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Molecular biology of 5-HT receptors

Jason Hannon, Daniel Hoyer*

Nervous System Research, WSJ.386.745, Novartis Institute for Biomedical Research, CH-4002 Basel, Switzerland

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ABSTRACT

Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter whose effects are mediated by at least 13 distinct G protein-coupled receptors (GPCRs) of the type A family which includes the monoamine receptors and a combination of ligand-gated ion channels (5-HT_3) of the Cys loop family which constitutes heteropentamers. 5-HT receptors are currently divided into seven classes (5-HT_1 to 5-HT_7), based on structural, transductional and operational features. While this degree of physical diversity clearly underscores the physiological importance of serotonin, evidence for an even greater degree of operational diversity is supported by the existence of a great number of splice and editing variants for several 5-HT receptors, their possible modulation by accessory proteins and chaperones, as well as their potential to form homo or heteromers both at the GPCR and at the ligand-gated channel level.

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) as a neurotransmitter acts via membrane receptors in the central nervous (CNS) and the peripheral nervous system (PNS), as well as in non-neuronal tissues (e.g. blood, gastro intestinal, endocrine, sensory and cardiovascular systems, to name a few). 5-HT is one of the oldest neurotransmitters/hormones in evolution, and its receptors are estimated to have appeared 700–800 Mio years in single cell eukaryotes such as paramecia; 5-HT receptors are found in such diverse species from planaria, c. Elegans, and drosophila to man are, and are rather well conserved. This may explain why 5-HT interacts with such a diversity of receptors of the G-protein-coupled family and the ligand-gated family, similarly to acetylcholine, GABA or glutamate, but with more receptor subtypes and a larger diversity at play. They may actually have been amongst the first rhodopsin-like receptors reacting to a chemical. The major classes of 5-HT receptors must have diverged about 750 millions years ago, long before cholinergic, adrenergic or dopaminergic receptors, although the GPCR family may date from >1 billion years. Serotonin was first described as enteramine, which was isolated from the gut in the 1930s by Ersparmer and colleagues and shown to cause contraction of the uterus. 5-HT was rediscovered in the 1940s by Irvin Page's group in the circulation and called serotonin, based on its vasoconstrictor features (although it also relaxes blood vessels); eventually Maurice Rapport purified, crystallized and characterized the molecule from vast amounts of blood. Rapport found that enteramine and serotonin covered the same entity, namely 5-hydroxytryptamine, which indeed contained an indole as already suggested by Ersparmer and eventually the two groups came to the same conclusion. The availability of synthetic 5-HT was the real start of the 5-HT saga in pharmacological terms.

The subdivision of 5-HT receptors started in the 1950s by Gaddum and colleagues, when they realised that in the guinea pig ileum, the effects of 5-HT could be blocked in part by morphine (M), and in part by dibenzyline (D). Gaddum and Picarelli proposed two receptor classes, 5-HT M and 5-HT D (1957). Although non-selective tools were used, the concept proved to be correct. In 1976, when after many painful attempts at monoamine receptor binding, the first radioligand-binding studies succeeded using [^{3}H]5-HT and [^{3}H]LSD, Fillion and colleagues suggested the existence of 5-HT receptors in brain labeled with [^{3}H]5-HT and [^{3}H]LSD (1976, 1977, 1978, 1979); however these papers did not get the deserved attention of the community. Then in 1979, Peroutka and Snyder described two classes of brain 5-HT receptors, using [^{3}H]-5-HT, [^{3}H]-spiperone (a dopaminergic ligand), and [^{3}H]-LSD called 5-HT_1 ([^{3}H]-5-HT binding) and 5-HT_2 ([^{3}H]-spiperone), with [^{3}H]-LSD labeling both classes. Interestingly, Gaddum's M receptor was still distinct from the 5-HT_1 and 5-HT_2 receptors in both function and distribution, whereas the D receptor resembled pharmacologically the 5-HT_2 binding site; it was also assumed for quite some time that the 5-HT M receptor was purely peripheral, as amply documented in functional studies, and most of the 5-HT stems from chromaffin cells in the gut, whereas in the brain much of the serotonin comes from the raphé nuclei. Thus, Phillip Bradley convened a party in charge of unifying the 5-HT receptor concept and nomenclature. Bradley et al. [1] proposed the existence three families of 5-HT receptors, named 5-HT_1-like (there was already suggestions

* Corresponding author. Tel.: +41 61 324 4209; fax: +41 61 324 4866.
E-mail address: daniel.hoyer@novartis.com (D. Hoyer).

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Table 1
5-HT_1 receptor nomenclature proposed by the NC-IUPHAR Subcommittee on 5-HT receptors

Nomenclature	5-HT_{1A}	5-HT_{1B}[a,b]	5-HT_{1D}[a]	5-ht_{1E}	5-HT_{1F}
Previous names	–	5-$HT_{1D\beta}$	5-$HT_{1D\alpha}$	–	5-$ht_{1E\beta}$, 5-HT_6
Selective agonists	8-OH-DPAT	Sumatriptan L 694247	Sumatriptan PNU 109291	–	LY 334370
Selective antagonists (pK_B)	(±)WAY 100635 (8.7)	GR 55562 (7.4) SB 224289 (8.5) SB 236057 (8.9)	BRL 15572 (7.9)	–	–
Radioligands	[^{3}H]WAY100635 [^{3}H]8-OH-DPAT	[^{125}I]GTI [^{125}I]CYP (rodent) [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{125}I]GTI [^{3}H]Sumatriptan [^{3}H]GR 125743	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]LY 334370
G protein effector	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Gene/chromosomal localization	*HTR1A*/5q11.2-q13	*HTR1B*/6q13	*HTR1D*/1p34.3-36.3	*HTR1E*/6q14-15	*HTR1F*/3p11-p14.1
Structural information	h421 P8908 m421 Q64264 r422 P19327	h390 P28222 m386 P28334 r386 P28564	h377 P28221 m374 Q61224 r374 P28565	h365 P28566 m366 Q02284 r366 P30940	h366 P30939

5-$HT_{2,3,4}$ receptor nomenclature proposed by the NC-IUPHAR Subcommittee on 5-HT receptors

Nomenclature	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}[e]	5-HT_3	5-HT_4
Previous names	D/5-HT_2	5-HT_{2F}	5-HT_{1C}	M	–
Selective agonists	DOI[c]	BW 723C86	Ro 600175	SR 57227 *m*-Chlorophenyl-biguanide	BIMU 8 RS 67506 ML 10302
Selective antagonists (pK_B)	Ketanserin (8.5-9.5) MDL 100907 (9.4)	SB 200646 (7.5)[d] SB 204741 (7.8)	Mesulergine (9.1) SB 242084 (9.0) RS 102221 (8.4)	Granisetron (10) Ondansetron (8–10) Tropisetron (10–11)	GR 113808 (9-9.5) SB 204070 (10.8) RS 100235 (11.2)
Radioligands	[^{125}I]DOI [^{3}H]Ketanserin [^{3}H]MDL 100907	[^{3}H]5-HT	[^{125}I]LSD [^{3}H]Mesulergine	[^{3}H](*S*)-Zacopride [^{3}H]Tropisetron [^{3}H]Granisetron [^{3}H]GR 65630 [^{3}H]LY 278584	[^{125}I]SB 207710 [^{3}H]GR 113808 [^{3}H]RS 57639
G protein effector	$G_{q/11}$	$G_{q/11}$	$G_{q/11}$	[f]	G_s
Gene/chromosomal Localization	*HTR2A*/13q14-q21	*HTR2B*/2q36.3-q37.1	*HTR2C*/Xq24	*HTR3*/11q23.1-q23.2	*HTR4*/5q31-33
Structural information	h471 P28223 m471 P35362 r471 P14842	h481 P41595 m504 Q02152 r479 P30994	h458 P28335 m459 P34968 r460 P08909	Multi-subunit[g] 5-HT_{3A}, 5-HT_{3B}, 5-ht_{3C}	h387 Y09756[AS] m387 Y09587[AS] r387 U20906[AS]

5-$HT_{5,6,7}$ receptor nomenclature proposed by the NC-IUPHAR subcommittee on 5-HT receptors

Nomenclature	5-ht_{5A}	5-ht_{5B}	5-HT_6	5-HT_7
Previous names	5-$HT_{5\alpha}$	–	–	5-HT_X 5-HT_1-like
Selective agonists	–	–	–	–
Selective antagonists (pK_B)	–	–	Ro 630563 (7.9) SB 271046 (7.8) SB 357134 (8.5)	SB 258719 (7.9) SB 269970 (9.0)
Radioligands	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]LSD [^{3}H]5-CT	[^{125}I]SB 258585 [^{125}I]LSD [^{3}H]5-HT	[^{125}I]LSD [^{3}H]SB 269970 [^{3}H]5-CT [^{3}H]5-HT
G protein effector	$G_{i/o}$	None identified	G_s	G_s
Gene/chromosomal localization	*HTR5A*/7q36.1	*htr5b*/2q11-q13	*HTR6*/1p35-36	*HTR7*/10q23.3-24.3
Structural information	h357 P47898 m357 P30966 r357 P35364	m370 P31387 r370 P35365	h440 P50406 m440 NP_067333 r438 P31388	h445 P34969[AS] m448 P32304 r448 P32305[AS]

Putative 5-ht_5 receptor retain lower case appellations in the absence of a clear functional roles.

[a] 5-HT_{1B} and 5-HT_{1D} receptor nomenclature has been revised; only the non-rodent form of the receptor was previously called 5-HT_{1D}.

[b] Displays a different pharmacology to the rodent form of the receptor. 5-ht_{1E} retains lower case in the absence of a clear functional role.

[c] Also activates the 5-HT_{2C} receptor.

[d] Non-selective blockade.

[e] Multiple isoforms of the 5-HT_{2C} receptor are produced by RNA editing.

[f] The 5-HT_3 receptor is a transmitter-gated cation channel that exists as a pentamer of 4TM subunits.

[g] Human, rat, mouse, guinea-pig and ferret homologues of the 5-HT_{3A} receptor have been cloned that exhibit interspecies variation in pahrmacology. A second 5-HT_3 receptor subunit, 5-HT_{3B}, imparts distinctive biophysical properties upon hetero-oligomeric (5-HT_{3A}/5-HT_{3B}) versus homo-oligomeric (5-HT_{3A}) recombinant receptors. Additional 5-HT_3 receptor subunits (5-$HT_{3C,\ 3D,\ 3E}$) have been reported, which may form heteroreceptors with 5-HT_{3A} or act as chaperones, but have not been reported to function as homomers.

for diversity of this group from radioligand binding and autoradiographic studies), 5-HT_2 and 5-HT_3, the latter corresponding to the M receptor. The proposal was based primarily on functional criteria, since radioligand binding was still not convincing to many colleagues, second messenger studies were less popular than today, and no GPCR had been cloned at the time that party congregated from 1984 on; nevertheless, the Bradley nomenclature represented a useful classification framework. However, with

the increasing use of radioligand binding in membranes and cells, autoradiography in brain slices and second messenger studies in cells and tissue, subtypes of 5-HT_1 receptor binding sites were further described (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-ht_{1E}). It became rapidly evident that the 5-HT_{1C} receptor found in the choroid plexus (although labeled with high affinity by [^{3}H]-5-HT), is closer the 5-HT_2 receptor family, due to similar pharmacological profiles and 2nd messenger features (stimulation of inositol phosphate production and calcium signalling), and this suggested the existence of 5-HT_2 receptor subtypes as well. Further, another 5-HT receptor which had been identified in the mid 1980's in the gastrointestinal tract, heart and brain, was termed 5-HT_4 by Saxena and colleagues (see [2]), but this proposal was initially rejected. Fortunately, in 1986–1988, the molecular biology era started with the cloning of the 5-HT_{1A} receptor (interestingly, G21 as the 5-HT_{1A} receptor was called when it was still an orphan, allowed the cloning of further beta adrenoceptors by homology, see [3,4]). Rapidly, most known but also some unsuspected 5-HT receptors were cloned. This work led to the identification of a number of 'new' receptors, devoid of immediate physiological counterparts. Tentatively termed 5-ht_{1E}, 5-HT_{1F}, 5-HT_{2F}, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6, and 5-HT_7, they required integration into an acceptable classification scheme. As is known now, all 5-HT receptors that belong to the GPCR are part of the type A family of GPCRs and show still significant sequence homology with rhodopsin, but also adrenergic and dopaminergic receptors, and contain the famous DRY motif in the third transmembrane spanning region. Based on these new findings and yet a constantly evolving field, the Serotonin Club Receptor Nomenclature Committee proposed a new nomenclature system based on operational, structural and transductional information ([5], see Table 1). These principles were subsequently applied to a number of receptor families by the newly created Receptor Nomenclature Committee of the International Union of Pharmacology (NC-IUPHAR), keeping in mind that some historical features will have to be acknowledged at least transiently (for instance the reclassification of opiate receptors met quite some resistance and we are left with the former mu, delta and kappa opiate receptor classes). The current classification ([6]; Hoyer et al. [214]) is flexible and intended to be adapted, as information from both recombinant and native systems becomes available; however it favours an alignment of nomenclature with the human genome to avoid species differences (see [7,8]). Seven families of 5-HT receptors form the basis of the classification, based on pharmacology, transduction and structure, although one could argue that structure must be predominant as it governs function and ultimately pharmacological signature. One orphan receptor, called 5-HT_{1P} by Gershon and co-workers, present in the gut [9], is not structurally characterized, and it remains to be seen whether this receptor is a homomer (new) or possibly a heterodimer composed of already known receptors. 5-HT_3 receptors are ligand-gated ion channels, thus the 5-HT receptor family is showing similar diversity as do the acetylcholine or glutamate receptors families, which act through both metabotropic receptors and some fast acting ligand-gated channels. The 5-HT system has long been known to regulate emotions, behavioral control and cognition in a very complex manner, as has become evident from a great number of animal and human studies (see [10]); and that complexity may not be too surprising given the number of players involved in the 5-HT system which includes a multiplicity of receptors, transporters and metabolizing enzymes.

2. 5-HT_1 receptors, a family of receptors coupled to $G_{i/o}$

Class A GPCRs can be subdivided depending on their coupling to second messengers via the G-proteins and 5-HT_1 receptors are mostly linked to $G_{i/o}$, which are pertussis toxin sensitive and couple negatively to adenylate cyclase; in cells, this may lead to membrane depolarization and inhibition of firing. The 5-HT_1 receptor class is composed of five receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1e} and 5-HT_{1F}) which, in humans, share 40–63% overall sequence identity and couple somewhat preferentially to $G_{i/o}$ to inhibit cAMP formation. The 5-ht_{1E} receptor has still a lower case appellation since the corresponding endogenous receptor has not yet been established. Actually 5-ht_{1E} may not be found in rodents, but has been reported in guinea-pig brain [210]. 5-HT_{1A}, 5-$HT_{1B,}$ 5-HT_{1D} and 5-HT_{1F} receptors have been demonstrated functionally in a variety of tissues. The 5-HT_{1C} receptor was renamed 5-$HT_{2C,}$ due to structural, operational and transductional similarities with the 5-HT_2 receptor subclass [6]; it was assigned to the 5-HT_1 group due to its high affinity binding of [^{3}H]-5-HT, a classic feature of 5-HT_1 receptors, but this has long been revised.

2.1. 5-HT_{1A} receptors

The human 5-HT_{1A} receptor is located on chromosome 5q11.2-q13. 5-HT_{1A} receptors are largely distributed throughout the CNS, but are also present in the PNS. They were initially characterized with [^{3}H]8-OH-DPAT as a selective radioligand, and corresponded roughly to the spiperone sensitive [^{3}H]5-HT binding in brain [11]. Receptor autoradiography confirmed that [^{3}H]8-OH-DPAT was identifying a subpopulation the sites labeled by [^{3}H]5-HT in brain.

The distribution of the 5-HT_{1A} receptor in brain has been mapped extensively by receptor autoradiography using a range of ligands including [^{3}H]-5-HT under appropriate conditions, but also with more subtype selective ligands such [^{3}H]-8-OH-DPAT, [^{3}H]-ipsapirone, [^{125}I]-BH-8-MeO-N-PAT, [^{125}I]-*p*-MPPI and [^{3}H]-WAY 100635. PET studies have used [^{11}C]-WAY 100635 to Image 5-HT_{1A} receptors in the living human brain. The density of 5-HT_{1A} binding sites is high in limbic brain areas, notably hippocampus, lateral septum, cortical areas (particularly cingulate and entorhinal cortex), and also the mesencephalic raphé nuclei. Levels of 5-HT_{1A} binding sites in the basal ganglia and cerebellum are extremely low if at all. 5-HT_{1A} receptors are located both postsynaptic to 5-HT neurones (as in forebrain regions), and also on the 5-HT neurones themselves at the level of the soma and dendrites in the mesencephalic and medullary raphé nuclei. At the cellular level, in situ hybridization and immunocytochemical studies demonstrate the presence of 5-HT_{1A} receptors in cortical pyramidal neurones as well as pyramidal and granular neurones of the hippocampus. The 5-HT_{1A} receptor is expressed in 5-HT-containing neurones in the raphé nuclei, cholinergic neurones in the septum and probably glutamatergic (pyramidal) neurones in cortex and hippocampus. The ultrastructural location of the 5-HT_{1A} receptor identifies synaptic membranes, as well as extrasynaptic structures. There are reports of 5-HT_{1A} receptors in brain glial cells, but this has not been confirmed. The receptor can be found in the periphery, especially in the GIT where it modulates Ach release. The distribution of mRNA encoding the 5-HT_{1A} receptor is very similar to that of 5-HT_{1A} binding sites, except for the usual mismatches between receptor proteins found on dendrites and mRNA in cells bodies. The pattern of 5-HT_{1A} receptor distribution is similar across species, although the laminar organization of the 5-HT_{1A} receptor in cortical and hippocampal areas of humans differs somewhat from that in rodents. Fortunately, the use of highly selective 5-HT_{1A} receptor antibodies has allowed to confirm and refine the results of autoradiographic studies.

In the raphé nuclei, 5-HT_{1A} receptors are somatodendritic and act as autoreceptors to inhibit cell firing; postsynaptic 5-HT_{1A} receptors are present in limbic structures, particularly the hippocampus. 5-HT_{1A} receptors mediate neuronal hyperpolarisation,

via G-protein coupled K^+ channels (GIRK channels) and ultimately inhibit neuronal firing, as well as they inhibit 5-HT release. In the gastrointestinal tract, 5-HT_{1A} receptors on the guinea pig myenteric plexus act as inhibitory modulators of fast excitatory postsynaptic potentials. The 5-HT_{1A} receptor mRNA is intronless and produces a single polypeptide chain of 421–422 a.a. that spans the membrane seven times (GPCR), with an extracellular amino terminus and an intracellular carboxyl terminus [4,12–14]. The receptor couples preferentially to $G_{i/o}$ to inhibit adenylate cyclase activity [15,16], although coupling to the IP3/PKC/calcium mobilization pathways has been described in recombinant systems; presumably this is an indirect effect mediated by activation of G_{β}, which will then directly interact with PLC [17]. The 5-HT_{1A} receptor has also been found to stimulate cAMP accumulation via G_{i2} and ACII [18], as also shown in rodent hippocampal membranes [19]. No splice variants are known and the gene is intronless. Two polymorphisms, $Gly^{22} \rightarrow Ser$ and $Ile^{28} \rightarrow Val$, have been found to alter the extracellular amino terminal region of the receptor [20]. The polymorphism $Arg^{219} \rightarrow Leu$ has been associated with Tourette's syndrome [21]. The human polymorphism $Ala^{50} \rightarrow Val$, occurring in transmembrane region 1, results in a loss of response to 5-HT [22]. 5-HT_{1A} receptor KO mice show increased responsiveness to stress [23,24]. They display a range of behaviors indicating elevated levels of anxiety [25,26], tend to avoid novel or fearful environments, and escape from stressful situations [27]. However, these anxiety-related effects seen in the 5-HT_{1A} receptor KO mouse appear to be developmentally related and conditional KO mice with receptor expression restored in the hippocampus and cortex during development, followed by gene inactivation in the adult, behave normally. Conditional expression in the raphé nuclei during development, however, does not rescue the anxiety phenotype of the knockout [28]. 5-HT_{1A} knockout mice also show an attenuated hypothermic response following administration of 5-HT_{1A} receptor agonists [24], an effect believed to be mediated by 5-HT_{1A} receptors in the hypothalamus [29]. It appears that short term memory is not affected by 5-HT_{1A} receptors, whereas 5-HT_{1A} antagonists facilitate long term memory [30,31].

2.2. 5-HT_{1B} receptors

The 5-HT_{1B} receptor and the 5-HT_{1D} receptor have a complex and debated history, due to their close structural features and pharmacological signatures. The non-spiperone sensitive [^{3}H]5-HT binding in brain was defined as 5-HT_{1B}, it was later recognized that [^{125}I]CYP, a ligand that labels also beta adrenoceptors, was able to recognize in brain a population of 5-HT_1 sites with low affinity for spiperone and 8-OH-DPAT, but with a clearcut 5-HT pharmacology. Interestingly, [^{125}I]CYP binding was well defined in rodents, and other species such as fish, opossum, but not guinea pig, pig, or man. Importantly, it was soon realised that some presynaptic receptors inhibiting 5-HT release in rodent brain had a 5-HT_{1B} pharmacology, as well as inhibition of cAMP production in rat substantia nigra. Thus, the 5-HT_{1B} receptor was originally defined according to operational criteria and thought to be a rodent-specific receptor, whereas 5-HT_{1D} was limited to non-rodents, since non-5-HT_{1A} binding was still to be found, but with variations in its pharmacological profile which was divergent from that of rodent 5-HT_{1B} sites. However, similarities in transductional features, function, and brain distribution led to the opinion that the rodent '5-HT_{1B}' and non-rodent '5-HT_{1D}' receptors were species homologues [32]. Again, presynaptic receptors inhibition of 5-HT release in non-rodent brain had a 5-HT_{1D} pharmacology, as well as inhibition of cAMP production in non-rodent substantia nigra. In addition, vascular effects could be attributed to 5-$HT_{1B/1D}$ receptors, depending on the species studied. The existence of two closely related receptors was then confirmed when these were cloned a few years later ([33–36], Hamblin et al. [37,38], Weinshank et al. [39,40]). However, to complicate matters, the pharmacologically defined human 5-HT_{1D} receptor was a composite of two subtypes, encoded by distinct genes, which were called 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (Weinshank et al. [37,38]). This notation reflected the fact that the operational profiles of these two receptors, were almost indistinguishable and except for a few specific compounds, the similarity of these receptors is still very present. Despite of overt differences in their pharmacological profiles, it became clear that 5-HT_{1B} and 5-$HT_{1D\beta}$ receptors are, respectively, rodent and non-rodent species homologues with 97% overall sequence homology. Interestingly, a single amino acid change accounts for these differences. Thus, the pharmacological features of these two homologues are attributed to the mutation of Asp^{123} to Arg^{123} [41]. The eventual identification of the 5-$HT_{1D\alpha}$ gene in rats confirmed that 5-$HT_{1B/1D}$ receptors represent two different classes, and the need to revise the receptor classification became evident. Thus, the 5-$HT_{1D\beta}$ receptor is now known as 5-HT_{1B}; consistent with the fact that it is the human homologue of the original rodent 5-HT_{1B} receptor. It has to be kept in mind that human 5-HT_{1B} receptors will not be labeled with [^{125}I]CYP in contrast to their rodent homologues.

Autoradiographic studies using [^{3}H]5-HT (in the presence of 8-OH-DPAT), [^{125}I]-cyanopindolol (in the presence of isoprenaline), or [^{125}I]GTI (serotonin-5-O-carboxymethyl-glycyl-[^{125}I]tyrosinamide) support the presence of a high density of 5-HT_{1B} sites in the basal ganglia (particularly the substantia nigra, globus pallidus, ventral pallidum, and entopeduncular nucleus), but also many other regions. With appropriate displacing agents, both [^{125}I]-cyanopindolol and [^{125}I]GTI allow discrimination of 5-HT_{1B}-binding sites from 5-HT_{1D}-binding sites in rodents. The discrimination of 5-HT_{1B} and 5-HT_{1D} receptors in both rodent and non-rodent species has been further facilitated with the availability of a new 5-$HT_{1B/D}$ radioligand, [^{3}H]GR-125743 as well as subtypes selective ligands which discriminate 5-HT_{1B} and 5-HT_{1D} receptors. Evidence from radioligand binding experiments using 5-HT neuronal lesions is equivocal regarding the synaptic location of the rat 5-HT_{1B} receptor, with some studies finding that the lesion causes an up-regulation of 5-HT_{1B}-binding sites and others finding a down-regulation in the same areas. In situ hybridization studies have located mRNA encoding the 5-HT_{1B} receptor in the dorsal and median raphé nuclei. Furthermore, 5-HT_{1B} receptor mRNA in the raphé nuclei is markedly reduced following 5-HT neuronal lesion. Thus suggesting that 5-HT_{1B} receptors are located both presynaptically and postsynaptically relative to 5-HT neurones. It is speculated that in some brain areas (including substantia nigra and globus pallidus), 5-HT_{1B}-binding sites may be located on non-5-HT nerve terminals, having been synthesized and then transported from cell bodies in other regions. Overall, the anatomical location of the 5-HT_{1B} receptor provides strong evidence to support the idea that the 5-HT_{1B} receptor has a role as both a 5-HT autoreceptor and 5-HT heteroreceptor, i.e., controlling transmitter release. In non-rodents, they exhibit the 5-HT_{1D} 'pharmacology'. Some forebrain areas with high levels of 5-HT_{1B}-binding sites (e.g., striatum) also express 5-HT_{1B} receptor mRNA, whereas other areas with high levels of 5-HT_{1B}-binding sites have little detectable mRNA (e.g., substantia nigra, globus pallidus, and entopeduncular nucleus). Similar mismatches between brain distribution of 5-HT_{1B} receptor mRNA and binding sites have been found in the primate and human brain. At the cellular level, in situ hybridization studies have localized 5-HT_{1B} receptor mRNA to granule and pyramidal cells within hippocampus, and medium spiny neurons of the caudate putamen, which are probably GABAergic. Immunocytochemical studies are necessary to reveal the synaptic location of the receptors. 5-HT_{1B} receptors are also found on cerebral

arteries and other vascular tissues, but no binding studies have shown this, although 5-HT_{1B} mRNA has been located to some blood vessels. Further, it seems that the receptor may be silent and only become responsive in conditions such as atherosclerosis. Further peripheral effects have been described, such as inhibition of noradrenaline release in vena cava and inhibition of plasma extravasation produced by trigeminal ganglion stimulation in guinea pigs and rats. 5-HT_{1B} receptors mediate contraction of rat caudal arteries.

The human 5-HT_{1B} receptor is located on chromosome 6q13, it is a GPCR with 386–390 a.a., negatively coupled to cAMP production, but which may also stimulate calcium release [42–45]. It is important to remember that, because the human receptor assumes pre-eminence, the operational characteristics of the 5-HT_{1B} class are those defined for the human receptor. There are no reported splice variants. All known antimigraine drugs of the sumatriptan series (triptans) act via the 5-HT_{1B} receptor (see [46,47]; Hou et al., 2000; [48]). SNPs in position 861 have linked the 5-HT_{1B} receptor with various disorders, such as alcoholism, substance abuse, ADHD, aggression and depression [49–53]. 5-HT_{1B} receptor KO mice have a hyperaggressive phenotype [54,55]. The aggressive behaviors in the 5-HT_{1B} KO mouse may actually be due, however, to an increase in impulsive behaviors and defects in impulsivity regulation rather than aggression per se [56,57]. 5-HT_{1B} receptors appear to modulate both short term and long term memory, as shown by facilatatory effects of 5-HT1B antagonist [30,31].

2.3. 5-HT_{1D} receptors

The 5-HT_{1D} receptor may not have been discovered without the power of molecular biology, indeed this receptor is expressed in very low levels, and given the similarities between 5-HT_{1B} and 5-HT_{1D} pharmacology, one can doubt that selective compounds would have been found in the absence of recombinant receptors expressed in high expression cell systems.

The 5-HT_{1D} receptor is located on chromosome 1p34.3-p36.3 and has 63 % overall sequence homology with the 5-HT_{1B} receptor. The receptor has 374–377 a.a. and is negatively coupled to cAMP production like 5-HT_{1B} ([34,37,38], Weinshank et al. [58]). Its expression levels are very low in comparison to those of 5-HT_{1B} receptors and it has been difficult to assign a functional role to 5-HT_{1D} [59–62]. The distinct features of the 5-HT_{1B} and 5-HT_{1D} subtypes are now well established, especially with the availability of new 5-$HT_{1B/1D}$ selective ligands, SB 216641 (h5-HT_{1B}) and BRL 15572 (h5-HT_{1D}), which allowed to document 5-HT_{1D} autoreceptors in the dorsal raphé nuclei [63–66]. 5-HT_{1D} receptors in human heart may modulate 5-HT release. The currently available anti-migraine drugs of the sumatriptan family (triptans) do not distinguish between 5-HT_{1B} and 5-HT_{1D} receptors. However, the selective 5-HT_{1D} receptor agonist, PNU 109291, plays a significant role in the suppression of meningeal neurogenic inflammation and trigeminal nociception in guinea pig, suggesting 5-HT_{1D} receptor to be involved in migraine headaches. These findings are in agreement with the 5-HT_{1D} receptor being detected on trigeminal fibers in the spinal trigeminal tract in the human brainstem as well as in other species. Actually, both 5-HT_{1B} and 5-HT_{1D} receptor immunoreactivity is found in human trigeminal ganglia, where the receptors co-localize with calcitonin gene-related peptide, substance P and nitric oxide synthase. However, selective 5-HT_{1D} agonists are devoid of vascular activity confirming that it is the 5-HT_{1B} receptor that mediates the vasoconstriction produced by sumatriptan and other triptans. PNU-142633, a selective 5-HT_{1D} agonist was not active in acute migraine trial. Altogether, these data would suggest that both the vascular and neuronal component are needed for the triptans to work in migraine, although this debate may last longer than the life of this paper.

In situ hybridization studies have detected 5-HT_{1D} mRNA in various rat brain regions including the caudate putamen, nucleus accumbens, olfactory cortex, dorsal raphé nucleus and locus coeruleus. The mRNA had low abundance in all regions especially in comparison to 5-HT_{1B} receptor mRNA. Interestingly, 5-HT_{1D} mRNA was undetectable in the globus pallidus, ventral pallidum and substantia nigra where 5-HT_{1D} binding sites appear to be present. These data are reminiscent of the findings with the 5-HT_{1B} receptor, and suggest the 5-HT_{1D} receptor to be located predominantly on axon terminals of both 5-HT and non-5-HT neurones. Probably absent in vascular smooth muscle, 5-HT_{1D} receptors are present in autonomic and trigeminal nerve terminals/ganglia. In relation to that low abundance, it has been difficult to determine the precise distribution of 5-HT_{1D} receptors, because protein levels appear to be low and there is a lack of radioligand capable of discriminating this receptor from the 5-HT_{1B} receptor. Receptor autoradiographic studies in rat utilizing [^{125}I]-GTI (in the presence of CP 93129 to mask the rat 5-HT_{1B} binding site) suggest that the 5-HT_{1D} site is present in various regions but especially the basal ganglia (particularly the globus pallidus, substantia nigra and caudate putamen) and also the hippocampus and cortex. The distribution of 5-HT_{1D} receptors in human brain, as defined by the ketanserin-sensitive component of [^{3}H]-sumatriptan binding site, detected their presence in the basal ganglia (globus pallidus and substantia nigra) as well as specific regions of the midbrain (periaqueductal grey) and spinal cord.

The 5-HT_{1D} receptor has 374–377 a.a. No splice variants have been reported. Polymorphism of the 5-HT_{1D} receptor have been linked to anorexia nervosa [67]. 5-HT_{1B} and 5-HT_{1D} receptors have been described to be modulated by 5-HT moduline (a tetrapeptide, LSAL, see [68,69]). 5-HT_{1B} and 5-HT_{1D} receptors may form homo or heterodimers [70,71], which is not entirely surprising given the rather strong co-localization that has been reported at the mRNA levels in various species, although the 5-HT_{1D} is always much lower. 5-HT_{1D} receptor antagonism does not affect long term memory (Meneses, 2007).

2.4. 5-ht_{1E} receptors

The putative 5-ht_{1E} receptor was identified in radioligand binding studies in human frontal cortex [72], but its wider distribution is still to be reported because of the absence of adequate tools; autoradiographic studies have also suggested its presence in the brain, but using rather non-selective tools, thus questioning the qualitative and especially quantitative aspects of such findings. There are indeed no selective 5-ht_{1E} ligands described so far and the apparent absence of the receptor in rodents is another major impediment in the study of the receptor's function. The 5-ht_{1E} receptor is a 365 a.a. protein, negatively linked to adenylate cyclase in recombinant cell systems [73–75], although positive modulation can be observed as well [76]. It is present on human chromosome 6q14-q15 [77]. The receptor is apparently not expressed in rats or mice, since the mRNA codes for a stop codon [75]. 5-ht_{1E} receptor mRNA and recognition sites with the pharmacological characteristics of the receptor have been mapped in monkey and human brain [59].

The 5-ht_{1E} receptor was first detected in radioligand binding studies that found that [^{3}H]5-HT, in the presence of blocking agents for other 5-HT_1 subtypes that were known at that time (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}), demonstrated a biphasic competition curve to 5-CT.The site with high affinity for 5-CT was thought to represent the 5-HT_{1D} receptor. The low affinity site had a novel pharmacology and was suggested to be a novel 5-HT receptor (5-ht_{1E}). Although 5-ht_{1E} binding sites have been described in rodents, the relevance of

these sites remains enigmatic as the receptor has yet to be cloned from rats or mice. A 5-CT-insensitive [^{3}H]5-HT binding site was found in cortex and caudate membranes of human as well as other species, e.g. guinea pig, rabbit, and dog. We now know that other 5-HT receptor subtypes also have high affinity for [^{3}H]5-HT, and are 5-CT insensitive (5-ht$_{1F}$, 5-HT$_6$) (and have probably contributed to the initially described 5-ht$_{1E}$ binding), Currently there are no available selective ligands or radioligands for the 5-ht$_{1E}$ receptor, autoradiographic studies have provided a picture of the distribution of non-5-HT$_{1A/1B/1D/2C}$ [^{3}H]5-HT binding sites e.g. in human brain. These binding sites are present in the cortex (particularly entorhinal cortex), caudate putamen, and claustrum, with detectable levels in other areas, e.g. hippocampus (subiculum) and amygdala. In the human and monkey brain, 5-ht$_{1E}$ mRNA is present in cortical areas (including entorhinal cortex) and the caudate and putamen, with lower levels in amygdala and hypothalamus.

A thorough characterization of the 5-ht$_{1E}$ receptor awaits the development of selective ligands. Because confirmation of a true physiological role for 5-ht$_{1E}$ receptors is still lacking, they retain their lower case appellation. No splice variants are known.

2.5. 5-HT$_{1F}$ receptors

Another example where molecular biology was essential, is the 5-HT$_{1F}$ receptor, since its existence was not suspected before cloning. The 5-HT$_{1F}$ receptor gene was originally found in the mouse on the basis of its sequence homology with the 5-HT$_{1B/1D}$ receptor subtypes; the human gene was identified and reported shortly later [78–81]. Initially, the receptor was designated 5-ht$_{1E\beta}$, due to its pharmacological profile closely related to that of the 5-ht$_{1E}$ receptor (including low affinity for 5-CT). 5-HT$_{1F}$ receptor mRNA distribution was quite different in the brain compared to 5-ht$_{1E}$ receptor mRNA [59]. The 5-HT$_{1F}$ receptor gene is located on chromosome 3p11 and codes for 366 amino acids. It is negatively linked to adenylate cyclase in recombinant cell systems [82], and most closely related to the 5-ht$_{1E}$ receptor with >70% sequence homology (see also [6,83]).

5-HT$_{1F}$ mRNA was first localized in the mouse and guinea pig brain using in situ hybridization. High expression was found in primary olfactory cortex, anterior olfactory nucleus, caudate-putamen, dentate gyrus, layers III-V of frontoparietal and cingulate cortex and claustrum. Significant in situ hybridization was also observed in the central, medial and basomedial amygdaloid nuclei, pyramidal cell layers of CA1, CA2 and CA3, periventricular and supraoptic hypothalamic areas, some thalamic nuclei, ventrolateral geniculate nucleus, entorhinal cortex, principal sensory nucleus of the spinal tract, ventral tegmental nucleus, lateral vestibular nucleus and cochlear nucleus. Autoradiography with [^{3}H]sumatriptan was used in the presence of 5-CT to label 5-HT$_{1F}$ binding sites in the guinea pig and rat brain. The distribution of 5-CT-insensitive [^{3}H]sumatriptan binding sites demonstrates a very good correlation with the distribution of 5-ht$_{1F}$ mRNA (in the guinea pig) with the highest levels of binding in cortical and hippocampal areas, claustrum, and the caudate nucleus. The brain distribution of 5-HT$_{1F}$ sites labeled by the selective 5-HT$_{1F}$ radioligand, [^{3}H]LY334370, was recently reported [84]. In rat brain, specific 5-HT$_{1F}$ binding was found in layers 4–5 of cortical regions, olfactory bulb and tubercle, nucleus accumbens, caudate putamen, parafascicular nucleus of the thalamus, medial mammillary nucleus, the CA3 region of the hippocampus, subiculum, and amygdaloid nuclei. Interestingly, rat brain autoradiography with [^{3}H]LY334370 and [^{3}H]sumatriptan showed labeling in the same brain regions when performed side by side. Some species differences in the distribution of the 5-HT$_{1F}$ receptor were noted between rat, guinea-pig, monkey and human brain. 5-HT$_{1F}$ receptors have not been found in blood vessels.

In addition to sumatritptan, naratriptan also has affinity for 5-HT$_{1F}$ receptors and it has been hypothesized that they might be a target for anti-migraine drugs, especially since 5-HT$_{1F}$ receptor mRNA has been detected in the trigeminal ganglia, stimulation of which leads to plasma extravasation in the dura, a component of neurogenic inflammation thought to be a possible cause of migraine [85,86]. 5-HT$_{1F}$ receptor stimulation would be devoid of vasomotor effects which would represent a major advantage with respect to the triptans [87]. LY 334370, a selective 5-HT$_{1F}$ receptor agonist, inhibits trigeminal stimulation-induced early activated gene expression in nociceptive neurones in the rat brainstem [85,88]. It seems that 5-HT$_{1B}$ and 5-HT$_{1F}$ receptors can co-express in neuronal and vascular tissues, and it appears that it is the 5-HT$_{1F}$ receptor which plays a major role in neurogenic inflammation in the guinea-pig, an animal model for migraine. However, clinical data are still missing to allocate the various roles of these receptors conclusively, although positive trials have been reported with LY 334370, but the further development was stopped. There is no report on 5-HT$_{1F}$ receptor KO mice.

3. 5-HT$_2$ receptors, a family of receptors coupled to Gq/$_{11}$

There are three types of 5-HT$_2$ receptors. 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors Exhibit 46–50% overall sequence identity and couple preferentially to Gq/$_{11}$ to increase inositol phosphates and cytosolic [Ca^{2+}] and in agreement with their long known role in muscle contraction and stimulation in the brain. 5-HT$_2$ receptors may also couple to G$_{12/13}$ which are known to mediate long term structural changes in cells. The 5-HT$_{2A}$ receptor refers to the classical D receptor initially described by Gaddum and Picarelli [89], and later defined as the 5-HT$_2$ receptor by Peroutka and Snyder [90]. 5-HT$_{2B}$ receptors were characterized almost 30 years ago to mediate the contractile action of 5-HT in the rat fundus, (but the 5-HT$_{2B}$ receptor had escaped any meaningful classification, our own group had tried to establish that the fundus receptor was of the 5-HT$_{2C}$ type, but the idea was abandoned). When cloned they were initially named 5-HT$_{2F}$ (for fundus). In human pulmonary artery endothelial cells, 5-HT$_{2B}$ receptor stimulation causes intracellular Ca^{2+} release and potent contraction. The third 5-HT$_2$ receptor subtype corresponds to the previously known 5-HT$_{1C}$ receptor, it was initially identified in the choroid plexus, where both [^{3}H]5-HT and [^{3}H]mesulergine showed high affinity binding, with superimposable profile (thus the initial 5-HT$_1$ appelation).

3.1. 5-HT$_{2A}$ receptors

The 5-HT$_{2A}$ receptor was not the first of the family to be cloned, it was 5-HT$_{2C}$. The 5-HT$_{2A}$ receptor gene is located on human chromosome 13q14-q21, and codes for 471 a.a. in rat, mouse, and humans ([91–94]; Fogu et al., 1992; [16,95,96]). The 5-HT$_{2A}$ gene, as that of the other members of the 5-HT$_2$ receptor family, has a complex genomic organization; the first receptor of the family to be cloned was the 5-HT$_{2C}$ receptor, and given the pharmacological and transductional homologies in the 5-HT$_2$ family, it was suggested that the receptors should have significant sequence similarities (Hoyer, 1998). Thus, it did not take long to identify the 5-HT$_{2A}$ receptor by homology screening [91,92].

We have reported 5-HT$_{2A}$ receptor binding using [^{3}H]ketanserin and other radioligands, and mRNA distribution in rat and human brain and other species: both signals are densely represented across the cerebral cortex, especially in laminae I and IV–Va, the piriform and entorhinal cortex. They are also concentrated in the claus-

trum, endopiriform nucleus and olfactory bulb/anterior olfactory nucleus. A number of brainstem nuclei show high signals: pontine, motor trigeminal, facial and hypoglossal nuclei. Intermediate levels were observed in the limbic system and in the basal ganglia e.g. caudate nucleus and accumbens. There were no transcripts in the cerebellum and thalamic nuclei, whereas hippocampal expression was low. Of note, in the human brain the distribution of mRNA is similar to rat, although mRNA was apparently absent from the striatum. Similar findings were made in monkey, where 5-HT_{2A} binding appears to be absent from the caudate putamen. However, the binding of [^{3}H]ketanserin in the caudate and putamen nuclei is not to 5-HT_{2A} sites, but to "so-called" tetrabenazine binding sites. Overall, there is a good agreement between in situ hybridization and binding data. Further, this distribution is compatible with immunocytochemical data using specific 5-HT_{2A} receptor antibodies. Thus, 5-HT_{2A} receptors are expressed in pyramidal cells and interneurones in the neo-cortex. A number of other 5-HT_{2A}-selective radioligands have been used in vitro and in vivo, one of the most promising being the PET ligand [^{11}C]-MDL 100907. 5-HT_{2A} receptors are obviously present in the periphery, primarily in blood vessels but also in the GIT.

5-HT_{2A} receptors mediate contractile responses in many vascular smooth muscle preparations, e.g., bronchial, uterine and urinary smooth muscle, and part of the contractile effects of 5-HT in the guinea-pig ileum. In addition, platelet aggregation and increased capillary permeability after exposure to 5-HT have been attributed to 5-HT_{2A} receptor-mediated functions. Prominent and problematic effects of 5-HT_{2A} receptor activation in the brain by LSD and 5-HT_{2A} agonists such as DOI, psylocibin and other psychedelics, are hallucinations and eventually psychosis. 5-HT_{2A} receptors, like the other 5-HT_2 receptors, couple preferentially via $G_{q/11}$ to the IP3/PKC/calcium pathway, although inhibition of cAMP production has been noticed. Non-functional splice variants have been reported, polymorphism in the promoter region has been associated with responses to clozapine in schizophrenics [97]. The 5-HT_{2A} receptor KO mouse shows behavioral sensitization to amphetamine [98], as well as paradoxical changes in sleep pattern opposite to what is seen with 5-HT_{2A} antagonists in WT mice [99]. It seems that 5-HT2A antagonism does not impact long term memory, however, the 5-HT2 receptor agonist DOI has negative effects on both short and long term memory [30,31].

3.2. 5-HT_{2B} receptors

The 5-HT_{2B} receptor has been known for many years, but its assignment to one class of 5-HT receptors or another was difficult; due to its high sensitivity 5-HT in the fundic smooth muscle contraction, it was suggested to be a 5-HT_1 like receptor, then to be similar to 5-HT_{2C} receptor, but this did not hold the test of time either. Due to operational features similar to those 5-HT_{2A} and 5-HT_{2C} receptor one was left with this option, but the lack of selective compounds and groups working one it left it as an orphan [92,100]. Eventually, the cloning of the rat, mouse and human 'fundic' receptors (also reported as 5-HT_{2F}) clarified the issue [101–109]. It is located on human chromosome 2q36.3-2q37.1. The receptor has 479–504 a.a. and no splice variants are known.

5-HT_{2B} receptor mRNA is found in rat fundus, gut, heart, kidney, lung and brain although the presence of the 5-HT_{2B} receptor in the brain (especially that of the rat) has been controversial. It appears that 5-HT_{2B} receptor mRNA and its protein product are very limited in quantities, (relative to 5-HT_{2A} and 5-HT_{2C}) but functionally important. The presence of 5-HT_{2B} receptor-like immunoreactivity has been reported in rat brain [110], restricted to a few brain regions, particularly cerebellum, lateral septum, dorsal hypothalamus and medial amygdala. The cells expressing 5-HT_{2B} receptor-like immunoreactivity have a neuronal and not astrocytic morphology.

Application of BW 723C86 into the medial amygdala produces anxiolytic effects in rats [111]. 5-HT_{2B} receptor activation has been implicated in mediating hyperphagia. 5-HT_{2B} receptor mRNA, in contrast to 5-HT_{2C}, is found in a number of blood vessels. 5-HT_{2B} receptors on endothelial cells of pig pulmonary arteries and in rat jugular vein mediate vasorelaxation via NO release [112]. 5-HT_{2B} receptors contract longitudinal muscle in human small intestine and when expressed in mouse fibroblast cells, cause mitogenesis linked to tumour transforming activity, via MAP kinase. Activation of the 5-HT_{2B} receptor, is most probably responsible for the valvulopathies reported for appetite suppressant preparations containing dex-fenfluramine [113], and receptor overexpression leads to ventricular hypertrophy by a mitochondrial mechanism [114]. The 5-HT_{2B} receptor appears also to play a role in pulmonary hypertension [115] with overexpression of these receptors in pulmonarty arteries. The 5-HT_{2B} receptor KO in mice is lethal and produce severe embryonic defects, whereas overexpression of 5-HT_{2B} receptor in mice has dramatic effects on the cardiovascular system (see [114,116,117]).

3.3. 5-HT_{2C} receptors

The 5-HT_{2C} receptor was one of the first 5-HT receptors to be cloned, although full-length sequences were difficult to obtain, due to a highly complex exon–intron structure ([101,102,118,119]; Stam et al., 1994; [120]). The receptor gene was mapped to human chromosome Xq24 and has 458–460 a.a. Given its similar pharmacological and transductional features with the 5-HT_{2A} receptor [121], the sequence of the latter was established based on homology cloning [91].

The initial perception that the 5-HT_{2C} receptor should be essentially the 'choroid plexus' receptor comes from radioligand binding studies which left this rather false impression [122], due 1) to the absence of really 5-HT_{2C} selective ligand and 2) to the very high concentration of binding sites in the plexus. Depending on the species though, it was clear that 5-HT_{2C} binding in brain was more widespread. In situ hybridization has corrected this unfortunate misrepresentation and confirmed that indeed 5-HT_{2C} receptors are not limited to the choroid plexus. 5-HT_{2C} binding and mRNA expression in the rodent brain identify the choroid plexus and limbic structures: hippocampus (CA3), amygdala, anterior olfactory and endopiriform nuclei, cingulate and piriform cortex. In addition, 5-HT_{2C} receptor mRNA is found in some thalamic nuclei, lateral habenula basal ganglia and particularly subthalamic nucleus and substantia nigra. Binding is in general comparable with mRNA, with a few exceptions e.g. subthalamic nucleus. Low levels of binding and in situ signals are present in medulla and brainstem. These results are further supported by the use of anti-5-HT_{2C} receptor peptide antibodies which allowed to confirm strong labeling in rat and human substantia nigra.

Although it has been demonstrated that 5-HT_{2C} receptors in the choroid plexus couple to PLC activity [123,124], additional functional correlates remain to be established [125]. The 5-HT_{2C} receptor is one of the very few GPCRs whose mRNA is undergoing editing [126–128], in addition to be spliced. Thus, there are four editing sites for adenine deaminases in rodents and 5 in humans. As a result, 32 different mRNAs and 24 different proteins of the 5-HT_{2C} receptor could result from mRNA editing. The 5-HT_{2C} receptor consists of a single polypeptide chain of 458-460 a.a. There are also two non-functional short splice variants [129] in addition to the multiple RNA editing variants. The different editing variants are characterized by decreasing degrees of affinity and intrinsic activity for 5-HT and other agonists, as well as reduced constitutive activ-

ity and altered coupling to G proteins as the editing becomes more complete [128,130–134]. A potential link between a 5-HT_{2C} receptor allele and vulnerability to affective disorders has been reported [135], and a number of antipsychotics have inverse agonist activity at 5-HT_{2C} receptors [130,134]; however, similar observations can be made for 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptors. A -759T allele within the promoter region of the 5-HT_{2C} gene has a higher transcriptional activity than the more common -759C allele, which may lead to higher basal expression of 5-HT_{2C} receptors and subsequent protection against antipsychotic-induced weight gain [136]. The 5-HT_{2C} receptor KO mouse is severely obese and shows defective in food intake regulation (see [137–139]) and can experience fatal seizures. 5-HT_{2C} antagonism does not affect short term memory, but facilates long term memory [30,31].

4. 5-HT_3 receptors, a ligand-gated ion channel receptor of the cys-loop channel family

5-HT_3 receptors equate with the M receptor of Gaddum and Picarelli [89]; they belong to the ligand-gated ion channel receptor superfamily, similarly to the nicotinic acetylcholine, glycine or GABA-A receptors and share electrophysiological and structural patterns with the Cys-loop transmitter-gated superfamily of ligand-gated ion channels [140]. There are antagonist ligands that share affinity at 5-HT_3 and nicotine receptors, such as tropisetron (ICS-205930). 5-HT_3 receptors are located on central and peripheral neurons, where they trigger rapid depolarization due to the opening of non-selective cation channels (Na^+, Ca^{++} influx, K^+ efflux) ([5,6]; Martin et al., 2000). The response desensitises and resensitises rapidly. The native 5-HT_3 receptor, as revealed by electron microscopy in neuroblastoma-glioma cells, is a pentamer with the 5 subunits surrounding a central channel [141]. To date, two genes have been recognized to encode 5-HT_3 receptors: the 5-HT_{3A} receptor subunit [142] has 487 amino acids and displays a high level of identity with other members of the Cys-Cys loop ligand-gated ion channel superfamily (e.g. nicotinic, $GABA_A$, and glycine receptors). A second subunit was isolated from various species, 5-$HT_{3B,}$ which codes for 441 amino acids in humans [143]: 5-HT_{3B} as a homopentamer is not active. 5-$HT_{3C,\ D,\ E}$ have been reported as sequences, but until recently no report confirmed any 5-HT_3 direct role of these subunits, although a chaperone function may well exit ([144], see below).

4.1. 5-HT_{3A} and 5-HT_{3B} receptors

A cDNA encoding a single subunit of the 5-HT_{3A} receptor was isolated from a neuroblastoma cell line, where the receptor had been identified previously both in electrophysiological and radioligand binding studies [142,145]. The human homologue maps to chromosome 11q23.1-q23.2. Two splice variants have been found in neuroblastoma-glioma cells (NCB-20, NG 108-15) and rat native tissue, with a similar distribution, pharmacological profile and electrophysiological characteristics when expressed as homomers.

Radioligand autoradiography has mapped the distribution of 5-HT_3 receptors in the central nervous system, with highest levels within the dorsal vagal complex (nucleus tractus solitarius, area postrema, and dorsal motor nucleus of the vagus nerve) involved in the initiation and coordination of the vomiting reflex; antagonism of 5-HT_3 receptors in these nuclei is likely to contribute to the antiemetic action of 5-HT_3 receptor antagonists. Relative to the dorsal vagal complex, 5-HT_3 receptor expression in the forebrain is low. Highest levels are expressed in regions such as the hippocampus, amygdala, and superficial layers of the cerebral cortex. The relative distribution of 5-HT_3 receptor recognition sites, within the forebrain, display species variations. In the human forebrain, relatively high levels of 5-HT_3 receptor recognition sites have been located within the caudate nucleus and putamen whereas relatively low levels are detected within cortical regions, in contrast to rodents. Most species express significant levels of 5-HT_3 receptors within the hippocampus relative to other forebrain regions. In situ hybridization studies indicate that 5-HT_{3A} receptor transcripts are similarly distributed, in the rodent brain, to radiolabeled 5-HT_3 receptor binding sites (e.g. piriform cortex, entorhinal cortex, hippocampus). Within the hippocampal formation, mRNA is detected primarily within interneurons; this distribution indicating that the 5-HT_3 receptor may mediate the indirect inhibition of excitatory pyramidal neurones via activation of GABAergic interneurons. 5-HT_3 receptor-like immunoreactivity is primarily associated with GABA containing neurones in the cerebral cortex and hippocampus that often co-localize with CCK (but not somatostatin) [146]. 5-HT_3 receptor binding has also been localized in dorsal root ganglia (DRG), trigeminal ganglia (TG) and peripheral nerves (including the vagus), in a number of species. In addition, the presence of 5-HT_3 receptors in the PNS and GIT are well documented functionally. There has been questions about the presence of 5-HT_{3B} subunit in the brain, but recent immunocytochemistry studies [147], have convincingly documented the presence and at times co-distribution of 5-HT_{3A} and 5-HT_{3B} subunits in brain, as well as in the DRG and TG.

In the periphery, 5-HT_3 receptors are located on pre- and post-ganglionic autonomic neurons and on neurons of the sensory nervous system [83,146,148]. 5-HT_3 receptor activation has pronounced effects on the cardiovascular system and regulates both motility and intestinal secretion throughout the entire gastrointestinal tract. Species differences rather then the existence of multiple receptor subtypes, provide the basis of the pharmacological heterogeneity reported in the GI tract. After extensive investigations, a second subunit, 5-HT_{3B}, has been cloned, and it is the heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits that apparently accounts for the full functional features of the native 5-HT_3 receptor ([149], Dubin et al. [150]): the 5-HT_{3A} subunit alone (in most cases) results in receptors with very low conductance and response amplitude ([151,152]; Peters et al., 2004). On the other hand, there is consensus that 5-HT_{3B} homomers have no functional channel activity [153]. However, it is not established that 5-HT_{3A} and 5-HT_{3B} are systematically co-expressed or always form heteropentamers in situ [154–161].

4.2. Other potential 5-HT_3 receptor subunits

The 5-HT_3 receptor, like other members of the ligand-gated ion channel receptor superfamily, possesses additional pharmacologically distinct recognition sites, subject to allosteric modulation. The patent literature reported the cloning of additional potential subunits, 5-HT_{3C},5-HT_{3D}, 5-HT_{3E}, and 5-HT_{3Ea} but no supportive information pertaining to their specific features as receptor or accessory proteins had been available for a number of years ([150,162]; Karnowski et al. [215]). Very recently, Niesler et al. [144] re-investigated whether these novel subunit candidates are able to form functional 5-HT_3 receptor complexes. Using immunofluorescence and immunoprecipitation, it was shown that each of 5-HT_{3C}, 5-HT_{3D}, 5-HT_{3E}, and 5-HT_{3Ea} coassembles with 5-HT_{3A}. Radioligand binding and calcium-influx studies in HEK 293 cells showed that 5-HT_{3C},5-HT_{3D}, 5-HT_{3E}, and 5-HT_{3Ea} subunits expressed alone are non-functional. Coexpression with 5-HT_{3A}, however, results in the formation of functional heteromeric complexes showing different 5-HT relative efficacies. 5-HT showed increased efficacy with at 5-$HT_{3A/3D}$ and 5-$HT_{3A/3E}$ heteromers, which is consistent with the

increased surface expression compared with 5-HT_{3A} receptors. In contrast, 5-$HT_{3A/3C}$ and 5-$HT_{3A/3Ea}$ heteromers exhibited decreased 5-HT efficacy. Thus, the novel 5-HT_3 subunits are able to form heteromeric 5-HT_3 receptors, which exhibit quantitatively different functional properties compared with homomeric 5-HT_{3A} receptors. On the other hand, apparent affinities for agonists and antagonists remained unaffected by the subunit composition. Further, work is required to confirm a contribution of these subunits to differences in pharmacology of 5-HT3 receptors as has been reported in e.g. the GIT (see [140]).

5. 5-HT receptors that preferentially couple to Gs

5-HT_4, 5-HT_6 and 5-HT_7 receptors all couple preferentially to G_s and promote cAMP formation, by activation of various adenylate cyclases. In turn, cAMP as an intracellular messenger interacts with various targets, the phosphorylating enzyme protein kinase A (PKA), but also cyclic nucleotide-gated ion channels, leading to the modulation of calcium ion flux and membrane excitability, other cellular processes. PKA phosphorylates cAMP-responsive transcription factors, such as the cAMP response element binding protein (CREB), which leads to changes in gene expression, and thus may promote long term changes in cellular responses. Further, cAMP seems to interact with a family of cAMP sensors called Epac (exchange proteins directly activated by cAMP). Epacs mediate PKA-independent signal transduction, e.g. activation of Rap and Ras GTPases, and possibly other important cellular proteins. 5-HT_4, 5-HT_6 and 5-HT_7 receptors are considered as distinct receptor classes because of their limited (<35%) overall sequence identities, which are much lower as those featured by either 5-HT_1 or 5-HT_2 receptors. This subdivision is arbitrary and may be subject to future modification. On the other hand, 5-ht_5 receptors form a class a part, as neither G-protein-coupling nor function are clearly defined.

5.1. 5-HT_4 receptors

The 5-HT_4 receptor was pharmacologically defined in cultured mouse colliculi neurones and guinea pig brain by Bockaert and coworkers using stimulation of adenylate cyclase activity with the help of a number of benzamides [163,164] and the 5-HT_3 antagonist ICS 205-930 (tropisetron), which lead to the initial suggestion that 5-HT_4 may be somewhat related to 5-HT_3 receptors, which obviously cannot be since there is no structural or functional relationship between GPCRs and ligand-gated channel receptors. 5-HT_4 receptor were also identified in the GI tract [165] where benzamides were known to produce functional effects, as well as in the heart [166], once it became evident that tropisetron can be used as a tool to block both 5-HT_3 and 5-HT_4 receptors. The ability of brain 5-HT receptors to stimulate adenylate cyclase had been reported for a number of years previous to the pharmacological definition of the 5-HT_4 receptor; thus, Fillion and coll. had reported 5-HT stimulated cAMP production in the brain in the late 1970s (1978, 1979): the nature of that receptor is unclear, as the relatively high concentration of methysergide (and some other compounds inactive at 5-HT_4 receptors) needed to antagonise these responses may rule out 5-HT_4 receptors, suggesting a possible involvement of 5-HT_6 and/or 5-HT_7 receptors.

The 5-HT_4 receptor had been well described in both central and peripheral tissues long before cloning. Confusion between 5-HT_M, 5-HT_3 and 5-HT_4 receptors occurred in earlier days (see [140]), especially in tissues expressing a combination of these receptors such as the guinea pig ileum. Cardiovascular receptors were also described as atypical or 5-HT_4 in the late 1980s e.g. by Pramod Saxena and colleagues.

Several useful radioligands were used to map and characterize the 5-HT_4 receptor (e.g. [^{3}H]GR113808, [^{3}H]RS 57639, [^{125}I]SB207710, [^{3}H]BIMU1). For instance using [^{125}I] SB 207710, rat and human brain showed a similar distribution: olfactory tubercle, islands of Calleja, substantia nigra, ventral pallidum, striatum, septum, hippocampus and amygdala. Thus, a consistent finding across the species investigated is the presence of relatively high levels of 5-HT_4 receptors in the nigrostriatal and mesolimbic systems. Brain 5-HT_4 receptors although widespread, belong to 2 main systems: the septo-hippocampo-habenulo-peduncular pathway and the striato-nigro-tectal pathway suggesting two types of functions: limbic and visuo-motor. There is evidence that 5-HT_4 receptors and cholinergic terminals co-localize, in line with electrophysiological findings made in hippocampus. Obviously 5-HT_4 receptor-mediated responses have been functionally documented throughout the GIT, as well as in the heart. Although the protein has not always been validated due to technical issues, its presence is clear as can been shown in Northern blots in most of these tissues. The brain distribution is in line with promnesic and cholinergic effects that have been reported with various 5-HT_4 receptor agonists (see [30,31,167]) which have also been shown to specifically affect LTP and LTD in the hippocampus [168].

The 5-HT_4 receptor was cloned ([169,209]), confirmed to be a GPCR and mapped to chromosome 5q31-33, initially with 2 splice variants. However, the 5-HT_4 receptor gene is highly complex: it has a multitude of exons and possible splices variants, in most species including man [170]. At least nine 5-HT_4 receptor splice variants have been reported so far (5-HT_{4a}–5-HT_{4n}), with some minor differences across species. Thus, the h5-HT_{4d} receptor isoform has not yet been described in other species: it is limited to the gut, whereas the other isoforms are more widely expressed.

So far, all 5-HT_4 receptor variants couple positively to adenylate cyclase and have almost overlapping pharmacological profiles (e.g. [171]), although differences in intrinsic activity of benzamides is a recurrent feature of native receptors along the GI tract. A possible important feature of the receptor is its level of constitutive activity, that can be observed at low receptor levels, which may well explain the variable intrinsic activity of a number of 5-HT_4 ligands seen in the GI system, depending on tissue and/or species. The pattern of expression of the human 5-HT_4 receptor isoforms is tissue specific. In addition to adenylate cyclase stimulation, direct coupling to potassium channels and voltage-sensitive calcium channels have been proposed.

The complex 5-HT_4 receptor gene (700Kb, 38 exons) generates a number of carboxy-terminal variants. Gerald and colleagues [169,172] were first to report the cloning of the 5-HT_4 receptor. Two isoforms identical between residues 1 and 359 and differing only in the C-terminus, generated by alternative splicing, were described and named 5-HT_{4L} and 5-HT_{4S} for the long and short isoforms. A third shorter isoform was then reported in rat (Claeysen et al., [212]) and the nomenclature for these receptors became r5-$HT_{4(b)}$, r5-$HT_{4(a)}$, and r5-$HT_{4(e)}$. In mouse, four isoforms were rapidly found: 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(e)}$, and 5-$HT_{4(f)}$ [173,174]. In 1997, several groups (Blondel et al., Clayesen et al., and Van den Wyngaert et al.) described the first human 5-HT_4 receptor isoform. Blondel et al. isolated the receptor from human atrium and named it h5-$HT_{4(a)}$, in reference to the short isoform described in rat. Several other splice variants were then cloned: h5-$HT_{4(b)}$, two different h5-$HT_{4(c)}$ isoforms, h5-$HT_{4(d)}$, h5-$HT_{4(e)}$ [175,176,176], but this isoform was to be renamed h5-$HT_{4(g)}$ [177,178], since another h5-$HT_{4(e)}$ isoform closely related to the mouse 5-$HT_{4(e)}$ receptor was found (Claeysen et al., [213]), h5-$HT_{4(f)}$, [177] and h5-$HT_{4(n)}$ [179]. Whether in mice, rats or humans, these splice variants (except 5-HT_{hb}) differ at their carboxyl termini after Leu 358, and the length and the composition of the rest of the C-terminal tail is specific for each

variant. In humans, the C-terminal end is very short for the 5-$HT_{4(d)}$ and 5-$HT_{4(n)}$ isoforms (respectively, only 2 and 1 amino acids after Leu 358) and the 5-$HT_{4(d)}$ isoform (only 2 after L358) and is 31 amino acids long for the 5-$HT_{4(b)}$ variant. The h5-$HT_{4(hb)}$ variant features a 14 residue insertion in the extracellular loop 2; 5-$HT_{4(i)}$ was also cloned from atrium [180]. Like 5-$HT_{4(hb)}$ with insert of exon h in the 5-$HT_{4(b)}$ mRNA [177], the 5-$HT_{4(i)}$ receptor mRNA has an additional exon (exon i) spliced in the 5-$HT_{4(b)}$ mRNA between the common sequence and exon b, starting at base 76574. The 5-$HT_{4(i)}$ receptor mRNA contains an open reading frame of 1287 bp encoding a protein of 428 amino acids. Even though the complete b-tail is present, this splice was named 5-$HT_{4(i)}$, instead of e.g. 5-$HT_{4(ib)}$ since the exon i is inserted in the C-terminal tail and to avoid several confusing double labels. The 5-$HT_{4(c)}$ isoform might be the target of protein kinases because several phosphorylation sites are present on its C-terminus tail. The tissue distribution revealed some degree of specificity. For instance, 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(c)}$, and 5-$HT_{4(g)}$ receptors are all expressed in the heart (atrium), brain, and intestine. The 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptor subtypes are the only receptors present in the bladder and kidney, respectively [175]. The 5-$HT_{4(d)}$ isoform is apparently only present in the intestine. The different splice variants when recombinantly expressed have a classical profile of the 5-HT_4 receptors, as known from the native receptors found in heart, brain or GI tract, and all seem to stimulate adenylyl cyclase activity. No major difference in affinity for agonists or antagonists have been reported so far. On the other hand, differences in apparent intrinsic activity of the agonists ML 10302 and renzapride became apparent. ML 10302, known as a full agonist in the GI tract in rat, is a weak partial agonist of each human isoform; renzapride is a full agonist on the 5-$HT_{4(b)}$ and 5-$HT_{4(d)}$ isoforms and a partial agonist on the 5-$HT_{4(a)}$ and 5-$HT_{4(g)}$ receptors [176,181]. These differences may explain the tissue-dependent efficacy reported for benzamides, which act as full or superagonists (Dumuis et al., 1988) in the mouse colliculus and as partial agonists [182] in other systems. On the other hand, our own work carried out with a variety of human splice variants has not revealed significant differences either in the pharmacological profile or the rank order of relative efficacy with a rather extensive range of ligands. Thus, the differences in apparent efficacy reported for various drugs along the GIT still remains to be explained. The 5-HT_4 receptor KO mouse, is behaviorally normal in a standard environment, but displays very low locomotor activity accompanied by hypophagia in response to novelty and stress. Furthermore, the 5-HT_4 KO mouse has increased sensitivity to the pro-convulsant pentylenetetrazole, which may be related to 5-HT_4 receptor expression on GABAergic neurons [183].

5.2. 5-ht_5 receptors

The function of 5-ht_5 receptors is not established, neither is their preferential coupling which may be to $G_{i/o}$ or possibly Gs. This is why they retain their lower case appellation. Two subtypes of putative 5-ht_5 receptor (5-ht_{5A} and 5-ht_{5B}), sharing 70% overall sequence identity, have been cloned in rodents. The 5-ht_{5A} receptor has 357 a.a. across species and is located on human chromosome 7q36.1 [184–187]. The transduction pathways have not been well established, and there is little evidence that endogenous 5-ht_5 receptors have ever been found in native cells or tissues. For these reasons, the putative receptors retain their lower case appellations. Human recombinant 5-ht_{5A} receptors inhibit forskolin-stimulated cAMP production, although the receptor may also couple positively to cAMP, Inositol phosphate production and to GiRK channels, but a physiological readout for this receptor is still missing 14 years after its cloning. The 5-ht_{5B} receptor gene has been mapped to human chromosome 2q11-q13; however it has been shown that the gene fails to encode a functional protein, due to the presence of stop codons in its coding sequence [185,187].

There have been no published reports concerning a physiological response, and specific binding to a 5-ht_5 recognition site has not been described. In situ hybridization studies suggest a widespread distribution of 5-ht_{5A} receptor mRNA in both mouse and rat brain. Within mouse brain, 5-ht_{5A} receptor transcripts were associated with neurones within the cerebral cortex, the dentate gyrus and the pyramidal cell layer within hippocampal fields CA1-3, the granule cell layer of the cerebellum and tufted cells of the olfactory bulb. Human brain 5-HT_{5A} mRNA expression is localized to the cerebral cortex, hippocampus, and cerebellum. In the neocortex, 5-ht_{5A} receptor mRNA was primarily distributed in layers II, III, V, and VI. The dentate gyrus and the pyramidal cell layer of the CA1 and CA3 hippocampal fields also express high levels of 5-ht_{5A} mRNA. 5-ht_{5A} mRNA also was detected in the cerebellum, with high expression in Purkinje cells, in the dentate nucleus and, at lower density, in the granule cells [188].

The 5-ht_{5A} receptor KO mouse shows increased exploratory activity in a novel environment, and is less reactive to LSD, which has high affinity to the receptor. A putative role for 5-ht_{5A} receptors in the acquisition of adapted behaviour under stressful situations has been postulated (see [211]), and indeed A-843277, a selective 5-ht_{5A} antagonist is reported to have antidepressant/antipsychotic properties in rodent models, in line with the distribution of the receptor in higher cortical and limbic regions (Garcia-Ladona et al., unpublished).

5.3. 5-HT_6 receptors

The 5-HT_6 receptor was suspected to exist in the brain for some time since a 5-HT-stimulated adenylate cyclase activity was detected in the striatum which did not fit with any of the know classes of 5-HT receptors; it was similar in profile to cAMP production in some glioblastoma cells which was sensitive to antipsychotics. The cloned receptor has 436–440 amino acids and is indeed positively coupled to adenylyl cyclase via G_s. The human gene has 89% sequence homology with the rat orthologue, and maps chromosome region 1p35-p36 [189].

Rat and human 5-HT_6 receptor mRNA is located in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, with a primarily post-synaptic localization, whereas it has not been found in peripheral tissues. The 5-HT_6 receptor can be labeled with [^{125}I]SB 258585 [190]. In adult animals, immunohistochemical studies have demonstrated highest receptor expression in the striatum, nucleus accumbens, olfactory tubercle, and cortex, with moderate expression in the amygdala, hypothalamus, thalamus, cerebellum, and hippocampus. [^{125}I]SB-258585 [191] detects in rat brain high levels of 5-HT_6 receptor in the corpus striatum, nucleus accumbens, Islands of Calleja, olfactory tubercle, and the choroid plexus. Moderate levels are found in the hippocampal formation and cerebral cortex, thalamus, hypothalamus, and substantia nigra, and very low levels in the globus pallidus, cerebellum, other mesencephalic regions, and the rhombencephalon. There appears to be negligible expression outside the CNS. Electron microscopic analysis of 5-HT_6 immunohistochemistry revealed receptor staining primarily on dendritic and cilia processes, with little expression on cell bodies.

A truncated, non-functional 5-HT_6 receptor with a 289 bp deletion of the region coding for transmembrane IV and third intracellular loop has been identified in the caudate and substantia nigra of the human brain. NCB 20 and N18TG2 cells and rat striatal neuronal cultures express a receptor which couples positively to adenylyl cyclase and displays an operational profile consistent with the recombinant 5-HT_6 receptor (see [192]). The 5-HT_6 recep-

tor was identified in pig caudate nucleus where cAMP accumulation has a 5-HT_6 receptor profile and was antagonised by clozapine and methiothepin. [^{3}H]clozapine binds with nM affinity to two distinct sites in rat brain; one site displays the operational 5-HT_6 receptor profile. Coupling to calcium can also be seen in recombinant systems.

The selective 5-HT_6 receptor antagonists, increase cholinergic neurotransmission (although the receptors are not located on cholinergic cells) and have positive effects on learning and memory (see e.g. [193]). A role for the 5-HT_6 receptor in the control of central cholinergic function, and thus a putative target for cognitive dysfunction such as Alzheimer's disease is thus suggested. On the other hand, antipsychotics (clozapine, olanzapine, fluperlapine and seroquel) and antidepressants (clomipramine, amitryptyline, doxepin and nortryptyline) act as 5-HT_6 receptor antagonists, but clearly with no selectivity. This attribute tempted speculation of an involvement of the 5-HT_6 receptor in psychiatric disorders. There is also evidence that 5-HT_6 receptor activation modulate glutamate, GABA, dopamine and noradrenaline release, and it is hoped that 5-HT_6 selective ligands will have application in Alzheimer's disease and schizophrenia, possibly depression (although it remains to be seen whether agonists or antagonists are needed) and obesity. Highly selective agonists are however still lacking.

5.4. 5-HT_7 receptors

The 5-HT_7 receptor has an interesting story: it has been known for quite some time that a vascular 5-HT receptor producing primarily relaxation was responding to 5-HT and 5-CT, but it did not fit with the known 5-HT_1 or 5-HT_2 receptor types, neither did it with any of the subsequently cloned receptors; for a number of years it was known as 5-HT_1–like and remained rather elusive, since no specific pharmacological tools were available. Eventually, the 5-HT_7 receptor was cloned from rat, mouse, guinea pig and human cDNA. It is located on human chromosome 10q21-q24. One of the interesting strategies consisted of homology cloning starting from a cAMP coupled drosophila receptor. Indeed, despite of having high interspecies homology (>90%; [194]), the receptor shares a low homology with other members of the 5-HT receptor family (<50%). The human receptor has 479 amino acids (but splice variants vary in the length of the C terminus) and modulates positively cAMP formation via G_S [79,80,195,196], as may have been anticipated from the cloning strategy. The receptor also activates the mitogen-activated protein kinase, ERK, in primary neuronal cultures [197]. The cDNA encoding the receptor contains two introns; one located in the second intracellular loop [195,198] and the second in the predicted intracellular carboxyl terminal [199]. Alternate splicing of this latter intron has been reported to generate at least four 5-HT_7 receptor isoforms (5-HT_{7a}-5-HT_{7d}), which differ in their C-termini [200] and vary amongst species. However, these isoforms, to date, have not been shown to differ in their respective pharmacology, signal transduction or tissue distribution [201,202]. A human 5-$HT_{7(a)}$ receptor polymorphism $Thr^{92} \rightarrow Lys$ exhibits reduced agonist binding affinities.

Functional studies have confirmed that the 5-HT_7 receptor has an extensive vascular distribution, and is responsible for the prominent, persistent vasodilator response to 5-HT in anaesthetised animals. Moreover the receptors are expressed in non-vascular smooth muscle such as the colon and throughout the brain, with a fairly selective expression in the suprachiasmatic nucleus. To et al. [194] performed side by side receptor autoradiography and in situ hybridization in guinea-pig brain. Autoradiographic data revealed a high density of sites in the medial thalamic nuclei and related limbic and cortical regions with lower levels in sensory relay nuclei, substantia nigra, hypothalamus, central grey and dorsal raphé nuclei. In general, there was a good correspondence between the areas and nuclei identified with the two methods, although there was the usual mismatch in some cases: for instance there were no mRNA transcripts seen in caudate-putamen, globus pallidus, ventral pallidum and substantia nigra, whereas 5-HT_7 binding was apparently present. Overall, since the limbic system is particularly well represented, a potential role in sensory and affective processes may be suggested for the 5-HT_7 receptor.

It has therefore been suggested that the receptor plays a role in sleep, circadian rhythmic activity and mood. [^{3}H]SB 269970 can be used as a selective radioligand for 5-HT_7 receptors [203] and it is now clear that this receptor is the orphan receptor originally described as the '5-HT_1-like' receptor mediating relaxation various vascular and non-vascular smooth muscles [1] and subsequently shown to mediate elevation of cAMP and relaxation in neonatal porcine vena cava. In addition, the receptor appears to play a role in thermoregulation as suggested by the effects produced by selective agonists and antagonist and changes in body temperature in KO mice. Application of 8-OH-DPAT (a 5-HT_{1A} agonist with 5-HT_7 affinity) under defined conditions, produces advance in phase shifts, but there are species differences. Antagonism at 5-HT_7 receptors has no effect on short term memory [30,31]. 5-HT_7 KO mice show reduced immobility in the forced swim test, suggesting an 'antidepressant-like' phenotype [204] in agreement with the effects of 5-HT_7 antagonists as long they are applied during the dark phase.

6. Conclusion

The 5-HT receptor family is complex, and one may ask as does Bryan Roth et al. [205] whether this is useless diversity (i.e. too much redundancy) or an embarrassment of the riches (i.e. many potential targets to choose from to affect normal or pathological function); molecular biology has largely confirmed, but also significantly enlarged the suspected diversity and stopped discussions about too much complexity or redundancy of 5-HT receptors. It still remains to be seen which functions some of the many subtypes play in health and/or disease (e.g. 5-ht_5 receptors). There are multiple links between 5-HT receptors and disease, as illustrated by a large list of medications active at one or the other receptors and/or 5-HT transporters or metabolizing enzymes, other drugs being active at several receptors at the time, as illustrated by various antipsychotics/antidepressants. The complexity of the system is probably even larger than suspected, as illustrated by the great number of splices variants for some receptors (e.g. 5-HT_4 or 5-HT_7) or even editing variants (e.g. 5-HT_{2C}). It is beyond the scope of this short review to address the impact of genetic variations on 5-HT receptor or transporter function in health and disease (see [206]). Similarly, complexity is also contributed by GPCR interacting proteins and other chaperones, endogenous modulating proteins such as 5-HT moduline [68] or oleamide, homo and/or heterodimerisation ([70]; see also Breitwieser, 2004) (which may explain some features of the still orphan receptors such as 5-HT_{1P}). There is room for more 5-HT_3 receptors, in light of the recent cloning of further subunits, especially when considering what nature has done with nicotine, GABA or glutamate receptors. Cross talk between some of these ligand-gated channel receptors cannot be excluded given structural similarities and especially since a number of drugs share similar affinities for 5-HT_3 and nicotine $alpha_7$ receptors. It will be interesting to revisit various 5-HT receptor models which have been proposed based on sequence alignments with rhodopsin, now that a high resolution structure/crystal of the $beta_2$ adrenoceptor has been reported by Kobilka and collaborators (see [207,208]).

References

[1] Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PPA, Middlemiss DN, et al. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 1986;25:563–76.

[2] Villalon CM, Denboer MO, Heiligers JPC, Saxena PR. Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-Ht4-receptor. Br J Pharmacol 1990;100:665–7.

[3] Kobilka BK, Frielle T, Collins S, Yangfeng T, Kobilka TS, Francke U, et al. An intronless gene encoding a potential member of the family of receptors coupled to guanine-nucleotide regulatory proteins. Nature 1987;329:75–9.

[4] Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ. The genomic clone G-21 which resembles a beta-adrenergic-receptor sequence encodes the 5-Ht1a Receptor. Nature 1988;335:358–60.

[5] Humphrey PPA, Hartig P, Hoyer D. A proposed new nomenclature for 5-Ht receptors. Trends Pharmacol Sci 1993;14:233–6.

[6] Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 1994;46:157–203.

[7] Hartig PR, Hoyer D, Humphrey PPA, Martin GR. Alignment of receptor nomenclature with the human genome: classification of 5-HT1B and 5-HT1D receptor subtypes. Trends Pharmacol Sci 1996;17:103–5.

[8] Hoyer D, Martin G. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 1997;36:419–28.

[9] Branchek TA, Mawe GM, Gershon MD. Characterization and localization of a peripheral neural 5-hydroxytryptamine receptor subtype (5-Ht1p) with a selective agonist, H-3-5 hydroxyindalpine. J Neurosci 1988;8:2582–95.

[10] Cools R, Roberts AC, Robbins TW. Serotoninergic regulation of emotional and behavioural control processes. Trends Cogn Sci 2008;12:31–40.

[11] Pedigo NW, Yamamura HI, Nelson DL. Discrimination of multiple [H-3]5-hydroxytryptamine binding-sites by the neuroleptic spiperone in rat-brain. J Neurochem 1981;36:220–6.

[12] Fujiwara Y, Nelson DL, Kashihara K, Varga E, Roeske WR, Yamamura HI. The cloning and sequence-analysis of the rat serotonin-1a receptor gene. Life Sci 1990;47:PL127–32.

[13] Albert PR, Zhou QY, Vantol HHM, Bunzow JR, Civelli O. Cloning, functional expression, and messenger-RNA tissue distribution of the rat 5-hydroxytryptamine-1a receptor gene. J Biol Chem 1990;265:5825–32.

[14] Charest A, Wainer BH, Albert PR. Cloning and differentiation-induced expression of a murine serotonin(1a) receptor in a septal cell-line. J Neurosci 1993;13:5164–71.

[15] Fargin A, Raymond JR, Regan JW, Cotecchia S, Lefkowitz RJ, Caron MG. Effector coupling mechanisms of the cloned 5-Ht1a receptor. J Biol Chem 1989;264:14848–52.

[16] Stam NJ, Vanhuizen F, Vanalebeek C, Brands J, Dijkema R, Tonnaer J, et al. Genomic organization, coding sequence and functional expression of human 5-Ht2 and 5-Ht1a receptor genes. Eur J Pharmacol: Mol Pharmacol Sect 1992;227:153–62.

[17] Boddeke H, Fargin A, Raymond JR, Schoeffter P, Hoyer D. Agonist antagonist interactions with cloned human 5-Ht1a receptors – variations in intrinsic activity studied in transfected Hela-cells. Naunyn-Schmiedebergs Arch Pharmacol 1992;345:257–63.

[18] Albert PR, Sajedi N, Lemonde S, Ghahremani MH. Constitutive G(i2)-dependent activation of adenylyl cyclase type II by the 5-HT1A receptor – inhibition by anxiolytic partial agonists. J Biol Chem 1999;274:35469–74.

[19] Markstein R, Matsumoto M, Kohler C, Togashi H, Yoshioka M, Hoyer D. Pharmacological characterisation of 5-HT receptors positively coupled to adenylyl cyclase in the rat hippocampus. Naunyn-Schmiedebergs Arch Pharmacol 1999;359:454–9.

[20] Nakhai B, Nielsen DA, Linnoila M, Goldman D. 2 Naturally-occurring amino-acid substitutions in the human 5-Ht1a receptor – glycine-22 to serine-22 and isoleucine-28 to valine-28. Biochem Biophys Res Commun 1995;210:530–6.

[21] Lam S, Shen Y, Nguyen T, Messier TL, Brann M, Comings D, et al. A serotonin receptor gene (5HT1A) variant found in a Tourette's syndrome patient. Biochem Biophys Res Commun 1996;219:853–8.

[22] Del Tredici AL, Schiffer HH, Burstein ES, Lameh J, Mohell N, Hacksell U, et al. Pharmacology of polymorphic variants of the human 5-HT1A receptor. Biochem Pharmacol 2004;67:479–90.

[23] Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 1998;95:14476–81.

[24] Toth M. 5-HT1A receptor knockout mouse as a genetic model of anxiety. Eur J Pharmacol 2003;463:177–84.

[25] Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P, Parsons LH, et al. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci USA 1998;95:15049–54.

[26] Heisler LK, Chu HM, Tecott LH. Epilepsy and obesity in serotonin 5-HT2C receptor mutant mice. Adv Serotonin Receptor Res 1998;861:74–8.

[27] Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin(1A) receptor. Proc Natl Acad Sci USA 1998;95:10734–9.

[28] Gross C, Zhuang XX, Stark K, Ramboz S, Oosting R, Kirby L, et al. Serotonin(1A) receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 2002;416:396–400.

[29] Blier P, Seletti B, Gilbert F, Young SN, Benkelfat C. Serotonin(1A) receptor activation and hypothermia in humans: lack of evidence for a presynaptic mediation. Neuropsychopharmacology 2002;27:301–8.

[30] Meneses A. Do serotonin1-7 receptors modulate short and long-term memory? Neurobiol Learn Mem 2007;87:561–72.

[31] Meneses A. Stimulation of 5-HT1A, 5-HT1B, 5-HT2A/2C, 5-HT3 and 5-HT4 receptors or 5-HT uptake inhibition: short- and long-term memory. Behav Brain Res 2007;184:81–90.

[32] Hoyer D, Middlemiss DN. Species-differences in the pharmacology of terminal 5-Ht autoreceptors in mammalian brain. Trends Pharmacol Sci 1989;10:130–2.

[33] Voigt MM, Laurie DJ, Seeburg PH, Bach A. Molecular-cloning and characterization of a rat-brain Cdna-encoding a 5-hydroxytryptamine1b receptor. EMBO J 1991;10:4017–23.

[34] Hamblin MW, Metcalf MA. Primary structure and functional—characterization of a human 5-Ht1d-type serotonin receptor. Mol Pharmacol 1991;40:143–8.

[35] Adham N, Romanienko P, Hartig P, Weinshank RL, Branchek T. The rat 5-hydroxytryptamine1B receptor is the species homologue of the human 5-hydroxytryptamine1D beta receptor. Mol Pharmacol 1992;41:1–7.

[36] Hartig PR, Branchek TA, Weinshank RL. A subfamily of 5-Ht1d receptor genes. Trends Pharmacol Sci 1992;13:152–9.

[37] Hamblin MW, McGuffin RW, Metcalf MA, Dorsa DM, Merchant KM. Distinct 5-Ht(1b) and 5-Ht(1d) serotonin receptors in rat – structural and pharmacological comparison of the 2 cloned receptors. Mol Cell Neurosci 1992;3:578–87.

[38] Hamblin MW, Metcalf MA, McGuffin RW, Karpells S. Molecular-cloning and functional—characterization of a human 5-Ht1b serotonin receptor – a homolog of the rat 5-Ht1b receptor with 5-Ht1d-like pharmacological specificity. Biochem Biophys Res Commun 1992;184:752–9.

[39] Jin H, Oksenberg D, Ashkenazi A, Peroutka SJ, Duncan AMV, Rozmahel R, et al. Characterization of the human 5-hydroxytryptamine-1b receptor. J Biol Chem 1992;267:5735–8.

[40] Maroteaux L, Saudou F, Amlaiky N, Boschert U, Plassat JL, Hen R. Mouse 5ht1b serotonin receptor – cloning, functional expression, and localization in motor control centers. Proc Natl Acad Sci USA 1992;89:3020–4.

[41] Adham N, Tamm JA, Salon JA, Vaysse PJJ, Weinshank RL, Branchek TA. A single-point mutation increases the affinity of serotonin 5-Ht1d-Alpha, 5-Ht1d-Beta 5-Ht1e and 5-Ht1f receptors for beta-adrenergic antagonists. Neuropharmacology 1994;33:387–91.

[42] Schoeffter P, Hoyer D. 5-Hydroxytryptamine 5-Ht-1b and 5-Ht-1d receptors mediating inhibition of adenylate-cyclase activity – pharmacological comparison with special reference to the effects of Yohimbine Rauwolscine and some beta-adrenoceptor antagonists. Naunyn-Schmiedebergs Arch Pharmacol 1989;340:285–92.

[43] Pauwels PJ, Wurch T, Amoureux MC, Palmier C, Colpaert FC. Stimulation of cloned human serotonin 5-HT1D beta receptor sites in stably transfected C6 glial cells promotes cell growth. J Neurochem 1996;66:65–73.

[44] Zgombick JM, Branchek TA. Native 5-HT1B receptors expressed in OH cells display dual coupling to elevation of intracellular calcium concentrations and inhibition of adenylate cyclase. Naunyn-Schmiedebergs Arch Pharmacol 1998;358:503–8.

[45] Lin SL, Setya S, Johnson-Farley NN, Cowen DS. Differential coupling of 5-HT1 receptors to G proteins of the G(i) family. Br J Pharmacol 2002;136:1072–8.

[46] MassenVanDenBrink A, Vergouwe MN, Ophoff RA, Saxena PR, Ferrari MD, Frants RR. 5-HT1B receptor polymorphism and clinical response to sumatriptan. Headache 1998;38:288–91.

[47] Lesage AS, Wouters R, Van Gompel P, Heylen L, Vanhoenacker P, Haegeman G, et al. Agonistic properties of alniditan, sumatriptan and dihydroergotamine on human 5-HT1B and 5-HT1D receptors expressed in various mammalian cell lines. Br J Pharmacol 1998;123:1655–65.

[48] Longmore J, Maguire JJ, MacLeod A, Street L, Schofield WN, Hill RG. Comparison of the vasoconstrictor effects of the selective 5-HT1D-receptor agonist L-775,606 with the mixed 5-HT1B/(1D)-receptor agonist sumatriptan and 5-HT in human isolated coronary artery. Br J Clin Pharmacol 2000;49:126–31.

[49] Hasegawa Y, Higuchi S, Matsushita S, Miyaoka H. Association of a polymorphism of the serotonin 1B receptor gene and alcohol dependence with inactive aldehyde dehydrogenase-2. J Neural Transmission 2002;109:513–21.

[50] Hawi Z, Dring M, Kirley A, Foley D, Kent L, Craddock N, et al. Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT1B receptor gene in 273 nuclear families from a multi-centre sample. Mol Psychiatry 2002;7:718–25.

[51] Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, et al. The serotonin 5-HT1B receptor gene and attention deficit hyperactivity disorder. Mol Psychiatry 2003;8:98–102.

[52] Soyka M, Preuss UW, Koller G, Zill P, Bondy B. Association of 5-HT1B receptor gene and antisocial behavior in alcoholism. J Neural Transmission 2004;111:101–9.

[53] Huang YY, Oquendo MA, Friedman JMH, Greenhill LL, Brodsky B, Malone KM, et al. Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology 2003;28:163–9.

[54] Saudou F, Amara DA, Dierich A, Lemeur M, Ramboz S, Segu L, et al. Enhanced aggressive-behavior in mice lacking 5-Ht1b receptor. Science 1994;265:1875–8.

[55] Ramboz S, Saudou F, Amara DA, Belzung C, Segu L, Misslin R, et al. 5-HT1B receptor knock out – behavioral consequences. Behav Brain Res 1995;73:305–12.
[56] Brunner D, Hen R. Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. Neurobiol Suicide 1997;836:81–105.
[57] Bouwknecht JA, Hijzen TH, van der Gugten J, Mass RAA, Hen R, Olivier B. Absence of 5-HT1B receptors is associated with impaired impulse control in male 5-HT1B knockout mice. Biol Psychiatry 2001;49:557–68.
[58] Weydert A, Cloeztayarani I, Fillion MP, Simonchazottes D, Guenet JL, Fillion G. Molecular-cloning of 2 partial serotonin 5-Ht(1d) receptor sequences in mouse and one in guinea-pig. Comptes Rendus Acad Sci Ser III: Sci Vie-Life Sci 1992;314:429–35.
[59] Bruinvels AT, Landwehrmeyer B, Probst A, Palacios JM, Hoyer D. A comparative autoradiographic study of 5-Ht1d binding-sites in human and guinea-pig brain using different radioligands. Mol Brain Res 1994;21:19–29.
[60] Castro ME, Pascual J, Romon T, DelArco C, DelOlmo E, Pazos A. Differential distribution of [H-3]sumatriptan binding sites (5-HT1B 5-HT1D and 5-HT1F receptors) in human brain: focus on brainstem and spinal cord. Neuropharmacology 1997;36:535–42.
[61] Varnas K, Hall H, Bonaventure P, Sedvall G. Autoradiographic mapping of 5-HT1B and 5-HT1D receptors in the post mortem human brain using [H-3]GR 125743. Brain Res 2001;915:47–57.
[62] Bonaventure P, Langlois X, Leysen JE. Co-localization of 5-HT1B- and 5-HT1D receptor mRNA in serotonergic cell bodies in guinea pig dorsal raphe nucleus: a double labeling in situ hybridization histochemistry study. Neurosci Lett 1998;254:113–6.
[63] Price GW, Burton MJ, Collin LJ, Duckworth M, Gaster L, Gothert M, et al. SB-216641 and BRL-15572 – compounds to pharmacologically discriminate h5-HT1B and h5-HT1D receptors. Naunyn-Schmiedebergs Arch Pharmacol 1997;356:312–20.
[64] Schlicker E, Fink K, Molderings GJ, Price GW, Duckworth M, Gaster L, et al. Effects of selective h5-HT1B (SB-216641) and h5-HT1D (BRL-15572) receptor ligands on guinea-pig and human 5-HT auto- and heteroreceptors. Naunyn-Schmiedebergs Arch Pharmacol 1997;356:321–7.
[65] Schlicker E, Fink K, Molderings GJ, Price GW, Middlemiss DN, Zentner J, et al. Effects of SB 216641 and BRL 15572 (selective h5-HT1B and h5-HT(1D)receptor antagonists, respectively) on guinea-pig and human 5-HT auto- and hetero-receptors. Br J Pharmacol 1997;120:P143–143.
[66] Middlemiss DN, Gothert M, Schlicker E, Scott CM, Selkirk JV, Watson J, et al. SB-236057, a selective 5-HT1B receptor inverse agonist, blocks the 5-HT human terminal autoreceptor. Eur J Pharmacol 1999;375:359–65.
[67] Bergen AW, van den Bree MBM, Yeager M, Welch R, Ganjei JK, Haque K, et al. Candidate genes for anorexia nervosa in the 1p33-36 linkage region: serotonin 1D and delta opioid receptors display significant association to anorexia nervosa. Biol Psychiatry 2003;53:165S–165S.
[68] Rousselle JC, Plantefol M, Fillion MP, Massot O, Pauwels PJ, Fillion G. Specific interaction of 5-HT-moduline with human 5-HT1b as well as 5-HT1d receptors expressed in transfected cultured cells. Naunyn-Schmiedebergs Arch Pharmacol 1998;358:279–86.
[69] Sarhan H, Grimaldi B, Hen R, Fillion G. 5-HT1B receptors modulate release of [H-3]dopamine from rat striatal synaptosomes: further evidence using 5-HT moduline, polyclonal 5-HT1B receptor antibodies and 5-HT1B receptor knock-out mice. Naunyn-Schmiedebergs Arch Pharmacol 2000;361:12–8.
[70] Xie XD, Lee SP, O'Dowd BF, George SR. Serotonin 5-HT1B and 5-HT1D receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Lett 1999;456:63–7.
[71] Lee SP, Xie ZD, Varghese G, Nguyen T, O'Dowd BF, George SR. Oligomerization of dopamine and serotonin receptors. Neuropsychopharmacology 2000;23:S32–40.
[72] Miller KJ, Teitler M. Quantitative autoradiography of 5-Ct-sensitive (5-Ht1d) and 5-Ct-insensitive (5-Ht1e) serotonin receptors in human brain. Neurosci Lett 1992;136:223–6.
[73] McAllister G, Charlesworth A, Snodin C, Beer MS, Noble AJ, Middlemiss DN, et al. Molecular-cloning of a serotonin receptor from human brain (5ht1e) – a 5th 5ht1-like subtype. Proc Natl Acad Sci USA 1992;89:5517–21.
[74] Zgombick JM, Schechter LE, Macchi M, Hartig PR, Branchek TA, Weinshank RL. Human gene S31 encodes the pharmacologically defined serotonin 5-hydroxytryptamine-1e receptor. Mol Pharmacol 1992;42:180–5.
[75] Amlaiky N, Ramboz S, Boschert U, Plassat JL, Hen R. Isolation of a mouse 5ht1e-like serotonin receptor expressed predominantly in hippocampus. J Biol Chem 1992;267:19761–4.
[76] Adham N, Vaysse PJJ, Weinshank RL, Branchek TA. The cloned human 5-Ht1e receptor couples to inhibition and activation of adenylyl-cyclase via 2 distinct pathways in transfected Bs-C-1 cells. Neuropharmacology 1994;33:403–10.
[77] Levy FO, Holtgrevegrez H, Tasken K, Solberg R, Ried T, Gudermann T. Assignment of the gene encoding the 5-Ht1e serotonin receptor (S31) (locus Htr1e) to human-chromosome 6q14-Q15. Genomics 1994;22:637–40.
[78] Adham N, Borden LA, Schechter LE, Gustafson EL, Cochran TL, Vaysse PJJ, et al. Cell-specific coupling of the cloned human 5-Ht(1f) receptor to multiple signal-transduction pathways. Naunyn-Schmiedebergs Arch Pharmacol 1993;348:566–75.
[79] Lovenberg TW, Baron BM, Delecea L, Miller JD, Prosser RA, Rea MA, et al. A novel adenylyl cyclase-activating serotonin receptor (5-Ht7) implicated in the regulation of mammalian circadian-rhythms. Neuron 1993;11:449–58.
[80] Lovenberg TW, Erlander MG, Baron BM, Racke M, Slone AL, Siegel BW, et al. Molecular-cloning and functional expression of 5-Ht1e-like rat and human 5-hydroxytryptamine receptor genes. Proc Natl Acad Sci USA 1993;90:2184–8.
[81] Guptan P, Dhingra A, Panicker MM. Multiple transcripts encode the 5-HT1F receptor in rodent brain. Neuroreport 1997;8:3317–21.
[82] Adham N, Kao H, Schechter LE, Bard J, Olsen M, Urquhart D, et al. Cloning of another human serotonin receptor (5-HT1F): a fifth 5-HT1 receptor subtype coupled to the inhibition of adenylate cyclase. Proc Natl Aacd Sci USA 1993;90:408–12.
[83] Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 2002;71:533–54.
[84] Lucaites VL, Krushinski JH, Schaus JM, Audia JE, Nelson DL. [3H]LY334370, a novel radioligand for the 5-HT1F receptor. II. Autoradiographic localization in rat, guinea pig, monkey and human brain. Naunyn-Schmiedeberg's Arch Pharmacol 2005;371:178–84.
[85] Johnson KW, Schaus JM, Durkin MM, Audia JE, Kaldor SW, Flaugh ME, et al. 5-HT1F receptor agonists inhibit neurogenic dural inflammation in guinea pigs. Neuroreport 1997;8:2237–40.
[86] Mitsikostas DD, del Rio MS, Moskowitz MA, Waeber C. Both 5-HT1B and 5-HT1F receptors modulate c-fos expression within rat trigeminal nucleus caudalis. Eur J Pharmacol 1999;369:271–7.
[87] Cohen ML, Schenck K. 5-Hydroxytryptamine(1F) receptors do not participate in vasoconstriction: lack of vasoconstriction to LY344864, a selective serotonin(1F) receptor agonist in rabbit saphenous vein. J Pharmacol Exp Therapeut 1999;290:935–9.
[88] Phebus LA, Johnson KW, Zgombick JM, Gilbert PJ, VanBelle K, Mancuso V, et al. Characterization of LY344864 as a pharmacological tool to study 5-HT1F receptors: binding affinities, brain penetration and activity in the neurogenic dural inflammation model of migraine. Life Sci 1997;61:2117–26.
[89] Gaddum JH, Picarelli ZP. 2 Kinds of tryptamine receptor. Br J Pharmacol Chemother 1957;12:323–8.
[90] Peroutka SJ, Snyder SH. Multiple serotonin receptors – differential binding of [5-hydroxytryptamine-H-3] [lysergic-H-3 acid diethylamide and [H-3]spiroperidol. Mol Pharmacol 1979;16:687–99.
[91] Pritchett DB, Bach AWJ, Wozny M, Taleb O, Daltoso R, Shih JC, et al. Structure and functional expression of cloned rat serotonin 5ht-2 receptor. EMBO J 1988;7:4135–40.
[92] Julius D, Huang KN, Livelli TJ, Axel R, Jessell TM. The 5ht2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors. Proc Natl Acad Sci USA 1990;87:928–32.
[93] Saltzman AG, Morse B, Whitman MM, Ivanshchenko Y, Jaye M, Felder S. Cloning of the human serotonin 5-Ht2 and 5-Ht1c receptor subtypes. Biochem Biophys Res Commun 1991;181:1469–78.
[94] Chen K, Yang W, Grimsby J, Shih JC. The human 5-Ht2 receptor is encoded by a multiple intron exon gene. Mol Brain Res 1992;14:20–6.
[95] Yang W, Chen K, Lan NC, Gallaher TK, Shih JC. Gene structure and expression of the mouse 5-Ht2 receptor. J Neurosci Res 1992;33:196–204.
[96] Garlow SJ, Chin AC, Marinovich AM, Heller MR, Ciaranello RD. Cloning and functional promoter mapping of the rat serotonin-2 receptor gene. Mol Cell Neurosci 1994;5:291–300.
[97] Arranz MJ, Munro J, Owen MJ, Spurlock G, Sham PC, Zhao J, et al. Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine. Mol Psychiatry 1998;3:61–6.
[98] Salomon L, Lanteri C, Godeheu G, Blanc G, Gingrich J, Tassin JP. Paradoxical constitutive behavioral sensitization to amphetamine in mice lacking 5-HT2A receptors. Psychopharmacology 2007;194:11–20.
[99] Popa D, Lena C, Fabre V, Prenat C, Gingrich J, Escourrou P, et al. Contribution of 5-HT2 receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT2A receptors. J Neurosci 2005;25:11231–8.
[100] Ullmer C, Boddeke H, Schmuck K, Lubbert H. 5-HT2B receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. Br J Pharmacol 1996;117:1081–8.
[101] Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, et al. Cloning and functional—characterization of the rat stomach fundus serotonin receptor. EMBO J 1992;11:3481–7.
[102] Foguet M, Nguyen H, Le H, Lubbert H. Structure of the mouse 5-Ht1c 5-Ht2 and stomach fundus serotonin receptor genes. Neuroreport 1992;3:345–8.
[103] Kursar JD, Nelson DL, Wainscott DB, Cohen ML, Baez M. Molecular-cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5-hydroxytryptamine2f) from rat stomach fundus. Mol Pharmacol 1992;42:549–57.
[104] Kursar JD, Nelson DL, Wainscott DB, Baez M. Molecular-cloning, functional expression, and messenger—RNA tissue distribution of the human 5-hydroxytryptamine(2b) receptor. Mol Pharmacol 1994;46:227–34.
[105] Loric S, Launay JM, Colas JF, Maroteaux L. New mouse 5-Ht2-like receptor – expression in brain, heart and intestine. FEBS Lett 1992;312:203–7.
[106] Schmuck K, Ullmer C, Engels P, Lubbert H. Cloning and functional—characterization of the human 5-Ht2b serotonin receptor. FEBS Lett 1994;342:85–90.

[107] Wainscott DB, Cohen ML, Schenck KW, Audia JE, Nissen JS, Baez M, et al. Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine2f receptor. Mol Pharmacol 1993;43:419–26.
[108] Wainscott DB, Lucaites VL, Kursar JD, Baez M, Nelson DL. Pharmacologic characterization of the human 5-hydroxytryptamine(2B) receptor: evidence for species differences. J Pharmacol Exp Therapeut 1996;276: 720–7.
[109] LeConiat M, Choi DS, Maroteaux L, Launay JM, Berger R. The 5-HT2B receptor gene maps to 2q36 3-2q37. 1. Genomics 1996;32:172–3.
[110] Bonhaus DW, Bach C, Desouza A, Salazar FHR, Matsuoka BD, Zuppan P, et al. The pharmacology and distribution of human 5-hydroxytryptamine(2b) (5-Ht2b) receptor gene-products – comparison with 5-Ht2a and 5-Ht2c receptors. Br J Pharmacol 1995;115:622–8.
[111] Duxon MS, Kennett GA, Lightowler S, Blackburn TP, Fone KCF. Activation of 5-HT2B receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. Neuropharmacology 1997;36:601–8.
[112] Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS. Mediation by 5-hydroxytryptamine(2b) receptors of endothelium-dependent relaxation in rat jugular-vein. Br J Pharmacol 1995;114:400–4.
[113] Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, et al. Possible role of valvular serotonin 5-HT2B receptors in the cardiopathy associated with fenfluramine. Mol Pharmacol 2000;57:75–81.
[114] Nebigil CG, Jaffre F, Messaddeq N, Hickel P, Monassier L, Launay JM, et al. Overexpression of the serotonin 5-HT2B receptor in heart leads to abnormal mitochondrial function and cardiac hypertrophy. Circulation 2003;107:3223–9.
[115] Launay JM, Herve P, Peoc'h K, Tournois C, Callebert J, Nebigil CG, et al. Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. Nat Med 2002;8:1129–35.
[116] Nebigil CG, Choi DS, Dierich A, Hickel P, Le Meur M, Messaddeq N, et al. Serotonin 2B receptor is required for heart development. Proc Natl Acad Sci USA 2000;97:9508–13.
[117] Nebigil CG, Hickel P, Messaddeq N, Vonesch JL, Douchet MP, Monassier L, et al. Ablation of serotonin 5-HT2B receptors in mice leads to abnormal cardiac structure and function. Circulation 2001;103:2973–9.
[118] Yu L, Nguyen H, Le H, Bloem LJ, Kozak CA, Hoffman BJ, et al. The mouse 5-Ht1c receptor contains 8 hydrophobic domains and Is X-linked. Mol Brain Res 1991;11:143–9.
[119] Boddeke H, Hoffman BJ, Palacios JM, Knot H, Hoyer D. Characterization of functional-responses in A9-cells transfected with cloned rat 5-Ht1c receptors. Naunyn-Schmiedebergs Arch Pharmacol 1993;347:119–24.
[120] Xie E, Zhu L, Zhao L, Chang L-S. The human serotonin 5-HT2C receptor: complete cDNA, genomic structure, and alternatively spliced variant. Genomics 1996;35:551–61.
[121] Hoyer D. Molecular pharmacolology and biology of 5-Ht1c receptors. Trends Pharmacol Sci 1988;9:89–94.
[122] Pazos A, Hoyer D, Palacios JM. The binding of serotonergic ligands to the porcine choroid-plexus – characterization of a new type of serotonin recognition site. Eur J Pharmacol 1984;106:539–46.
[123] Conn PJ, Sandersbush E, Hoffman BJ, Hartig PR. A unique serotonin receptor in choroid-plexus is linked to phosphatidylinositol turnover. Proc Natl Acad Sci USA 1986;83:4086–8.
[124] Hoyer D, Waeber C, Schoeffter P, Palacios JM, Dravid A. 5-Ht1c receptor-mediated stimulation of inositol phosphate production in pig choroid-plexus – a pharmacological characterization. Naunyn-Schmiedebergs Arch Pharmacol 1989;339:252–8.
[125] Lucaites VL, Nelson DL, Wainscott DB, Baez M. Receptor subtype and density determine the coupling repertoire of the 5-HT2 receptor subfamily. Life Sci 1996;59:1081–95.
[126] Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, SandersBush E, et al. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 1997;387:303–8.
[127] Fitzgerald LW, Iyer G, Conklin DS, Krause CM, Marshall A, Patterson JP, et al. Messenger RNA editing of the human serotonin 5-HT2C receptor. Neuropsychopharmacology 1999;21:S82–90.
[128] Price RD, Weiner DM, Chang MSS, Sanders-Bush E. RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G(13) protein. J Biol Chem 2001;276:44663–8.
[129] Canton H, Emeson RB, Barker EL, Backstrom JR, Lu JT, Chang MS, et al. Identification, molecular cloning, and distribution of a short variant of the 5-hydroxytryptamine(2C) receptor produced by alternative splicing. Mol Pharmacol 1996;50:799–807.
[130] Berg KA, Cropper JD, Niswender CM, Sanders-Bush E, Emeson RB, Clarke WP. RNA-editing of the 5-HT2C receptor alters agonist-receptor-effector coupling specificity. Br J Pharmacol 2001;134:386–92.
[131] Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. J Biol Chem 1999;274:9472–8.
[132] Herrick-Davis K, Grinde E, Niswander CM. Serotonin 5-HT2C receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. J Neurochem 1999;73:1711–7.
[133] Herrick-Davis K, Grinde E, Teitler M. Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine2C receptors. J Pharmacol Exp Therapeut 2000;295:226–32.
[134] Rauser L, Savage JE, Meltzer HY, Roth BL. Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. J Pharmacol Exp Therapeut 2001;299:83–9.
[135] Lerer B, Macciardi F, Segman RH, Adolfsson R, Blackwood D, Blairy S, et al. Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 2001;6:579–85.
[136] Buckland PR, Hoogendoorn B, Guy CA, Smith SK, Coleman SL, O'Donovan MC. Low gene expression conferred by association of an allele of the 5-HT2C receptor gene with antipsychotic-induced weight gain. Am J Psychiatry 2005;162:613–5.
[137] Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, et al. Eating disorder and epilepsy in mice lacking 5-Ht2c serotonin receptors. Nature 1995;374:542–6.
[138] Tecott LH, Abdallah L. Mouse genetic approaches to feeding regulation: serotonin 5-HT2C receptor mutant mice. CNS Spectrums 2003;8:578-+.
[139] Tecott LH. Genetic approaches to the serotonergic regulation of food and drug ingestion. Behav Pharmacol 2003;14:S21–121.
[140] Hoyer D. Serotonin 5-Ht3, 5-Ht4, and 5-Ht-M receptors. Neuropsychopharmacology 1990;3:371–83.
[141] Boess FG, Beroukhim R, Martin IL. Ultrastructure of the 5-hydroxytryptamine(3) receptor. J Neurochem 1995;64:1401–5.
[142] Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D. Primary structure and functional expression of the 5ht3 receptor, a serotonin-gated ion channel. Science 1991;254:432–7.
[143] Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, et al. The pharmacological and functional characteristics of the serotonin 5-HT3A receptor are specifically modified by a 5-MT3B receptor subunit. J Biol Chem 1999;274:30799–810.
[144] Niesler B, Walstab J, Combrink S, Moller D, Kapeller J, Rietdorf J, et al. Characterization of the novel human serotonin receptor subunits 5-HT3C, 5-HT3D, and 5-HT3E. Mol Pharmacol 2007;72:8–17.
[145] Hoyer D, Neijt HC. Identification of serotonin 5-Ht3 recognition sites by radioligand binding in Ng108-15 neuroblastoma-glioma cells. Eur J Pharmacol 1987;143:291–2.
[146] Morales M, Bloom FE. The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. J Neurosci 1997;17:3157–67.
[147] Doucet E, Latremoliere A, Darmon M, Hamon M, Emerit MB. Immunolabelling of the 5-HT3B receptor subunit in the central and peripheral nervous systems in rodents. Eur J Neurosci 2007;26:355–66.
[148] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology 1999;38:1083–152.
[149] Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG, et al. The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 1999;397:359–63.
[150] Brady CA, Stanford IM, Ali I, Lin L, Williams JM, Dubin AE, et al. Pharmacological comparison of human homomeric 5-HT3A receptors versus heteromeric 5-HT3A/3B receptors. Neuropharmacology 2001;41:282–4.
[151] Brown AM, Hope AG, Lambert JJ, Peters JA. Ion permeation and conduction in a human recombinant 5-HT3 receptor subunit (h5-HT3A). J Physiol (London) 1998;507:653–65.
[152] Hapfelmeier G, Haseneder R, Lampadius K, Rammes G, Rupprecht R, Zieglgansberger W. Cloned human and murine serotonin(3A) receptors expressed in human embryonic kidney 293 cells display different single-channel kinetics. Neurosci Lett 2002;335:44–8.
[153] Boyd GW, Low P, Dunlop JI, Robertson LA, Vardy A, Lambert JJ, et al. Assembly and cell surface expression of homomeric and heteromeric 5-HT3 receptors: the role of oligomerization and chaperone proteins. Mol Cell Neurosci 2002;21:38–50.
[154] Hapfelmeier G, Tredt C, Haseneder R, Zieglgansberger W, Eisensamer B, Rupprecht R, et al. Co-expression of the 5-HT3B serotonin receptor subunit alters the biophysics of the 5-HT3 receptor. Biophys J 2003;84:1720–33.
[155] Fletcher S, Lindstrom JM, McKernan RM, Barnes NM. Evidence that porcine native 5-HT3 receptors do not contain nicotinic acetylcholine receptor subunits. Neuropharmacology 1998;37:397–9.
[156] Hanna MC, Davies PA, Hales TG, Kirkness EF. Evidence for expression of heteromeric serotonin 5-HT3 receptors in rodents. J Neurochem 2000;75: 240–7.
[157] Monk SA, Desai K, Brady CA, Williams JM, Lin L, Princivalle A, et al. Generation of a selective 5-HT3B subunit-recognising polyclonal antibody; identification of immunoreactive cells in rat hippocampus. Neuropharmacology 2001;41:1013–6.
[158] Morales M, McCollum N, Kirkness EF. 5-HT3-receptor subunits A and B are co-expressed in neurons of the dorsal root ganglion. J Compar Neurol 2001;438:163–72.
[159] Morales M, Wng SD. Differential composition of 5-hydroxytryptamine(3) receptors synthesized in the rat CNS and peripheral nervous system. J Neurosci 2002;22:6732–41.
[160] Stewart A, Davies PA, Kirkness EF, Safa P, Hales TG. Introduction of the 5-HT3B subunit alters the functional properties of 5-HT3 receptors native to neuroblastoma cells. Neuropharmacology 2003;44:214–23.
[161] van Hooft JA, Yakel JL. 5-HT3 receptors in the CNS: 3B or not 3B? Trends Pharmacol Sci 2003;24:157–60.

[162] Niesler B, Frank B, Kapeller J, Rappold GA. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003;310:101–11.
[163] Dumuis A, Bouhelal R, Sebben M, Cory R, Bockaert J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate-cyclase in the central nervous-system. Mol Pharmacol 1988;34:880–7.
[164] Bockaert J, Sebben M, Dumuis A. Pharmacological characterization of 5-hydroxytryptamine4(5-Ht4) receptors positively coupled to adenylate-cyclase in adult guinea-pig hippocampal membranes – effect of substituted benzamide derivatives. Mol Pharmacol 1990;37:408–11.
[165] Ford A, Baxter GS, Eglen RM, Clarke DE. 5-Hydroxytryptamine stimulates cyclic-Amp formation in the tunica muscularis mucosae of the rat esophagus via 5-Ht(4) receptors. Eur J Pharmacol 1992;211:117–20.
[166] Kaumann AJ, Sanders L, Brown AM, Murray KJ, Brown MJ. A 5-hydroxytryptamine receptor in human atrium. Br J Pharmacol 1990;100: 879–85.
[167] Mohler EG, Shacham S, Noiman S, Lezoualc'h F, Robert S, Gastineau M, et al. VRX-03011, a novel 5-HT4 agonist, enhances memory and hippocampal acetylcholine efflux. Neuropharmacology 2007;53:563–73.
[168] Kemp A, Manahan-Vaughan D. Hippocampal long-term depression: master or minion in declarative memory processes? Trends Neurosci 2007;30:111–8.
[169] Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, Schechter LE, et al. The 5-Ht4 receptor – molecular-cloning and pharmacological characterization of 2 splice variants. EMBO J 1995;14:2806–15.
[170] Bockaert J, Claeysen S, Sebben M, Dumuis A. 5-HT4 receptors: gene, transduction and effects on olfactory memory. Adv Serotonin Receptor Res 1998;861:1–15.
[171] Bach T, Syversveen T, Kvingedal AM, Krobert KA, Brattelid T, Kaumann AJ, et al. 5-HT4(a) and 5-HT4(b) receptors have nearly identical pharmacology and are both expressed in human atrium and ventricle. Naunyn-Schmiedebergs Arch Pharmacol 2001;363:146–60.
[172] Adham N, Gerald C, Schechter L, Vaysse P, Weinshank R, Branchek T. [H-3]5-Hydroxytryptamine labels the agonist high affinity state of the cloned rat 5-HT4 receptor. Eur J Pharmacol 1996;304:231–5.
[173] Claeysen S, Sebben M, Journot L, Bockaert J, Dumuis A. Cloning, expression and pharmacology of the mouse 5-HT4L receptor. FEBS Lett 1996;398:19–25.
[174] Claeysen S, Sebben M, Becamel C, Bockaert J, Dumuis A. Novel brain-specific 5-HT4 receptor splice variants show marked constitutive activity: role of the C-terminal intracellular domain. Mol Pharmacol 1999;55:910–20.
[175] Blondel O, Gastineau M, Dahmoune Y, Langlois M, Fischmeister R. Cloning, expression, and pharmacology of four human 5-hydroxytryptamine(4) receptor isoforms produced by alternative splicing in the carboxyl terminus. J Neurochem 1998;70:2252–61.
[176] Mialet J, Berque-Bestel I, Eftekhari P, Gastineau M, Giner M, Dahmoune Y, et al. Isolation of the serotoninergic 5-HT4(e) receptor from human heart and comparative analysis of its pharmacological profile in C6-glial and CHO cell lines. Br J Pharmacol 2000;129:771–81.
[177] Bender E, Pindon A, van Oers I, Zhang YB, Gommeren W, Verhasselt P, et al. Structure of the human serotonin 5-HT4 receptor gene and cloning of a novel 5-HT4 splice variant. J Neurochem 2000;74:478–89.
[178] Medhurst AD, Lezoualc'h F, Fischmeister R, Middlemiss DN, Sanger GJ. Quantitative mRNA analysis of five C-terminal splice variants of the human 5-HT4 receptor in the central nervous system by TaqMan real time RT-PCR. Mol Brain Res 2001;90:125–34.
[179] Vilaro MT, Domenech T, Palacios JM, Mengod G. Cloning and characterization of a novel human 5-HT4 receptor variant that lacks the alternatively spliced carboxy terminal exon RT-PCR distribution in human brain and periphery of multiple 5-HT4 receptor variants. Neuropharmacology 2002;42:60–73.
[180] Brattelid T, Kvingedal AM, Krobert KA, Andressen KW, Bach T, Hystad ME, et al. Cloning, pharmacological characterisation and tissue distribution of a novel 5-HT4 receptor splice variant 5-HT4(i). Naunyn-Schmiedebergs Arch Pharmacol 2004;369:616–28.
[181] Mialet J, Berque-Bestel I, Sicsic S, Langlois M, Fischmeister R, Lezoualc'h F. Pharmacological characterization of the human 5-HT4(d) receptor splice variant stably expressed in Chinese hamster ovary cells. Br J Pharmacol 2000;131:827–35.
[182] Ouadid H, Seguin J, Dumuis A, Bockaert J, Nargeot J. Serotonin increases calcium current in human atrial myocytes via the newly described 5-hydroxytryptamine4 receptors. Mol Pharmacol 1992;41:346–51.
[183] Compan V, Zhou MM, Grailhe R, Gazzara RA, Martin R, Gingrich J, et al. Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT4 receptor knock-out mice. J Neurosci 2004;24:412–9.
[184] Erlander MG, Lovenberg TW, Baron BM, Delecea L, Danielson PE, Racke M, et al. 2 Members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat-brain. Proc Natl Acad Sci USA 1993;90:3452–6.
[185] Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, Muscatelli F, et al. Mouse 5-hydroxytryptamine5a and 5-hydroxytryptamine5b receptors define a new family of serotonin receptors – cloning, functional expression, and chromosomal localization. Mol Pharmacol 1993;43:313–9.
[186] Schanen NC, Scherer SW, Tsui LC, Francke U. Assignment of the 5-hydroxytryptamine (serotonin) receptor 5A gene (HTR5A) to human chromosome band 7q36.1. Cytogenet Cell Genet 1996;72:187–8.
[187] Grailhe R, Grabtree GW, Hen R. Human 5-HT5 receptors: the 5-HT5A receptor is functional but the 5-HT5B receptor was lost during mammalian evolution. Eur J Pharmacol 2001;418:157–67.
[188] Pasqualetti M, Ori M, Nardi I, Castagna M, Cassano GB, Marazziti D. Distribution of the 5-HT5A serotonin receptor mRNA in the human brain. Mol Brain Res 1998;56:1–8.
[189] Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, et al. Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. J Neurochem 1996;66:47–56.
[190] Hirst WD, Minton JAL, Bromidge SM, Moss SF, Latter AJ, Riley G, et al. Characterization of [I-125]-SB-258585 binding to human recombinant and native 5-HT6 receptors in rat, pig and human brain tissue. Br J Pharmacol 2000;130:1597–605.
[191] Roberts JC, Reavill C, East SZ, Harrison PJ, Patel S, Routledge C, et al. The distribution of 5-HT6 receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT6 receptor antagonist [125I]SB-258585. Brain Res 2002;934:49–57.
[192] Sebben M, Ansanay H, Bockaert J, Dumuis A. 5-Ht6 receptors positively coupled to adenylyl-cyclase in striatal neurons in culture. Neuroreport 1994;5:2553–7.
[193] Meneses A, Manuel-Apolinar L, Castillo C, Castillo E. Memory consolidation and amnesia modify 5-HT6 receptors expression in rat brain: an autoradiographic study. Behavioural. Brain Res 2007;178:53–61.
[194] To ZP, Bonhaus DW, Eglen RM, Jakeman LB. Characterization and distribution of putative 5-Ht(7) receptors in guinea-pig brain. Br J Pharmacol 1995;115:107–16.
[195] Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA, Weinshank RL. Cloning of a novel human serotonin receptor (5-Ht7) positively linked to adenylate-cyclase. J Biol Chem 1993;268:23422–6.
[196] Adham N, Zgombick JM, Bard J, Branchek TA. Functional characterization of the recombinant human 5-hydroxytryptamine(7(a)) receptor isoform coupled to adenylate cyclase stimulation. J Pharmacol Exp Therapeut 1998;287:508–14.
[197] Errico H, Crozier RA, Plummer MR, Cowen DS. 5-HT7 receptors activate the mitogen activated protein kinase extracellular signal related kinase in cultured rat hippocampal neurons. Neuroscience 2001;102:361–7.
[198] Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW, Sibley DR. Molecular-cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. J Biol Chem 1993;268:18200–4.
[199] Ruat M, Traiffort E, Leurs R, Tardivellacombe J, Diaz J, Arrang JM, et al. Molecular-cloning, characterization, and localization of a high-affinity serotonin receptor (5-Ht(7)) activating camp formation. Proc Natl Acad Sci USA 1993;90:8547–51.
[200] Heidmann DEA, Metcalf MA, Kohen R, Hamblin MW. Four 5-hydroxytryptamine(7) (5-HT7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization. J Neurochem 1997;68:1372–81.
[201] Jasper JR, Kosaka A, To ZP, Chang DJ, Eglen RM. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT7 receptor (h5-HT7(b)). Br J Pharmacol 1997;122:126–32.
[202] Heidmann DEA, Szot P, Kohen R, Hamblin MW. Function and distribution of three rat 5-hydroxytryptamine(7) (5-HT7) receptor isoforms produced by alternative splicing. Neuropharmacology 1998;37:1621–32.
[203] Thomas DR, Atkinson PJ, Ho M, Bromidge SM, Lovell PJ, Villani AJ, et al. [H-3]-SB-269970 – a selective antagonist radioligand for 5-HT7 receptors. Br J Pharmacol 2000;130:409–17.
[204] Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, et al. Genetic knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. Neuropharmacology 2005;48:492–502.
[205] Roth BL, Lopez E, Patel S, Kroeze WK. The multiplicity of serotonin receptors: uselessly diverse molecules or an embarrassment of riches? Neuroscientist 2000;6:252–62.
[206] Tang C-M, Insel PA. Genetic variation in G-protein-coupled receptors – consequences for G-protein-coupled receptors as drug targets. Expert Opin Therapeut Targets 2005;9:1247–65.
[207] Rosenbaum DM, Cherezov V, Hanson MA, Rasmussen SGF, Thian FS, Kobilka TS, et al. GPCR engineering yields high-resolution structural insights into {beta}2 adrenergic receptor function. Science 2007:1150609.
[208] Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SGF, Thian FS, Kobilka TS, et al. High-resolution crystal structure of an engineered human {beta}2-adrenergic G protein coupled receptor. Science 2007:1150577.
[209] Adham N, Gerald C, Schechter L, Vaysse P, Weinshank R, Branchek T. [H-3]5-Hydroxytryptamine labels the agonist high affinity state of the cloned rat 5-HT4 receptor. Euro J Pharm 1996;304:231–5.
[210] Bai F, Yin T, Johnstone EM, Su C, Varga G, Little SP, et al. Molecular cloning and pharmacological characterization of the guinea pig 5-HT1E receptor. Euro J Pharm 2004;484:127–39.
[211] Branchek TA, Zgombick JM. Molecular biology and potential functional role of 5-ht5, 5-HT6 and 5-HT7 receptors. In: Baumgarten HG, Gothert M, editors. Handbook of experimental pharmacology. Serotonin neurons and 5-HT receptors in the CNS. Berlin: Springer-Verlag; 1997. p. 475–97.

[212] Claeysen S, Sebben M, Journot L, Bockaert J, Dumuis A. Cloning, expression and pharmacology of the mouse 5-HT4L receptor. Febs Letters 1996;398:19–25.
[213] Claeysen S, Sebben M, Becamel C, Bockaert J, Dumuis A. Novel brain-specific 5-HT4 receptor splice variants show marked constitutive activity: Role of the C-terminal intracellular domain. Mol Pharm 1999;55:910–20.
[214] Hoyer D, Martin GR, Andrade R, Barnes N, Baxter G, Branchek T, et al. 5-Hydroxytryptamine receptors – IUPHAR Receptor database. 2006; doi:10.1786/543800763668.
[215] Karnovsky AM, Gotow LF, McKinley DD, Piechan JL, Ruble CL, Mills CJ, et al. A cluster of novel serotonin receptor 3-like genes on human chromosome 3. Gene 2003;319:137–48.