impaired attention and perturbed task performance in $Htr_{2C}^{-/-}$ mice. Additionally, in a spatial reversal-learning task, $Htr_{2C}^{-/-}$ mice failed to improve their performance over a series of reversals, indicating that intact 5-HT_{2C} receptor signalling is required to accurately respond to repeated changes in reward contingencies. In contrast to the $Htr_{2C}^{-/-}$ mice phenotype in the 5-CSRTT, WT mice treated with the 5-HT_{2C} receptor antagonist SB242084 exhibited a diminished response inhibition, suggesting different effects between acute pharmacological blockade and constitutive loss of 5-HT_{2C} receptor activity (Pennanen et al., 2013). These findings suggest that impaired 5-HT_{2C} receptor signalling during development may predispose the organism to executive function disorders.

3.2.5. Psychosis

The head-twitch behavioural response (HTR-rapid lateral movements of the head) in rodents is reliably elicited by a variety of psychedelics (e.g., DOI, DOM, DOB, mescaline, LSD, and psilocin). In 1982, it was proposed that the mescaline-induced HTR is mediated by the 5-HT_{2A} receptor based on the fact that the relative potency of 5-HT antagonists to block this behaviour is correlated with their affinity to 5-HT_{2A}. Furthermore, non-hallucinogenic 5-HT_2 receptor agonists such as lisuride and ergotamine do not induce this behavioural response. Thus, HTR seems to serve as a mouse bioassay that can predict the hallucinogenic-specific signalling and effects of 5-HT_{2A} receptor agonists in humans. Numerous studies have shown that MDL100907 blocks the HTR induced by hallucinogens. Mice lacking the 5-HT_{2A} receptor gene do not produce HTRs in response to mescaline, DOI, DOM, LSD, DMT, 5-MeO-DMT, psilocin, or 1-methylpsilocin (Halberstadt, 2015), although the response can be rescued by selectively restoring the 5-HT_{2A} receptor gene to cortical regions.

Interestingly, NMDA receptor antagonists including phencyclidine (PCP)-like drugs also induce an HTR in rodents and enhance the 5-HT_{2A}-receptor-mediated HTR in mice (Nabeshima et al., 1987). In animals, clozapine, ritanserin, amesergide, and ketanserin block PCP-dependent locomotion and HTRs, which indicates a potential role of circuits functioning through the 5-HT_2 receptors to mediate the behavioural responses induced by NMDA antagonism. Similar results were reported with the selective 5-HT_{2A} receptor antagonist MDL100907. Atypical antipsychotics reverse the cellular responses produced by PCP-like drugs in cortical pyramidal neurons and block the release of 5-HT and glutamate in the prefrontal cortex. mGlu2 receptor activation abolishes the hallucinogen-specific signalling activated by LSD binding to the 5-HT_{2A}/mGlu2 receptor complex. Microdialysis experiments in the cortex have demonstrated that extracellular glutamate levels increase more slowly than extracellular 5-HT levels after intraperitoneal administration of PCP-like drugs. These observations support that 5-HT and glutamate signalling through the 5-HT_{2A}/mGlu2 receptor complex might each be responsible for different LSD-like and PCP-like symptoms of schizophrenia (Gonzalez-Maeso et al., 2008). Serotonergic and glutamatergic drugs bind to the 5-HT_{2A}/mGlu2 receptor heterocomplex, which then balances Gi- and Gq-dependent signalling. The 5-HT_{2A}/mGlu2 receptor-mediated changes in Gi and Gq activity predict the psychoactive behavioural effects of a variety of pharmacological compounds (Fribourg et al., 2011). Prefrontal 5-HT_{2A} receptor activation by serotonin also enhances NMDA transmission and gates the induction of temporal-dependent plasticity mediated by NMDA receptors at thalamocortical synapses as observed in acute slices. Using a viral gene-delivery approach, expressing 5-HT_{2A} receptors in the mediodorsal thalamus of 5-HT_{2A} receptor-deficient mice rescued the otherwise absent potentiation of NMDA transmission, induction of temporal plasticity, and deficit in associative memory (Barre et al., 2016).

In a Finnish cohort of impulsive patients (Bevilacqua et al., 2010), early onset schizophrenia was more prevalent in HTR_{2B} Q*20 carriers. Recently, it was shown that domains related to the positive, negative, and cognitive symptom clusters of schizophrenia are affected in $Htr_{2B}^{-/-}$ mice as shown by deficits in sensorimotor gating, selective attention, social interactions, and processes involving learning and memory. In addition, $Htr_{2B}^{-/-}$ mice present with an enhanced locomotor response to the NMDA receptor antagonist dizocilpine and the psychostimulant amphetamine, with robust alterations in sleep architecture. Importantly, selected schizophrenic-like phenotypes and endophenotypes are rescued by chronic haloperidol treatment (Pitychoutis et al., 2015). Owing to its relatively low expression level in the brain, the 5-HT_{2B} receptor has not received much of the limelight as a possible regulator of the DOI-induced HTR. The effects of DOI on HTR induction are increased in 5-HT_{2B} receptor knockout mice (Luc Maroteaux, unpublished). Interestingly, although the ergoline analogue lisuride has 5-HT_{2A} receptor agonist activity, it does not produce the HTR and is a potent antagonist of 5-HT_{2B} receptors. It is worth considering the 5-HT_{2B} receptor as a potential modulator of the behavioural effects of DOI. For example, 5-HT_{2B} receptor affinity of hallucinogenic phenylisopropalamines is significantly correlated with their human hallucinogenic potency (Nelson et al., 1999). Studies have shown that the 5-HT_{2B} receptor is required for the releasing effects of the 5-HT releasers DF and MDMA, and other findings argue that brain 5-HT_{2B} receptors may be involved in the cognitive and/or behavioural effects of psychoactive drugs (Canal & Morgan, 2012).

There is a consensus in the literature that the ability of DOI to induce the HTR is not blocked by selective 5-HT_{2C} receptor antagonists. There is some evidence that 5-$HT_{2B/2C}$ sites may play a modulatory role in this behaviour. The non-selective 5-$HT_{2B/2C}$ receptor agonists Ro 60-0175, MK-212, and mCPP do not induce the HTR in rats unless administered in combination with the 5-HT_{2C} antagonist SB242084. There is also evidence that the ability of DOI to induce the HTR is significantly attenuated by pretreatment with 5-$HT_{2B/2C}$ agonists such as Ro 60-0175 and mCPP. These findings indicate that 5-HT_{2C} receptor activation reduces the manifestation of the HTR (Halberstadt, 2015). Likewise, DOI produces a biphasic dose–response curve, and SB242084 reportedly shifts the descending arm of the DOI response to the right (Fantegrossi et al., 2010). Both the 5-$HT_{2B/2C}$ receptor selective antagonist/inverse agonist SB206553 and the 5-HT_{2C} receptor antagonist SB242084 attenuate the HTR elicited by DOI in mice by 50%, and the DOI-induced HTR was attenuated by ~50% in $Htr_{2C}^{-/-}$ mice relative to their WT littermates (Canal & Morgan, 2012; Canal et al., 2010).

In conclusion, both the 5-HT_{2B} and 5-HT_{2C} receptors modulate antipsychotic responses. Atypical antipsychotics behaving as 5-HT_{2A} receptor inverse agonists might have efficacy against negative symptoms without evoking motor perturbation (Meltzer, 2013). Finally, with the goal of exploiting ligand-directed signalling, 5-HT_{2A}-receptor agonists favouring Gq versus Gi activation and devoid of hallucinogenic properties are attractive possibilities for future drug discovery.

3.2.6. 3.2.5. Prader–Willi syndrome

Prader–Willi syndrome (PWS) is caused by the loss of function of paternally expressed genes in the 15q11-q13 region. This region contains the small nucleolar RNA (snoRNA) HBII-52, which exhibits sequence complementarity to the alternatively spliced exon Vb of the 5-HT_{2C} receptor. HBII-52 regulates the alternative splicing of the 5-HT_{2C} receptor by binding to a silencing element in exon Vb. Prader–Willi syndrome patients do not express HBII-52 and have different 5-HT_{2C} receptor mRNA isoforms compared to healthy individuals (Kishore & Stamm, 2006). Using whole genome microarrays to analyse gene expression and quantitative RT-PCR analysis, alterations in the expression of serotonin receptor genes (e.g., *HTR2B*) and genes involved

in eating behaviour and obesity (e.g., *ADIPOR2, MC2R, HCRT, OXTR*) were noted. Other genes of interest with reduced expression in PWS subjects included STAR (a key regulator of steroid synthesis) and SAG (an arrestin family member that desensitizes G protein-coupled receptors). Quantitative RT-PCR data of *SAG, OXTR, STAR, HCRT*, and *HTR2B* levels using RNA isolated from the lymphoblastoid cells and available brain tissue (frontal cortex) from either individuals with PWS or control subjects were normalized to *GAPDH* gene expression levels and validated the microarray gene expression data (Bittel et al., 2007).

3.3. Cardiovascular system

Expression of the 5-HT_{2C} receptors in the cardiovascular system is undetectable.

3.3.1. Heart failure and cardiac hypertrophy

Both the 5-HT_{2A} and 5-HT_{2B} receptors have been implicated in cardiac hypertrophy and failure and are expressed on the cardiomyocyte cell surface. 5-HT_{2A} receptor activation triggers positive ionotropic responses (Brattelid et al., 2007). Noteworthy, despite a similar coupling to the 5-HT_{2A} receptor subtype, 5-HT_{2B} receptor stimulation does not elicit any contractile response, although the response to the β-adrenergic receptor agonist dobutamine in $Htr_{2B}^{-/-}$ cardiomyocytes was impaired (Nebigil et al., 2001). Serotonin binding to the Gq-coupled 5-HT_{2B} receptor protects cardiomyocytes against serum deprivation-induced apoptosis, which is detected by DNA fragmentation, nuclear chromatin condensation, and TUNEL labelling. Serotonin prevents cytochrome c release and activation of caspase-9 and caspase-3 after serum deprivation via cross-talk between the phosphatidylinositol-3 kinase/Akt and extracellular signal-regulated kinase ERK1/2 signalling pathways. Serotonin binding to the 5-HT_{2B}-receptor activates ERK kinases to inhibit the expression of Bax induced by serum deprivation. Serotonin signalling via the phosphatidylinositol-3 kinase/Akt pathway can activate NF-kB, which is required for the regulation of the mitochondrial adenine nucleotide translocator (ANT-1). Parallel to these observations, ultrastructural analysis of $Htr_{2B}^{-/-}$ mouse hearts revealed pronounced mitochondrial defects in addition to altered mitochondrial enzyme activities (i.e., cytochrome oxidase and succinate dehydrogenase) and ANT-1 and Bax expression levels. These findings identify 5-HT as a novel survival factor targeting the mitochondria in cardiomyocytes via 5-HT_{2B} receptors (Nebigil et al., 2003).

A cardioprotective role of the 5-HT_{2A} receptor blockade was also recently suggested by Blasco-Fontecilla et al. (2010). These authors emphasized that atypical antipsychotic drugs could reduce the risk of cardiovascular events in patients with schizophrenia. In fact, the cardiovascular risk is increased in these patients due to cigarette smoking, metabolic disorders and cardiac arrhythmias caused by QT prolongation. Independent of lifestyle, some of these issues can be favoured by antipsychotics themselves but interestingly, Tiihonen et al. (2009) suggested that long-term exposure to antipsychotics could reduce overall cardiovascular mortality, with the best profile obtained for antipsychotic drugs having higher 5-HT_2 receptor affinity, i.e., clozapine, quetiapine, olanzapine, and thioridazine. It is quite difficult to identify a mechanism in such epidemiological studies, but a reduction of platelet aggregation and thrombus formation combined with a limitation of coronary spasms, both of which are linked with 5-HT-mediated 5-HT_{2A} receptor activation, could contribute to cardiovascular protection in these patients.

Another role of cardiac 5-HT_2 receptors is myocardial hypertrophy. Genetic studies failed to show any genetic polymorphisms in the 5-HT_{2A} receptor gene in patients with hypertrophic cardiomyopathy either of genetic origin or due to hypertension. Conversely, the expression of this receptor is increased in patients with cardiac hypertrophy, and pharmacological inhibition can prevent the development of cardiac hypertrophy induced by transverse aortic constriction in mice (Lairez et al., 2013). Caveolin-3 (Mialet-Perez et al., 2012) and the calcineurin/NFAT (Vindis et al., 2010) pathway could be involved in these regulatory actions by providing a pathophysiological role of 5-HT in cardiac hypertrophy. In adult humans, 5-HT_{2B} receptors were also found to be overexpressed in the hearts of patients with congestive heart failure, and this overexpression was positively correlated with cytokine and norepinephrine plasma levels (Jaffre et al., 2009). Serotonin plasma levels are also increased in patients with heart failure and in animal models of cardiac hypertrophy induced by aortic constriction. These findings may indicate that 5-HT induces cardiac hypertrophy or heart failure through the 5-HT_{2B} receptor. The 5-HT_{2B} receptor has 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_{2B} receptors significantly increased. 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 upregulation of 5-HT_{2B} receptor protein expression in rats after aortic banding. Following in vitro mechanical stretching of cardiomyocytes and incubation with 5-HT, the level of 5-HT_{2B} receptors and BNP protein increased in a temporal-dependent manner. When transfected with specific siRNA against 5-HT_{2B} receptors, cardiomyocytes showed a reversal of the 5-HT-induced increase in NF-κB translocation and BNP protein as well as in the mechanical stretching (Liang et al., 2006).

By mimicking sympathetic stimulation in vivo, mice globally lacking serotonin 5-HT_{2B} receptors did not develop isoproterenol-induced left ventricular hypertrophy (Jaffré et al., 2004). The exact cardiac cell type(s) expressing 5-HT_{2B} receptors (cardiomyocytes versus non-cardiomyocytes) involved in this pathological cardiac hypertrophy was addressed in vivo: mice with conditional expression of 5-HT_{2B} receptor in cardiomyocytes, such as global 5-HT_{2B} receptor-null mice, are resistant to isoproterenol-induced cardiac hypertrophy and dysfunction as well as to isoproterenol-induced increases in plasma cytokine levels (Jaffre et al., 2009), which points to a fibroblast effect. In primary cultures of cardiac fibroblasts, angiotensin II and isoproterenol stimulated NOX activity that was prevented by a selective antagonist (SB215505). 5-HT_{2B} receptor blockade by SB215505 prevented the increase in cardiac superoxide generation and hypertrophy in two mouse models of cardiac hypertrophy, i.e., angiotensin II and isoproterenol infusions (Monassier et al., 2008). A functional interaction between the AT1 and 5-HT_{2B} receptors via a transinhibitory mechanism that may involve heterodimeric receptor complexes was shown to trigger cytokine release in cardiac fibroblasts (Jaffre et al., 2009) and could be a new therapeutic target.

The involvement of 5-HT_{2B} receptors was reported in the generation of apoptotic events associated with cardiac remodelling during increased adrenergic stimulation (Bai et al., 2010). Based on these data, the effect of a chronic 5-HT_{2B} receptor blockade by the selective antagonist RS127445 was investigated in spontaneously hypertensive rats showing left ventricular hypertrophy in conjunction with diastolic dysfunction and an apparently normal ejection fraction (Ayme-Dietrich et al., 2015; Marzak et al., 2014). In this model, the 5-HT_{2B} receptor is overexpressed in the left ventricle, but the antagonist did not improve either cardiac function or the hypertrophy. An increase in subendocardial ventricular fibrosis was observed and was reproduced by 5-HT injections in WT mice but amplified in $Htr_{2B}^{-/-}$ animals. Therefore, in hypertensive cardiomyopathy, 5-HT_{2B} receptors could also be associated with cardioprotection through endothelial-mediated coronary vasodilatation (Ayme-Dietrich et al., 2015).

These data revealed a dual role of 5-$HT_{2A/2B}$ receptors on both cardiomyocytes and cardiac fibroblasts in regulating cardiac hypertrophy in vivo.

3.3.2. Pulmonary arterial hypertension

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 has been associated with the pathogenesis of PAH (Chan & Loscalzo, 2008). Therapeutic drugs that exert PAH as a side effect, such as the amphetamine derivative and anorexigen dexfenfluramine, can act as potent 5-HT releasers acting at SERT and/or agonists at 5-HT_{2B} receptors (Weir et al., 2008). Blockade of 5-HT_{2B} receptors using independent approaches (i.e., genetic ablation ($Htr_{2B}^{-/-}$) or pharmacologic inactivation (5-HT_{2B} receptor antagonist RS127445)) completely prevented the development of hypoxia-induced pulmonary hypertension in mice, including the increase in pulmonary blood pressure and lung remodelling, the increase in vascular proliferation, increased elastase activity and elevated transforming growth factor-β-1 (TGFβ1) levels (Launay et al., 2002). Using the monocrotaline-induced pulmonary hypertension model, recent studies confirmed that other 5-HT_{2B} receptor antagonists (e.g., terguride, PRX-08066, and 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_{2B} receptor expression in the pulmonary arteries of rodents and humans (Launay et al., 2002; Dumitrascu et al., 2011). Therefore, activation of 5-HT_{2B} receptors appears to be a limiting step in the development of pulmonary hypertension. Recently, the restricted expression of 5-HT_{2B} receptors to bone marrow cells was shown to be necessary and sufficient for the development of pulmonary hypertension in response to either hypoxia or monocrotaline (Launay et al., 2012).

These findings reveal a limiting role of 5-HT_{2B} receptors in PAH development and shift the contribution of 5-HT to PAH to an extrapulmonary, haematopoietic event and open new possibility for therapeutic interventions.

3.3.3. Vascular tone of systemic and coronary arteries

The pharmacology of 5-HT in systemic arteries is complex. 5-HT can elicit vasoconstriction through its interaction with 5-HT_{2A} receptors on vascular smooth muscle cells but can also induce vasodilatation via a nitric oxide-dependent mechanism involving 5-$HT_{1B/2B}$ receptors. In the coronary circulation, the effects of the 5-HT_{2A} receptor-selective antagonist sarpogrelate were investigated in dogs subjected to an acute reduction of the coronary blood flow (30% of the baseline flow) in the anterior wall (Fujita et al., 2004). Sarpogrelate amplified 5-HT release in the myocardium and 5-HT_{1B} receptor-mediated dilatation via NO synthase. Such a mechanism was also suggested in an ischaemic hind limb model in diabetic mice in which sarpogrelate restored perfusion through 5-HT_{1B} receptor-mediated stimulation of the eNOS/Akt pathway (Iwabayashi et al., 2012). Nevertheless, an effect in the absence of 5-HT could also occur due to the inverse agonist action of this drug (Muntasir et al., 2006; Hossain et al., 2012). In humans, a single 200 mg oral dose of sarpogrelate also increased the basal and maximal (in response to adenosine triphosphate) average peak velocity of coronary blood flow in patients with coronary artery disease. These changes in vasodilatation were also observed in rabbit cerebral arteries (Kawamura et al., 2013) and in experimental vein grafts (Kodama et al., 2009). The mechanisms of these sarpogrelate effects are not fully understood. A novel vasodilation response linked to 5-HT-induced 5-HT_{1B} receptor stimulation is one possible explanation, but other mechanisms have been proposed. In rats chronically treated with sarpogrelate, serotonergic stimulation of the 5-HT_7 and 5-HT_{1D} receptors counteracts the pressure response elicited by sympathetic nervous system stimulation (Garcia-Pedraza et al., 2014). This phenomenon involves smooth muscle cell hyperpolarization and the COX2 pathway. Interestingly, 5-HT can induce 5-HT_{2A} receptor-mediated COX2-dependent prostacyclin synthesis in smooth muscle cells via the PKC/Src/MAPK pathway. This biological effect could be involved in vasodilatation and implicates the 5-HT_{2A} receptor because this effect is antagonized by sarpogrelate (Machida et al., 2011).

Therefore, in some conditions, a 5-HT_{2A} receptor stimulation could sensitize smooth muscle cells towards vasodilation triggered by other serotonergic receptors.

Aside from its contribution to vasomotion, 5-HT_{2A} receptors also appear to be key contributors to atherosclerosis and arterial wall remodelling. In rabbits fed a high cholesterol diet, sarpogrelate reduced the extent of atherosclerotic deposits in the aorta in parallel to induced endothelial nitric oxide synthase expression (Hayashi et al., 2003). These chronic effects combined with a reduction of 5-HT- and angiotensin II-induced vascular smooth muscle cell proliferation (Sharma et al., 2001; Watanabe et al., 2001) could limit vascular remodelling. This effect is now clear in peripheral arterial disease, as sarpogrelate improves the outcome of symptomatic patients (Ren et al., 2013), but this effect remains to be demonstrated after endovascular therapy. Retrospective studies showed a reduction of major clinical endpoints such as amputation or death from any cause after endovascular therapy for patients with critical hind limb ischaemia (Takahara et al., 2014), and the first preliminary results of a recent prospective study suggest that sarpogrelate coadministered with aspirin is as efficacious as sarpogrelate coadministered with clopidogrel for the prevention of restenosis following femoropopliteal arterial stenting (Chen et al., 2015).

The reduction of neointimal hyperplasia in combination with the improvement of endothelial function after sarpogrelate treatment (Miyazaki et al., 2007) highly encourages its use in patients with peripheral arterial disease and emphasizes the contribution of the 5-HT_{2A} receptor in chronic artery disease.

Diabetes mellitus is a pathological situation that puts patients at high risk for cardiovascular events. In an experimental model of type 2 diabetes, 5-HT increases plasma epinephrine and glucose levels, making rats insulin resistant. This resistance involves 5-HT_{2A} receptors because these increases are blocked by sarpogrelate pretreatment (Takishita et al., 2004). The insulin-sensitizing effect of sarpogrelate has been confirmed in diabetic patients (Kokubu et al., 2006) and in mice when combined with the PPAR-γ agonist pioglitazone (Iizuka et al., 2009). High glucose levels promote endothelial dysfunction, and 5-HT has been suggested as a key pathophysiological player in the vascular complications of diabetes in the context of metabolic syndrome. When rat aortic rings are incubated in a solution with a high glucose concentration, the endothelium-dependent vasodilatation triggered by acetylcholine is reduced, whereas the endothelium-independent dilatation induced by an NO donor is unaffected. Interestingly, sarpogrelate can restore endothelial NO production and, as a consequence, NO-mediated dilatation (Sun et al., 2011). Nevertheless, these effects were obtained at high sarpogrelate concentrations (10 μM) in a culture medium in which the concentration of 5-HT was not measured. This work emphasizes that blocking 5-HT_{2A} receptors could protect endothelial function in diabetic patients. In a rat model of type 1 diabetes, sarpogrelate reduced blood glucose levels and endothelial PECAM-1 overexpression, thereby limiting 5-HT-induced thrombosis (Yamada et al., 2012). Interestingly, this receptor also affects neointimal proliferation and restenosis following arterial stenting, although the 5-HT_{2A} receptor is not expressed on endothelial cells. Either a paracrine mechanism involving cytokines released by smooth muscle cells or the regulation of the homing of circulating cells could explain some of the vascular beneficial effects following 5-HT_{2A} receptor blockade.

Taken together, these data all argue in favour of a major contribution of the 5-HT_{2A} receptor in the regulation of vasomotor tone and in the interaction between endothelial and smooth muscle cells trough a subtle equilibrium involving other receptors.

5-HT_{2B} receptors are also involved in vascular responsiveness during hypertension. Mesenteric arteries from deoxycorticosterone (DOCA)-salt hypertensive rats predominantly contract via 5-HT_{2B} receptors. PCR analyses indicate an increase in 5-HT_{2B} receptor mRNA levels in the mesenteric arteries of DOCA-salt hypertensive arteries, suggesting an increase in receptor number (Watts et al., 1996). The endothelium-denuded isolated superior mesenteric arteries of DOCA-salt rats display

a marked increase in maximum contraction to the 5-HT_{2B} receptor agonist BW723C86 compared with the response of arteries from sham rats, confirming that the 5-HT_{2B} receptor plays a greater role in 5-HT-induced contraction in the arteries from DOCA-salt rats. In chronically instrumented rats, the 5-HT_{2B} receptor antagonist LY272015 significantly reduced the mean blood pressure (Watts & Fink, 1999). LY272015 produced a 4-fold rightward shift to 5-HT in the aortas from hypertensive rats exposed to the nitric-oxide synthase inhibitor *N*(omega)-nitro-L-arginine indicating that 5-HT_{2B} receptors are overexpressed and contribute to the high blood pressure in these animals (Russell et al., 2002).

These findings reveal that the 5-HT_{2B} receptor plays an important role in 5-HT-induced contraction in arteries of hypertensive individuals who could be targeted for therapeutic purposes.

3.3.4. Valvular heart disease

Carcinoid heart disease occurs in over 65% of patients with carcinoid syndrome and is characterized by fibrous thickening of cardiac valves, leading to heart failure (Roth, 2007). A correlation of high plasma 5-HT levels with valvular abnormalities detected by cardiac catheterization and echocardiography has been reported. Thus, 5-HT overproduction has been proposed to be responsible for cardiac valvular disease in patients with carcinoid tumours (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 5-HT 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). Dexfenfluramine was approved in the United States for long-term use as an appetite suppressant until it was reported to be associated with valvular heart disease. The valvular changes (e.g., myofibroblast proliferation) are histopathologically indistinguishable from those observed either in patients with carcinoid disease or after long-term exposure to 5-hydroxytryptamine 5-HT_2-preferring ergot drugs (e.g., ergotamine, methysergide). The amphetamine derivative MDMA and its metabolite MDA each preferentially bind to and activate human recombinant 5-HT_{2B} receptors and, similar to fenfluramine and its *N*-demethylated metabolite norfenfluramine, elicit mitogenic responses in human valvular interstitial cells via activation of 5-HT_{2B} receptors (Setola et al., 2003).

Based on strikingly similar echocardiographic and histopathological features, it is now considered that ergot-derived dopamine agonists may cause valvular heart diseases nearly identical to that observed in patients with carcinoid syndrome (Horvath et al., 2004). Population studies of patients with Parkinson's disease compared to 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 a high affinity for the 5-HT_{2B} receptor. The frequency of moderate-to-severe regurgitation in at least one heart valve was higher in patients receiving either cabergoline or pergolide than in patients taking either non-ergot agonists or controls, and the incidence of new-onset valvulopathy was high in patients taking ergot-derived drugs (Antonini & Poewe, 2007; Roth, 2007). Simultaneous mitral bioprostheses hypertrophic scaring and native aortic valve fibrosis was recently reported during benfluorex therapy in a 40-year-old woman. The bioprostheses and aortic valves exhibited similar histopathological lesions. Thickening and plaque deposits made by smooth muscle α-actin- and vimentin-positive cells in a glycosaminoglycan matrix were observed, which supported that activation by the 5-HT_{2B} receptor agonist norfenfluramine (also a metabolite of benfluorex) triggers the development of drug-induced heart disease (Ayme-Dietrich et al., 2012). 5-HT_{2B} and 5-HT_{2A} receptor transcripts are easily detected in heart valves while no 5-HT_{2C} receptor transcript is detectable. Preferential stimulation of valvular 5-HT_{2B} receptors (with or without accompanying 5-HT_{2A} 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_{2B} receptors and SERT in pigs (Cremer et al., 2015a). Canine myxomatous mitral valve disease was associated with higher expression of 5-HT_{2B} receptors in the mitral valve (Cremer et al., 2015b).

These findings suggest that 5-HT_{2B} signalling links vascular damage and platelet activation to tissue remodelling and identifies 5-HT_{2B} as a novel therapeutic target to treat valvular heart diseases.

3.3.5. Migraine

A role for 5-HT in migraines has been supported by changes in the circulating levels of 5-HT and its metabolites during the phases of a migraine attack. A migraine headache is thought to be transmitted from the meninges and their associated blood vessels by the trigeminal nerve. Correlation of the receptor affinities with the potencies used in migraine prophylaxis showed significant correlations for the 5-HT_{2B} receptor. Various human meningeal tissues express 5-HT_{2B} mRNA (Schmuck et al., 1996). The 5-HT_{2B} receptor can activate the release of NO and induce the relaxation of the cerebral arteries and jugular vein. 5-HT_{2B} receptors located in the 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_{2B} receptors as a gene susceptible to migraine (Corominas et al., 2010).

Endothelial 5-HT_{2B} receptors may thus trigger the dilation of meningeal blood vessels which, by activating sensory trigeminovascular afferents, induces head pain.

3.4. Cancer

In addition to its numerous physiological functions, 5-HT has been shown to be a mitogenic factor in a wide range of normal and tumour cells. The following section will emphasize the role of 5-HT_2 receptors in cancer.

3.4.1. Carcinoid tumours

5-HT_{2B} receptor expression was observed in spontaneous human carcinoid tumours along with coupling to $p21^{ras}$ activation, ERK1/2 activation, and proliferation (Launay et al., 1996; Nebigil et al., 2000). The proliferative activity of small intestinal neuroendocrine tumours (including cell growth and the development of desmoplasia) is associated with the particular microenvironment in the peritoneum, and tumour cells support this necessary milieu through the secretion of profibrotic/angiogenetic factors (Svejda et al., 2010), but autocrine activity of 5-HT/5-HT_{2B} receptors is likely.

3.4.2. Breast tumours

The increased biosynthetic capacity of 5-HT accompanied by multiple changes in 5-HT receptor expression and signalling favours the malignant progression of human breast cancer cells (e.g., stimulated proliferation, inappropriate cell survival). Expression levels of the 5-HT_{1F}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5A}, and 5-HT_7 receptors show an overall increase in breast cancer. Among these receptors, 5-HT_{2B} receptor expression was found to be increased in breast cancers, and *HTR_{2B}* mRNA is also expressed in untransformed human mammary epithelium (Pai et al., 2009). *HTR_{2B}* mRNA expression was found to be lower in oestrogen receptor (ER)-negative basal tumours compared with luminal tumours, which are most commonly ER-positive. *HTR_{2B}* mRNA was elevated in carcinomas, increased with tumour stage, and concomitantly higher in lymph node-positive tumours compared to lymph node-negative tumours. This observation was supported by a study showing that c-Myc transformation induced an increase in *HTR_{2B}* expression (Pai et al., 2009). In human breast cancer, an analysis revealed a significant correlation of *HTR_{2B}* with ER-α (Kopparapu et al.,

2013). Although 5-HT_{2A} receptor expression was detected in a human breast cancer cell line (MCF-7) and pegged as responsible for the mitogenic effect of 5-HT, these preliminary data have not been confirmed (Sonier et al., 2006).

3.4.3. Melanomas

Uveal (ocular) melanoma is an aggressive cancer that often forms undetectable micrometastases before diagnosis of the primary tumour. High increases in *HTR_{2B}* mRNA transcript levels were found in all uveal melanomas with monosomy 3 compared with low expression in all tumours with disomy 3. As monosomy 3 is associated with metastatic disease, *HTR_{2B}* expression has been proposed as a marker to identify patients with a poor prognosis (Tschentscher et al., 2003). The 5-HT_{2B} receptor signals through the heterotrimeric GTPase *GNAQ*, which is mutated in half of uveal melanomas (Onken et al., 2010). The 5-HT_{2B} receptor is one of the highest overexpressing genes in class 2 uveal melanomas (van Gils et al., 2008). A PCR-based 15-gene assay comprising 12 discriminating genes including *HTR_{2B}* 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_{2B}*, help to distinguishing whether uveal melanomas contain liver metastases and thus aid in the diagnosis and prevention of uveal melanoma liver metastases based on the differential expression patterns (Zhang et al., 2014).

3.4.4. Prostate cancer

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_{2B} receptor was found to be upregulated in tumours relative to benign glands (Magee et al., 2001). Overstimulation of receptors in response to neuroendocrine cell products has been suggested to contribute to the development of hormone-refractory prostate cancer. Immunostaining for the 5-HT_{2B} receptor was observed in low-grade and high-grade tumours, prostatic intra-epithelial neoplastic and benign prostatic hyperplasia cells, and vascular endothelial cells. Antagonists at the 5-HT_{2B} receptor have been reported to inhibit the proliferation of prostate cancer cells in a dose-dependent manner (Dizeyi et al., 2005).

3.4.5. Adrenocortical carcinoma

Interestingly, gene expression profiles of adrenocortical tumours identified the underexpression of *HTR_{2B}* mRNA as a marker of malignant adrenocortical carcinoma (Fernandez-Ranvier et al., 2008). Analysis of the biomarkers of malignant adrenocortical cancers in a meta-analysis has revealed that the combination of overexpressed anillin (*ANLN*) and underexpressed *HTR_{2B}* mRNA levels appeared to be the best predictor of malignancy (Zsippai et al., 2011). However, in adrenocorticotropin-dependent adrenal hyperplasias, the mechanisms responsible for the ectopic adrenal expression of glucose-dependent insulinotropic peptide (GIP) receptor (*GIPR*) in GIP-dependent Cushing's syndrome are unknown. Chronic adrenal stimulation by GIP in GIP-dependent ACTH-independent macronodular adrenal hyperplasia leads to a significant induction of *GPR54*, *HTR_{2B}*, *GPR4*, and the endothelial differentiation sphingolipid receptor *EDG8* (Lampron et al., 2006).

3.4.6. Hepatocellular carcinoma

Among the 64 genes for which mRNA expression levels differed between hepatitis C-type hepatocellular carcinoma (HCC) and either the non-hepatitis B-type or non-hepatitis C-type, the most affected gene found was *HTR_{2B}* (Iizuka et al., 2004). The function of 5-HT as a survival factor of HCC cells was recently demonstrated: activation of the 5-HT_{2B} receptor leads to sustained phosphorylation of the two downstream targets of mTOR, p70S6K and 4E-BP1, thereby facilitating survival and inhibiting autophagy. Inhibiting the 5-HT_{2B} receptor reduced cancer cell growth in vitro and in vivo. The presence of 5-HT_{2B} receptors in HCC and the activation of autophagy-related mechanisms demonstrate novel insights of 5-HT activity in cancer biology and suggest 5-HT-mediated signalling as a therapeutic target (Soll et al., 2010). The 5-HT_{1B} and 5-HT_{2B} receptors were expressed, respectively, in 32% and 35% of patients with hepatocellular cancer. Both receptors were associated with an increased proliferation index (Soll et al., 2012). The 5-HT_{2B} receptor mediates 5-HT-induced proliferation in serum-deprived HCC Huh7 cells. Additionally, inhibition of the 5-HT_{2B} receptor in Huh7 cells using SB204741, a selective 5-HT_{2B} receptor antagonist, significantly decreased the expression of FOXO3a, demonstrating that FOXO3a was a target of 5-HT in Huh7 cells (Liang et al., 2013).

3.4.7. Choriocarcinoma

This gestational trophoblastic disease is due to abnormal growth of trophoblast cells. 5-HT_{2A} receptor expression was found in human choriocarcinoma cell lines such as JEG-3 and BeWo as well as in normal human placental tissue. 5-HT stimulation leads to a concentration-dependent increase in proliferation of choriocarcinoma cell lines with a maximal increase in JEG-3 cell proliferation of 25.6 ± 6.8% with 40 μM of 5-HT and 11.1 ± 4% with 20 μM of 5-HT for BeWo cells compared to untreated control cells (Sonier et al., 2006). The same research group confirmed the role of 5-HT_{2A} receptor in cell viability and cell cycle progression by treating JEG-3 and BeWo cells with the 5-HT_2 receptor agonist DOI and reversing the concentration-dependent increase in cell viability with ketanserin, a 5-HT_{2A} receptor-selective antagonist. Moreover, stimulation of 5-HT_2 receptors by DOI in these two choriocarcinoma cell lines activated both the MEK-ERK1/2 and JAK2-STAT3 signalling pathways (Oufkir et al., 2010).

3.4.8. Glioma

Human malignant gliomas are particularly invasive, which makes a complete surgical resection very difficult. Therefore, pharmacological approaches are necessary. Merzak et al. (1996) identified 5-HT_2 receptor expression by RT-qPCR in eight glioma cell lines and weak expression in normal foetal astrocytes originating from the cerebellum and the left hemisphere. Moreover, these authors showed that 5-HT stimulates the proliferation, migration, and invasion of glioma cells. Nevertheless, they were unable to clearly establish the receptor subtype involved. Other in vitro experiments performed in rat astrocytes and 9L glioma cells detected 5-HT_{2A} receptor expression by RT-qPCR among the twelve 5-HT receptor subtypes explored (Sarrouilhe et al., 2015). In this work, 5-HT stimulation of C6 glioma cells increased glial fibrillary acidic protein (GFAP) expression at the mRNA and protein levels, suggesting that 5-HT could induce the differentiation of these cells (Morita et al., 2006). Finally, stimulation of C6 glioma cells with 5-HT increased the release of glial cell line-derived neurotrophic factor (GDNF), an effect that was blocked by treatment with either ketanserin or cyproheptadine. These results suggest that 5-HT_{2A} receptors could be involved in the survival, proliferation and migration of glioma cell lines via GDNF release (Hisaoka et al., 2004; Tsuchioka et al., 2008).

3.4.9. T cell leukaemia

The proteasome inhibitor bortezomib could be a potential therapeutic agent in treating adult T cell leukaemia (ATL) patients. A network that includes *HTR_{2B}* was identified and converges on the secreted protein acidic and rich in cysteine (*SPARC*) gene, a tumour-invasiveness related gene, which may act as a possible modulator of bortezomib-induced cell death in ATL cells (Ohyashiki et al., 2008), although the putative role of 5-HT was not investigated.

3.4.10. Osteosarcoma

Htr_{2A} mRNA is found in anaplastic osteoblasts as well as in differentiated and matured osteoblasts, whereas *Htr_{2B}* mRNA is only expressed in differentiated and matured osteoblasts (Westbroek et al., 2001; Hirai et al., 2009). The 5-HT_{2A} receptor has been shown to regulate MC3T3-E1 osteoblast cell proliferation by activating the ERK pathway. This effect is

reversed by the 5-HT_{2A} receptor-selective antagonist ketanserin (Hirai et al., 2009, 2010). Bracha et al. (2013) reported the serotonergic signalling pathway triggered by the 5-HT_{2A} receptor in non-neoplastic canine osteoblasts and in an osteosarcoma cell line. This group demonstrated that the intracellular second messenger signal is different between normal and malignant osteoblast cells: ritanserin, a 5-HT_2 receptor antagonist, decreased ERK phosphorylation and cell viability in non-neoplastic osteoblasts cell lines, whereas this compound decreased cell viability via CREB phosphorylation in osteosarcoma cell lines. The lack of selectivity of the 5-HT_2 antagonists used precludes any conclusion about the exact receptor involved in this observed activity.

3.4.11. Myosarcoma

In the pathogenesis of uterine leiomyosarcoma, a cDNA microarray analysis identified a fourfold overexpression of *HTR2B* among the most overexpressed genes (Arslan et al., 2005; Matsumura et al., 2006).

3.4.12. Tumour angiogenesis

In tumour-infiltrating macrophages, 5-HT does not enhance colon cancer tumour cell proliferation but may act as a regulator of angiogenesis by reducing the expression of MMP-12, which results in 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_{2B} 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_{2B} receptor resulted in the reduction of tumour angiogenesis and growth through the inhibitory effect of ERK1/2 and eNOS (Asada et al., 2009). Therefore, the possibility that 5-HT_{2B} receptors participate in tumour angiogenesis is likely but requires further evaluation in other tumour subtypes.

In conclusion, the contribution of 5-HT_2 receptors in tumours remains to be determined but is dependent on the tumour type.

3.5. Skeleton

3.5.1. Bone development and osteoporosis

Although *Htr2B* mRNA is undetected in anaplastic osteoblasts, it appears in differentiated and matured osteoblasts (Bliziotes et al., 2001; Westbroek et al., 2001). The differentiation and maturation of osteoblasts might thus be regulated by activation of the 5-HT_{2B} receptor (Hirai et al., 2009). Of interest, $Htr_{2B}^{-/-}$ female mice displayed significantly reduced bone density from the age of 4 months, which was intensified at 12 and 18 months. This finding histomorphometrically confirmed that osteopenia was due to reduced bone formation since (i) the alkaline phosphatase-positive colony-forming unit capacity of bone marrow precursors was markedly reduced in $Htr_{2B}^{-/-}$ mice from 4 to 12 months of age, (ii) ex vivo primary osteoblasts from $Htr_{2B}^{-/-}$ mice exhibited reduced proliferation and delayed differentiation, and (iii) calcium incorporation was markedly reduced in osteoblasts after 5-HT_{2B} receptor depletion (produced either genetically or by pharmacological inactivation) (Collet et al., 2008). Using the osteoprogenitor cell line C1, blockade of the intrinsic activity of the 5-HT_{2B} receptor was reported to affect the mineralization efficiency by decreasing calcium incorporation. Optimal bone matrix mineralization involves both NO and PLA2 signalling pathways, and the 5-HT_{2B} 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_{2B} receptor contributed to osteogenic differentiation in an autocrine manner (Locker et al., 2006). A functional link between the 5-HT_{2B} receptor and the activity of tissue non-specific alkaline phosphatase (TNAP) was established. Agonist stimulation of the receptor increased TNAP activity during the initial mineralization phase. Indeed, inhibition of intrinsic 5-HT_{2B} receptor 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_{2B} receptor coupled to the PLA2 pathway and prostaglandin production at the beginning of mineral deposition. The 5-HT_{2B} receptor also controlled leukotriene synthesis via PLA2 during the terminal stages of differentiation. These two 5-HT_{2B} receptor-dependent eicosanoid productions delineate distinct snapshots of TNAP regulation during osteogenic programming. Finally, either 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 $Htr_{2B}^{-/-}$ mice were shown to exhibit defects in TNAP activity (Baudry et al., 2010). Brain 5-HT was proposed to indirectly favour the accrual of bone mass following activation of 5-HT_{2C} receptors on ventromedial hypothalamic neurons and 5-HT_{2B} receptors on arcuate neurons (Yadav et al., 2009). Compared to control osteoblasts, the lack of 5-HT_{2B} receptors was associated with a 10-fold overproduction of prostacyclin (PGI_2). Additionally, a specific prostacyclin synthase inhibitor (U51605) completely rescued osteoblast aggregation and matrix mineralization in $Htr_{2B}^{-/-}$ osteoblasts without having any effect on WT osteoblasts. Prostacyclin is the endogenous ligand of PPAR-β/δ, and its inhibition in $Htr_{2B}^{-/-}$ cells completely rescued the mRNA levels of alkaline phosphatase and osteopontin, cell–cell adhesion, and matrix mineralization. The absence of 5-HT_{2B} receptors leads to the overproduction of prostacyclin, which results in reduced osteoblast differentiation due to peroxisome proliferator-activated receptor (PPAR)-β/δ-dependent target regulation and defective cell–cell adhesion and matrix mineralization (Chabbi-Achengli et al., 2013). *HTR2A* was also found to be expressed only in osteoblasts, whereas *HTR2B* expression increased from precursor to mature osteoclasts (Hodge et al., 2013). A recent study highlights the contribution of the 5-HT_{2A} receptor in bone metabolism. This receptor appears involved in osteoblast differentiation and bone mineral density. Blocking 5-HT_{2A} receptor signalling by MDL11939 decreased bone mass and led to skeletal fragility in mice. Moreover, the pharmacological blockade of 5-HT_{2A} receptors significantly decreased cellular differentiation in MC3T3-E1 cells and osteoblast primary cultures from the right femur of mice (Tanaka et al., 2015).

All these results drive to the hypothesis that 5-HT and the 5-$HT_{2A/2B}$ receptor signalling pathways may contribute to bone formation and cellular differentiation.

3.5.2. Tooth development

Periodontal diseases occur in patients treated with antidepressants such as SSRIs (e.g., Prozac), which target SERT. In the molars of $Htr_{2B}^{-/-}$ mice, rod curvatures and twisting were altered compared to WT mice, suggesting the involvement of *Htr2B* at early stages of enamel formation. The volume of the $Htr_{2B}^{-/-}$ enamel layer was also reduced, with smaller crystallite thickness. The outer aprismatic enamel border was 1.5- to 2-fold larger in $Htr_{2B}^{-/-}$ mice compared to WT mice. Finally, although no noticeable difference was observed in dentin, the three-dimensional pulp reconstruction suggested a decrease in both the length and width of dentin formation in the root canals of the $Htr_{2B}^{-/-}$ mice versus WT mice (Dimitrova-Nakov et al., 2014).

Therefore, 5-HT_{2B} receptors may mediate some harmful effects of the long-term use of SSRIs on bone and teeth regeneration.

4. Emerging research and new therapeutic opportunities

4.1. Haematopoiesis

In addition to its role as a neurotransmitter, 5-HT has been shown to regulate inflammation and tissue repair via a set of receptors whose expression pattern varies among cell lineages. Previous studies have shown that 5-HT is a growth factor for haematopoietic stem/progenitor cells. 5-HT_{2B} receptor expression was identified in the megakaryocytic cell lineage. Serotonin promoted the proliferation of megakaryocytes (MKs) and reduced cell apoptosis via activation of the 5-HT_{2B} receptor and the Akt pathway (Liu & Fanburg, 2006). Serotonin increased

proplatelet-bearing MKs and polymerized actin levels via ERK1/2 (Ye et al., 2014). Mice deficient in peripheral 5-HT (*Tph1*$^{-/-}$) displayed morphological and cellular features of ineffective erythropoiesis. The central event occurred in the bone marrow, where the absence of 5-HT hampered the progression of erythroid precursors that express 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors towards terminal differentiation. In addition, red blood cells from 5-HT-deficient mice were more sensitive to macrophage phagocytosis and have a shortened in vivo half-life (Amireault et al., 2011). Furthermore, 5-HT$_{2B}$ receptor expression was detected in c-kit + bone marrow cells (Launay et al., 2012). The 5-HT$_{2B}$ receptor antagonist RS127445 decreased colony-forming capacity by inhibiting both CFU-GEMM and BFU-E formation; this results can be attributed to a reduction of cell proliferation and/or an apoptotic effect. By contrast, 5-HT significantly enhanced the expansion of CD34+ cells to early stem/progenitors (CFU-GEMM) and committed progenitors (BFU/CFU-E) (Yang et al., 2007). Treatment with aggregated (1–40 or 1–42) and oligomeric (1–42) amyloid β (Aβ, a hallmark of Alzheimer's disease) promoted the differentiation of bone marrow-derived mesenchymal stem cells without toxic effects. The effect of Aβ was shown to be mediated by GPCRs, neuropeptide Y1 and the 5-HT$_{2B}$ receptor via PI3K-dependent activation of the MAPK/ERK1/2 pathway (Kim et al., 2009).

In human macrophages, 5-HT was reported to inhibit the lipopolysaccharide-induced release of proinflammatory cytokines, to upregulate the expression of M2 polarization-associated genes and to reduce the expression of M1-associated genes. The 5-HT$_{2B}$ receptor mediates the pro-M2 skewing effect of 5-HT. In fact, blocking signalling through this receptor during in vitro monocyte-to-macrophage differentiation preferentially modulates the acquisition of M2 polarization markers. *Htr$_{2B}$* mRNA was found to be preferentially expressed in anti-inflammatory M2 (M-CSF) macrophages and was detected in vivo in both liver Kupffer cells and tumour-associated macrophages (de Las Casas-Engel et al., 2013). Recently, expression of the 5-HT$_{2B}$ receptor was reported on postnatal microglia, supporting the notion that 5-HT participates in the temporal and spatial synchronization of microglial function (Kolodziejczak et al., 2015).

Htr$_{2B}$ mRNA expression was found in the spleen, thymus, and peripheral blood lymphocytes (Stefulj et al., 2000). Study of the expression of serotonergic receptors in human dendritic cells showed that immature dendritic cells expressed *Htr$_{2B}$* mRNA. Moreover, 5-HT$_{2B}$ receptor stimulation induced intracellular Ca^{2+} mobilization in immature but not mature dendritic cells. Serotonin stimulated different signalling pathways in dendritic cells in a maturation-dependent manner (Idzko et al., 2004). A proper balance between different T helper (Th) cell subsets is necessary for normal functioning of the adaptive immune system. In human umbilical cord blood, T helper cells cultured in the absence and presence of cytokines promoting either Th1 or Th2 differentiation were found to specifically express *HTR$_{2B}$* among 50 differentially expressed Th2 genes (Aijö et al., 2012). In gene expression profiles during human CD41 T cell differentiation, *HTR$_{2B}$* was found to be SP4-specific (~10-fold) among the sixteen transcripts that were expressed in SP4 thymocytes at levels 3-fold or higher; this level expression was greater than in any other isolated T cell subpopulation (Lee et al., 2004).

Rheumatoid arthritis is a chronic disease that results in a disabling and painful condition as it progresses to the destruction of the articular cartilage and ankylosis of the joints. Although the cause of the disease is still unknown, evidence has argued that autoimmunity plays an important part. There are increasing views regarding 5-HT as being associated with the activation of immunoinflammatory pathways and the onset of autoimmune reactions. In *Tph1*$^{-/-}$ mice with arthritis, a significant increase in osteoclast differentiation and bone resorption was observed with an increase in IL-17 levels in the paws and in the number of Th17 lymphocytes in the draining lymph nodes, whereas the number of T-regulatory cells was dampened. Ex vivo 5-HT and agonists at the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors restored IL-17 secretion from splenocytes and Th17 cell differentiation in *Tph1*$^{-/-}$ mice. These findings indicate that serotonin plays a fundamental role in arthritis by regulating the Th17/T-regulatory cell balance and osteoclastogenesis (Chabbi-Achengli et al., 2016).

Therefore, 5-HT signalling via the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors mediates the balance among various haematopoietic lineages.

4.2. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the major adult onset motor neuron disease and represents the third most frequent neurodegenerative disease after Alzheimer's disease and Parkinson's disease. ALS is characterized by the selective degeneration of upper motor neurons in the cerebral cortex and lower motor neurons in the spinal cord and brainstem and leads to progressive paralysis and death within 3–5 years after onset. Spinal cord injury leads to an initial phase of hyporeflexia followed by hyperreflexia, often referred to as spasticity. Spasticity is a common and disabling symptom observed in patients with ALS. A rat tail-spasticity model with a caudal spinal transection identified that the expression of 5-HT$_{2B}$ receptors was downregulated at 21 days post-injury (Wienecke et al., 2010). Motoneurons, which help recover from denervation, function autonomously by exhibiting large persistent calcium currents that help with the functional recovery and contribute to uncontrolled muscle spasms. Application of agonists to 5-HT$_{2B}$ receptors (including BW723C86) significantly increased these persistent calcium currents. 5-HT$_{2B}$ receptors on motoneurons ultimately contribute to the recovery of motoneuron function and the emergence of spasms (Murray et al., 2011). In ALS, spasticity is traditionally thought to be the result of degeneration of the upper motor neurons in the cerebral cortex, although the degeneration of other neuronal types (serotonergic neurons in particular) might also represent a cause of spasticity. In SOD1 (G86R) mice, which are a transgenic model of ALS, 5-HT levels were decreased in the brainstem and spinal cord before the onset of motor symptoms. Furthermore, there was noticeable atrophy of 5-HT neuronal cell bodies along with neuritic degeneration at the initial disease onset. In SOD1 (G86R) mice, spastic-like contractions in the tail muscle were observed at the end-stage. Importantly, these contractions were abolished by treatment with the 5-HT$_{2B/2C}$ receptors inverse agonist SB206553. In line with this result, 5-HT$_{2B}$ receptor expression was strongly increased at disease onset (Dentel et al., 2013).

In summary, 5-HT$_{2B}$ receptors in motoneurons may ultimately contribute to the recovery of motoneuron function and the emergence of spasms.

Microglia are the resident mononuclear phagocytes of the central nervous system and have been implicated in the pathogenesis of neurodegenerative diseases such as ALS. During neurodegeneration, microglial activation is accompanied by infiltration of circulating monocytes, leading to the production of multiple inflammatory mediators in the spinal cord. Degenerative alterations in mononuclear phagocytes are commonly observed during neurodegenerative diseases. In mutant SOD1 mice, 5-HT$_{2B}$ receptor upregulation was observed but was restricted to cells positive for CD11b, a marker of mononuclear phagocytes including microglia. Ablation of the 5-HT$_{2B}$ receptor in transgenic ALS mice expressing mutant SOD1 resulted in increased degeneration of mononuclear phagocytes, as evidenced by the fragmentation of Iba1-positive cellular processes, which was accompanied by not only decreased expression of key neuroinflammatory genes but also a loss of the expression of homeostatic microglial genes. Importantly, the dramatic effect of 5-HT$_{2B}$ receptor ablation on mononuclear phagocytes was associated with the acceleration of disease progression (El Oussini et al., 2016). A study on polymorphisms in the human *HTR$_{2B}$* gene (which encodes the 5-HT$_{2B}$ receptor) in a large cohort of ALS patients showed that the C allele of SNP rs10199752 in the *HTR$_{2B}$* gene was associated with longer survival. Moreover, patients carrying one copy of the C allele of SNP rs10199752 showed increased *HTR$_{2B}$* mRNA expression in the spinal cord and displayed less pronounced degeneration of

Iba1-positive cells than patients carrying two copies of the more common A allele (El Oussini et al., 2016).

Thus, the 5-HT_{2B} receptor limits the degeneration of spinal cord mononuclear phagocytes (most likely microglia) and slows the disease progression of ALS.

4.3. Obesity/metabolic syndrome

The serotonergic system affects feeding behaviour not only at the central nervous system level but also at the peripheral level by regulating glucose and lipid metabolism (McGlashon et al., 2015). A strong lactogen-dependent upregulation of 5-HT biosynthesis has been shown to occur in a subpopulation of mouse islet β-cells during pregnancy (Schraenen et al., 2010). This subpopulation of pancreatic islet cells expresses genes that encode all of the products necessary for synthesizing, packaging, and secreting 5-HT, including tryptophan hydroxylases. Transcriptome analysis of islets isolated from pregnant mice identified a very strong upregulation of two paralogous genes that encode tryptophan hydroxylase, Tph1 and Tph2 (Goyvaerts et al., 2016); this upregulation is dependent on the activation of prolactin receptors. In β-cells, Pet1 can bind to the serotonergic genes but also to a conserved insulin gene regulatory element. In *Tph1*$^{-/-}$ mice, the absence of 5-HT leads to impaired insulin secretion and, as a consequence, reduced glucose tolerance (Ohta et al., 2011). The mechanism could be impaired serotonylation of the small GTPases Rab3a and Rab27a, which are two key players for insulin exocytosis (Paulmann et al., 2009). Nevertheless, it remains debatable whether blocking 5-HT receptor signalling in pregnant mice also blocks β-cell expansion and causes glucose intolerance (Goyvaerts et al., 2016; Kim et al., 2010). The differential expression of the 5-HT receptor genes in human islets derived from non-diabetic and type 2 diabetic patients highlighted the overexpression of *HTR_{1D}* and *HTR_{2A}* mRNA in diabetic subjects. In fact, the effects of 5-HT in the pancreas are complex and could vary depending on the pathophysiological status. 5-HT was found to inhibit insulin and glucagon secretion in islets from non-diabetic subjects whereas 5-HT increased insulin release in response to glucose in islets from type 2 diabetic patients (Bennet et al., 2015).

Taken together, these results suggest that 5-HT regulates insulin secretion in non-diabetic islets; however, the stimulation of overexpressed 5-HT_{1D} and 5-HT_{2A} receptors contributes to islet dysfunction in type 2 diabetic patients.

On the other hand, gut-derived 5-HT favours lipolysis and liver neoglucogenesis and inhibits glucose uptake in liver and adipose tissue by signalling through the 5-HT_{2B} receptor. Inhibition of peripheral 5-HT synthesis and blockade of 5-HT_{2B} receptor signalling improves hyperglycaemia in a mouse model of type 2 diabetes (Sumara et al., 2012). In a high-throughput RNA screen with human primary (pre)adipocytes, 110 genes were found to be regulated at the gene expression level during adipogenesis. Among these, *HTR_{2B}* mRNA was one of the top hits identified. Adipocytes cultured in the presence of the 5-HT_{2B} receptor-selective antagonist RS127445 exhibited a significant increase in neutral lipid levels compared to control cells (Söhle et al., 2012). A recent study highlighted the role of peripheral 5-HT in brown adipose tissue thermogenesis. Tph1-deficient mice are protected from obesity and insulin resistance by elevated brown adipose tissue activity. This mechanism implies inhibition of β-adrenergic signalling by 5-HT (Crane et al., 2015). These studies suggest that peripheral 5-HT regulates glucose and lipid metabolism, fat accumulation, and obesity. The last major organ involved in glucose metabolism is the muscle. Recently, in mice fed a high-fat diet, Watanabe et al. (2016) observed a

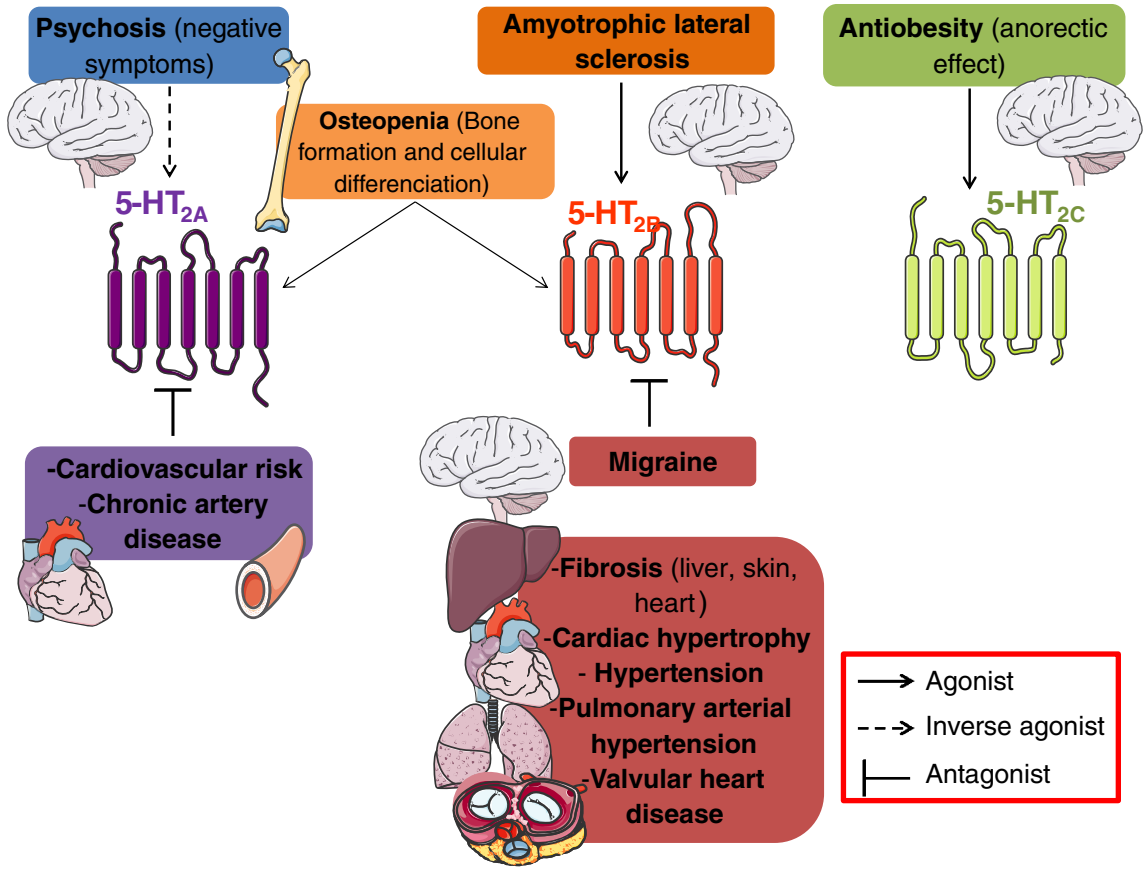

Fig. 1. Serotonin 5-HT_2 receptors as therapeutic targets for central nervous system and cardiovascular diseases. Blocking or stimulating 5-HT_2 receptors could have clinical impact in many fields. In this figure, the main areas are emphasized. We also show which type of receptor should be blocked or stimulated.

reduction of weight gain, hyperglycaemia, and insulin resistance after intraperitoneal injections of high doses of 5-HT (0.1 mg, 0.5 mg or 1 mg twice a week). The research team simultaneously observed a shift in the metabolic profile in the muscle fibre of the soleus muscle. 5-HT injections induced an increase the mRNA expression of PPAR coactivator 1α in the soleus muscle, which was inhibited by antagonists against either the 5-HT_{2A} or 5-HT_7 receptors.

These data suggest important roles for the 5-HT_{2A} and 5-HT_{2B} receptors in controlling energy homeostasis.

5. Conclusions and prospects

After many years of investigations in various fields such as fibrosis, the central nervous system and the cardiovascular system, new paradigms of the pathophysiological contributions of 5-HT_2 receptors have been discovered and clarified. In parallel, pharmacochemistry identified new selective ligands and highlighted multiple 5-HT_2 receptor intracellular signalling pathways. At that step, translational research will be required to determine clinical applications. Nevertheless, the observed cardiovascular side effects have ignited the interest for agonists. With regard to this obstacle, the search for allosteric modulators and biased ligands will be of great importance. On the other hand, the use of highly selective 5-HT_2 receptor antagonists seems to be safe and could prevent and/or treat many diseases (Cf Fig. 1).

Conflict of interest

The authors declare that there are no conflicts of interest.

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