



HAL
open science

5-HT4 receptors

Joël Bockaert, S. Claeysen, Valerie Compan, Aline Dumuis

► **To cite this version:**

Joël Bockaert, S. Claeysen, Valerie Compan, Aline Dumuis. 5-HT4 receptors. *Current Drug Targets -CNS and Neurological Disorders-*, 2004, 3 (1), pp.39-51. 10.2174/1568007043482615 . hal-01667891

HAL Id: hal-01667891

<https://hal.science/hal-01667891>

Submitted on 20 Feb 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

5-HT₄ RECEPTORS

J. Bockaert*, S. Claeysen, V. Compan, and A. Dumuis

UPR CNRS 2580 – CCIPE, 141 rue de la Cardonille, 34094 Montpellier CEDEX 5, France

* Corresponding author

Email: joel.bockaert@ccipe.cnrs.fr

Phone: 33 4 67 14 29 30, Fax: 33 4 67 54 24 32

UPR CNRS 2580, 141 rue de la Cardonille, 34094 Montpellier CEDEX 5, France

Abstract

Serotonin 4 receptors (5-HT₄Rs) were discovered 15 years ago. They are coded by a very complex gene (700Kb, 38 exons) which generates eight carboxy-terminal variants (a, b, c, d, e, f, g, n). Their sequences differ after position L³⁵⁸. Another variant is characterized by a 14 residue insertion within the extracellular loop 2. Highly selective potent 5-HT₄ receptor antagonists and partial agonists which cross the blood-brain barrier have been synthesized, but a specific full agonist for brain studies is still missing.

Based on physiological and behavioral experiments, 5-HT₄Rs may be targets to treat cognitive deficits, abdominal pain and feeding disorders. One 5-HT₄R-directed drug (SL65.0155) is already in phase II to treat patients suffering from memory deficits or dementia.

Key words: 5-HT₄ receptors; splice variants; G proteins; neurotransmitter release; cognition; pain; anxiety; feeding; CNS drug targets

I. Historical overview

There are several distinct periods in the history of 5-HT₄ receptors (5-HT₄Rs): A short **"Naughty but Nice"** period (1988-1989) (see [1]) characterized by a clear scepticism among many serotonergic specialists about the existence of the receptor. Its birth certificate was difficult to obtain [2]. 5-HT₄R was first described in colliculi and hippocampal neurons as a cAMP stimulatory 5-HT receptor, insensitive to classical 5-HT₁, 5-HT₂ and 5-HT₃ receptor antagonists [3]. The only exception was tropisetron, a potent 5-HT₃R antagonist, which is also a weak 5-HT₄R antagonist (pA₂ = 6.5). Benzamides, such as metoclopramide, renzapride, cisapride and zacopride were rapidly recognized as being potent agonists [4]. This formed a link between these 5-HTRs and an unclassified prokinetic 5-HT receptor in guinea-pig ileum. The latter was insensitive to classical 5-HT₁, 5-HT₂ and 5-HT₃ receptor antagonists, stimulated by benzamides and blocked by μ M concentrations of tropisetron (for a review see [5]).

Then came a **"Flourishing Pharmacological period"** (1990-1997). This was characterized by the description of the distribution of 5-HT₄ receptors in brain and peripheral organs, the analysis of some of their cellular and physiological functions as well as by the synthesis of very specific agonists and antagonists (for reviews see [6, 7]).

An **"Intense Molecular biology period"** (1995-2002) was defined by the cloning of the rodent [8-10] and human [11-14] 5-HT₄R gene. These studies carried out a structural analysis of ligand binding sites [15] and recognized the existence of multiple allosteric conformations which were stabilized by agonists, antagonists and inverse agonists [10, 16]. The 5-HT₄R gene is localized on chromosome 5, bands q31-q33 with both dopamine D₁ and α ₂-adrenergic receptor genes [17] (Fig. 1). There are 38 exons in the 5-HT₄R gene and nine splice variants have been cloned. They represent an important if not unique discovery amongst genes encoding 5-HT receptors [10, 18, 19] (Fig. 1). A common splicing site produces eight splice variants with an identical DNA sequence up to the codon encoding Leu³⁵⁸. On the contrary, the DNA sequence encoding the C-terminus is specific to each splice variant. One of the important issues will be to analyze the functional importance of these splice variants and their potential use as therapeutic targets.

The as yet **"Unwritten period"** needs to be characterized by an extensive analysis of the cellular and physiological functions of 5-HT₄Rs so that their potential for the treatment of

memory deficit, depression, addiction, epilepsy or eating disorders can be accurately assessed. Some 5-HT₄R-directed drugs used to be (cisapride), or are on the market (metoclopramide, tegaserod, which is on the US market under the name of Zelnorm™). Tegaserod is used to treat functional gastro-intestinal disorders (FGIDs) including Irritable Bowel Syndrome (IBS). The introduction of 5-HT₄R drugs for psychiatric and/or neurological diseases seems to be a reasonable possibility.

II. Cloning and localization

II-1. Cloning

The cloning of the rat 5-HT₄R gene was performed in 1995 by Gerald *et al* [8]. They isolated two cDNAs from rats that they called 5-HT_{4S} (387 residues) and 5-HT_{4L} (406 residues) receptors. According to the present NC-IUPHAR recommendations, they are now called 5-HT_{4a} and 5-HT_{4b}. Moreover, Van den Wyngaert *et al.* were the first to correct a sequencing error in the 5-HT_{4b} (388 residues) receptor which is, in fact, only one residue longer than 5-HT_{4a} whatever the species considered, including human [13]. From then on, several laboratories isolated 5-HT₄R cDNA from rat, mouse and human tissues. Alignment of the putative C-termini amino-acid sequences predicted from all their cDNA (except one, see hereafter), revealed that the divergence in their sequence starts after position L³⁵⁸, their last common amino acid. Four C-terminal splice variants have been cloned in mouse, (a, b, e, f) [9, 10], three in rat (a, b, e) [8, 10] and eight C-terminal splice variants in human (a, b, c, d, e, f, g, n) [11, 12, 14, 20-23] (see Fig. **1A**). The 5-HT₄R isoforms are indeed splice variants generated from the same gene, as revealed by the human genome sequencing. The human 5-HT₄R gene is one of the longest the G protein-coupled receptor genes (700kb) and consists of at least 38 exons (Fig. **1B**). Its 5' non-translated region is exceptionally long (>500 kb) and complex compared to other 5-HTR genes [19]. The human 5-HT₁R family has intronless genes [24, 25], the human 5-HT_{2A} gene (>20 kb) consists of three exons [26] and the human 5-HT_{2C} gene (>230 kb) consists of six exons [27]. It must be noted that the gene of the β₂-adrenergic receptor, a prototypal GPCR, is located in the longest intron 20 (Fig. **1B** and [19]). The 5-HT₄R promoter contains potential binding sites for transcription factors (Sp-1, AP-2, AP-4 and GATA) but lacks TATA- and CAAT- boxes.

Variants e, f, and g overlap whereas variants e and f are generated as shorter versions of variant g due to internal 3'-acceptor sites in exon 35 (see Table 1 and insert in Fig. 1B). Note that Bender *et al.* published the correct sequence of 5-HT_{4c} [18]. These authors also revealed the existence of a splice variant within the hepta-helical structure of the receptor, the 5-HT_{4h} receptor [18]. This variant is a result of the insertion of 14 amino acids (exon 30) into the second extracellular loop of the receptor (Fig. 1). This variant is only found in the 5-HT_{4b} isoform and therefore is called the 5-HT_{4hb} variant.

The recent description of a 5-HT_{4n} isoform containing 359 amino-acids (Fig. 1A) can be explained by two different 5'-donor sites at the end of exon 31 (Table 1) [22]. Thus, exon 31' can be generated and contains an in frame stop codon leading to the shorter C-terminal variant (see insert in Fig. 1B).

II-2. Localization

The distribution of 5-HT₄R in the brain has been extensively studied using the labeled antagonists [³H]-GR 113808 and [¹²⁵I]-SB 207710 in rat, mouse, guinea-pig, pig, calf, monkey and human (for a review see [28]). 5-HT₄Rs are expressed in limbic brain structures (hypothalamus, nucleus accumbens, amygdala), islands of Calleja, olfactory tubercle, fundus striati, ventral pallidum, septum, hippocampus, basal ganglia (striatum, globus pallidus) and substantia nigra.

In mice and rats, the [³H]-GR 113808 binding site density is higher in the nucleus accumbens shell than in the core [29]. The olfactory tubercles and fundus striati, like the striatum tail in the next posterior level, also exhibit strong labeling in mice. There are a few differences in the rat striatum where the density is identical in the rostral and caudal part, but higher in its lateral than medial part [29]. In the rat cerebral cortex, there is an average concentration of binding sites with a predominant labeling of the cingulate (prefrontal cortex) as compared to the frontoparietal regions. A laminar pattern was observed in the hippocampal formation. The various hypothalamic and thalamic nuclei show moderate density of receptors. The density is weak in the dorsal and medial raphe nuclei in rodents [28, 30].

We know the cellular and subcellular localizations of 5-HT₄Rs thanks to a diversity of methodological approaches: comparison between 5-HT₄R mRNA (localized in cell bodies) and 5-HT₄R binding site distribution, lesion and electrophysiological studies, as well as single

cell PCR studies. The general observation is that 5-HT₄Rs have a neuronal rather than a glial localization. In the striato-nigral pathways, 5-HT₄Rs are mainly expressed in somato-dendritic compartments of intrinsic striatal neurons, mainly GABAergic, but their presence on cholinergic neurons is also likely. Some 5-HT₄Rs are also present on terminals of striatal GABAergic neurons which project to globus pallidus and substantia nigra [29, 31]. In post-mortem putamen of patients suffering from Huntington's disease [32] there is a 50% loss of 5-HT₄Rs.

In the hippocampus, electrophysiological data indicate an expression in CA1 neurons [33-35] but also in granule cells and other neurons in the dentate gyrus [36]. In the prefrontal cortex, 60% of glutamatergic pyramidal neurons express 5-HT₄Rs as determined by electrophysiological responses [37] and single cell PCR [38]. Much data indicates an effect of 5-HT₄Rs activation on acetylcholine release in cortex [39], and also in hippocampus [35]. This suggests a localization on cholinergic neurons, although indirect effects cannot be excluded.

To better understand the putative differential functions of the different 5-HT₄R splice variants, it will be important to know their differential regional and cellular expression in the brain. Using specific oligonucleotides, but not quantitative PCR, three studies report expression of specific splice variant isoforms. In mouse brain, Claeysen *et al.* found an expression of 5-HT_{4a, b, e, f} isoforms. The 5-HT_{4e, f} isoforms were not detected in the periphery [10]. Bender *et al.* found a complex pattern of expression of human 5-HT₄R isoforms, which was dependent not only on the brain region studied, but also on each individual brain. This raises the possibility that there is an individual polymorphism in the 5-HT₄R variant pattern of expression in man [18]. Using non-quantitative PCR Vilaro *et al.* described a high expression of the 5-HT_{4n} isoform in human brain [22]. A quantitative analysis of mRNA levels has been performed by Medhurst *et al.* using human brain [40]. Expression levels observed for the 5-HT_{4b} splice variant were virtually identical to those obtained with primers that amplified all 5-HT₄ splice variants. The results indicate that 5-HT_{4b} was the dominant variant, followed by the 5-HT_{4a, c, g} variants. Interestingly the pattern of expression of the different variants was brain region specific [40].

III. Multiple structural conformations, pharmacological differences between splice variants, coupling to G proteins, and tool molecules

III-1. Multiple structural conformations

Transfections of the different splice variants in several cell lines revealed that 5-HT₄Rs have a high constitutive activity even when expressed at low and physiological densities (<500 fmol/mg) [10]. At a similar density, they show a much higher constitutive activity than the native and mutated α 2-adrenergic receptors. Therefore, 5-HT₄Rs have a high capacity to isomerize from an inactive (R) to an active (R*) isoform, even in the absence of agonist. We found that 16% of the WT receptors are under the R* form. Interestingly, the short C-terminal isoforms (5-HT_{4e, f} receptors) have a higher constitutive activity than the long C-terminal isoforms (5-HT_{4a, b} receptors). Progressive truncation of the C-terminal further increases the constitutive activity [10]. Several cysteines (Cys³²⁸, Cys³²⁹, and Cys³⁸⁶) of the C-terminal have been shown to be palmitoylated. Cysteine palmitoylation is stimulated by receptor activation [41]. The palmitoylated cysteine 386 is only present in the 5-HT_{4a} receptors splice variant, whereas Cys³²⁸ and Cys³²⁹ are present in all splice variants. Mutations of Cys³²⁸, Cys³²⁹ into Ser, significantly increases the capacity of receptors to convert from the R to the R* form [41, 42]. When tested on constitutively active mutants some 5-HT₄R antagonists have been found to be neutral antagonists (for example, RS 100235 and GR 113808), partial agonists (for example SB 204070) or inverse agonists (for example GR 125487, SB 207266 or RS 116-0086) [10, 43]. Inverse agonists have been proposed to stabilize the R form of GPCRs and therefore to reduce their constitutive activity. We found that 5-HT₄R inverse agonists stabilize a third "silent" form. A specific transmembrane (TM) network, implicated in the stabilization of this "silent" form but not the stabilization of the R* form, has been described. It includes, the Asp¹⁰⁰(3.32) in TMIII, Phe²⁷⁵(6.51) in TMVI and Trp²⁷²(6.48) in TMVI [16]. D100 is highly conserved within the biogenic amine receptor family and is indeed necessary for the binding and activity of 5-HT, tryptamines and small indole carbazimidamides. However, D100 is not necessary for the binding of benzamides and other non-indole 5-HT₄ agonists and antagonists. These ligands have affinities in the range of nanomolar concentrations for a mutated 5-HT₄R D100A [44]. More interestingly, two antagonists of native 5-HT₄Rs are agonists of this mutated Gs-coupled receptor [44]. The mutated 5-HT₄R D100A can be added to a class of mutated GPCRs called RASSLs (Receptors Activated Solely by Synthetic Ligands). The idea is to engineer receptors (RASSLs) that would be insensitive to their endogenous ligands but can be fully activated by

synthetic ligands [45]. Heterogeneously expressed in tissues, they can be used to control signal transduction and cellular physiology in a research or therapeutic perspective.

III-2. Pharmacology and coupling to G-proteins

Several reviews describe in detail the pharmacology of 5-HT₄Rs [6, 46]. We will only discuss the possible difference in pharmacology of the splice variants and the "non G α s", described signaling pathways (see Table 2).

All the 5-HT₄R splice variants have the transmembrane domain core and therefore the same primary binding site for 5-HT as well as for most of the synthetic ligands. In binding assays, the pharmacology of the variants (including the 5-HT_{4hb}) [18] are not significantly different. However, when functional studies are considered, some differences have been noted in their signaling properties: 1) as already discussed, the constitutive activity of the variants depends on the sequence of their C-terminal [10]; 2) the classical 5-HT₄R neutral antagonist, GR 113808, is a partial agonist on the 5-HT_{4hb} variant [18]; 3) renzapride is a full agonist on the 5-HT_{4b} and 5-HT_{4d} isoforms and a partial agonist on the 5-HT_{4a} and 5-HT_{4e/g} isoforms [20]; 4) 5-methoxytryptamine and prucalopride are more potent on the 5-HT_{4b} variant, whereas SDZ-HTF 919 and SB 204070 are more potent on the 5-HT_{4a} variant [47]; 5) in CHO cells, a selective benzodioxanoxadiazolone (SL 65.0155) is an inverse agonist on 5-HT_{4a}R but a partial agonist on 5-HT_{4b} and 5-HT_{4e} variants [48]; 6) a difference in desensitization of the splice variants is reported in CHO cells, the 5-HT_{4d} variant is more rapidly uncoupled to adenylyl cyclase than the 5-HT_{4e} variant [49]. In these cells, the desensitization is PKA-dependent. Note that in colliculi neurons, the desensitization of 5-HT₄Rs is homologous and thus results in an action of G-protein kinase (GRK), independent on PKA activity [50]; 7) an interesting set of data reports that in HEK 293 cells, 5-HT_{4b} but not 5-HT_{4a} variants are coupled to G α i/G α o [47] (Table 2); 8) the 5-HT_{4a} variant is much more potent than 5-HT_{4b} in increasing membrane bound Ca²⁺ channel activity in HEK 293 cells [47] (Table 2); 8) in COS cells, 5-HT_{4b} variant activates the ras and rap1 pathways, via PKA [51] (the other variants have not been tested). The activation of ras but not rap1 led to the downstream activation of ERK1/2 (Table 2); 9) finally, the 5-HT_{4a} variant (the other variants have not been tested) has been shown to be coupled to G α 13 in Sf9 cells, but also in mammalian cells, including the neuroblastoma x glioma NIE-115 and fibroblasts NIH 3T3 (Table 2). In NIE-115 cells, 5-HT_{4a}Rs provoke a RhoA-dependent neurite retraction and cell rounding [52].

Although interesting, all of the described experiments were performed in heterologous systems and often used overexpression. They indicate that differences in coupling and pharmacology are "possible" but need to be demonstrated in native cells and tissues before their true relevance can be judged.

In a recent series of experiments in brain, we were able to identify GPCR interacting proteins (GIPs) using the different C-termini of 5-HT_{4a, b, e} splice variants as bait. The last C-terminus residues of 5-HT_{4a}R (SCF) and 5-HT_{4e}R (PVPV) correspond to one of the PDZ (type I and type II ligands, respectively [53]). On the contrary, the 5-HT_{4b} C-terminus (SQS) is not typical of a PDZ ligand. Interestingly, proteins associated with the different C-termini of different variants suggest that some functional differences mediated via these GIPs may exist (L. Joubert *et al.* in preparation).

IV. 5-HT₄ receptor ligands

Structure–activity relationship studies (SAR) of 5-HT₄ receptor ligands have been recently reported (for reviews, see [46, 54]). Here, we describe the significant progress made over the last decade in discovering potent and selective 5-HT₄R agonists, antagonists and inverse agonists and point out those that are commercially available and those whose potential use in CNS disorders has yet to be determined (Table 3).

As previously noted, the 5-HT₄R ligands are currently related, with some exceptions, to five main chemical classes: the indoles (including analogues of 5-HT, the indole carboxylates and the indole carboxamides), the benzamides, the benzoates, the aryl ketones and the benzimidazolones.

IV-1. Agonists

The first class of 5-HT₄R agonists includes the substituted tryptamines or 5-HT analogues, as well as the substituted indole carbazimidamides. 5-Methoxytryptamine is an interesting tool because it lacks affinity for the 5-HT₃R [55]. From a series of indole carbazimidamide derivatives, HTF 919 (tegaserod) was found to be a potent partial agonist with high affinity at 5-HT₄R. It is 40- to 80-fold selective over the 5-HT_{1A} receptor, 25-fold selective over the 5-HT_{2C} and has no appreciable affinity for 5-HT₃ or dopamine receptors [56, 57]. *In vivo* studies

show that tegaserod enhances gastrointestinal motor activity and normalizes impaired motility throughout the gastrointestinal tract. Tegaserod maleate or Zelnorm™ is prescribed for IBS [58]. Further pharmacokinetic investigations in animals revealed a negligible penetration into the brain and so do not represent a useful tool for CNS research. The second class of 5-HT₄R agonists includes the first generation benzamides bearing the 2-methoxy-4-amino-5-chloro substitution [4]. These benzamides are found to be non-selective and always have antagonistic activity at the 5-HT₃ receptor (zacopride, renzapride and metoclopramide). Structural modification of the basic chain of the benzamides, cisapride SB 205149 and SC 53116 lead to the identification of more potent 5-HT₄R agonists with lower affinity for 5-HT₃ than the first generation benzamides [59-61]. Nevertheless, cisapride has appreciable affinity for 5-HT_{2A} and α_1 -adrenergic receptors.

Interestingly, newer benzamides with large substitutions in their basic chain, such as prucalopride, mosapride and Y-34959, are highly selective but show varying degrees of intrinsic activity depending on the *in vitro* preparations of the 5-HT₄R used [62-64].

When the amide linkage in benzamide was substituted by an ester, this led to benzoate derivatives with an increased affinity for 5-HT₄R. The first of this series was ML 10302 with a high affinity for 5-HT₄ receptors and a weak affinity for 5-HT₃ receptors [65]. ML 10302 was also inactive at other 5-HT receptor subtypes. ML 10302 was characterized as being the first potent and selective 5-HT₄R partial agonist but its susceptibility to hydrolysis limited its utility for *in vivo* investigation. Recently, Moser and coworkers synthesized SL65.0155, a novel compound with high affinity for 5-HT₄R related to the benzodioxanes[48]. This compound acts as a partial agonist with a remarkable selectivity over other receptor subtypes, except for the σ receptor and α_{1D} -adrenoceptor.

In the fourth class, the benzimidazolones, BIMU 8 and BIMU 1 are potent and efficacious 5-HT₄R agonists [66, 67] that enter the CNS. However, their high affinity for the 5-HT₃R and σ_2 recognition site limits their use for CNS applications [68, 69].

The aryl ketones, related to the 5-HT₄R ester ligands, were prepared in an attempt to overcome their metabolic lability: replacing the ester linkage of the antagonist RS 23597 produced RS 17017 which has a similar affinity for the 5-HT₄R but has partial agonist activity [70].

Increasing the size of the alkyl group led to a progressive increase in agonist activity with maximal efficacy being achieved with RS 67333 and RS 67506 [71]. They were found to have good selectivity and bioavailability. The incorporation of an n-butyl group on the piperidine ring increases hydrophobicity and, consequently, brain penetration [72, 73]. However, the compounds also bind to σ_1 and σ_2 receptors [71].

IV-2. Antagonists

In the indole class, GR 113808 was the first 5-HT₄R antagonist with a high affinity for 5-HT₄R and a low affinity for 5-HT₃R [74]. This indole ester provided a pharmacophore model that assisted the search for other selective 5-HT₄R antagonists. Furthermore, [³H]GR 113808 was the first commercially available radioligand for 5-HT₄R-binding studies [75]. The indole ester analogue, GR 125487 is more potent in the CNS and in the periphery, and also has higher bioavailability, presumably because the ester linkage is more stable [76]. SB 203186 is an indole ester with potent and selective 5-HT₄R antagonist properties in various physiological assays but its short half-life limits its use *in vivo* [77]. In contrast, SB 207266 is a highly potent and selective antagonist with a long duration of action after oral administration [78]. SB 207266 is a very useful tool for research in the CNS [79, 80]. Recently, the indazole carboxamide, LY 353433 was also found to be a potent and selective 5-HT₄R antagonist with clinically suitable pharmacokinetics [81, 82].

Surprisingly, there are no 5-HT₄ receptor antagonists among the benzamides which only display agonistic activity [4]. However, it is interesting to note that when the amide is substituted by an ester, the compound becomes an antagonist. The first example was the 5-HT₄R antagonist SDZ 205557 which is the ester analogue of metoclopramide [83]. Increasing the size of the substituent results in an increase in the affinity and selectivity of the drugs. RS 23597, ML 10375 and SB 204070 are good example [86]. The corresponding iodinated compound [¹²⁵I] SB 207710 is an excellent radioligand for binding studies with a remarkable selectivity (>5000-fold) over the other receptor subtypes [87]. The metabolic lability of the ester function leads, however, to limitations with respect to *in vivo* studies. Lack of selectivity of the benzimidazolone derivatives (DAU 6215, DAU 6285) compromised their utility for 5-HT₄R antagonist studies [88, 89]. New benzimidazole carboxamides and carboxylates have recently been synthesized by Lopez Rodriguez *et al.* [90, 91]. Some of them exhibit high affinity (sub-nanomolar) for the 5-HT₄ binding site and no significant affinity for the 5-HT₃R.

The aryl ketone class contains molecules that present several advantages; first the replacement of the ester by a ketone provides excellent oral bioavailability. Secondly they enter the CNS following systemic administration. Over the last five years, Roche Bioscience has achieved higher affinity, selectivity and bioavailability as shown by RS 39604 which has subnanomolar affinity and is now commercially available [92] together with RS 100235 and RS 100302 [54, 93].

A new series of tricyclic benzonaphthyridine and azepinoquinoline derivatives has been synthesized (e.g. ATBI 221). They have a very high affinity for the 5-HT₄R and a high selectivity over other 5-HTR subtypes and non-serotonergic receptors. The connection of the acyl group with the piperidine ring through an iminoether linkage provides high bioavailability and brain penetration [94].

IV-3. Inverse agonists.

Three specific, high affinity 5-HT₄R antagonists (GR 125487, SB 207266 and ML 10375) have been reported to exert inverse agonist activity [43, 95]. Recently Roche Bioscience have synthesized three potent 5-HT₄R inverse agonists, RO 116-2617, RO 116-0086 and RO 116-1148 [16]. RO 116-0086 is the des-amino, des-chloro derivative of the antagonist, SB 204070, and RO 116-1148 is the corresponding amide.

V. Control of neuronal ion and receptor channel activity and synaptic transmission

5-HT₄Rs have been shown to regulate several ion currents and channels in native neurons (Table 2). In colliculi neurons and in CA1 pyramidal neurons of hippocampus, 5-HT₄Rs reduce the after-hyperpolarization (AHP) that follows action potentials [96-98]. In colliculi neurons, a concomitant broadening of action potentials and a reduction of spike accommodation were observed [98]. These activities are mediated by cAMP and PKA activation and contribute to increased neuronal excitability. They can be explained by inhibition of K⁺ channels including Ca²⁺-activated K⁺ channels [33, 98]. In CA1 neurons, a decrease in the calcium-induced calcium release from intracellular stores contributes to the action of cAMP on AHP [33]. In colliculi neurons, a remarkable observation was that short term application of 5-HT₄ receptor agonists or 8-Br-cAMP produces a long lasting inhibition of K⁺ channels due to a long lasting blockade of phosphatases [98]. In addition to a reduction

in AHP, 5-HT₄Rs also increase depolarization in hippocampal CA1 neurons; one group has provided evidence for an activation of the hyperpolarization-activated current I_h by 5-HT₄Rs as a mechanism of this depolarization (in mice) [34]. All these electrophysiological effects could influence neuronal excitability and facilitation of neurotransmitter release (see section VI).

Recently, two reports described the effects of 5-HT₄Rs on GABA_A receptor activity and GABAergic synaptic transmission. In prefrontal cortex pyramidal neurons, 5-HT₄Rs have a bi-directional (activation and inhibition) regulation of GABA_A receptor channels and GABAergic synaptic transmission (measured by the amplitude of the spontaneous GABA_A receptor mediated inhibitory postsynaptic potentials (IPSPs)) [37]. The authors present evidence for a role of PKA as well as AKAP (A Kinase Anchoring Proteins) in both effects. In guinea-pig dentate gyrus neurons, including granule cells, 5-HT₄Rs have a different effect on GABAergic transmission. Their activation increases the frequency of GABA_A-mediated IPSPs [36]. This is a pre-synaptic effect. The latter can be explained by the effects on K⁺ channels described above and by data showing a stimulation of GABA release by 5-HT₄R agonists (see section VI).

A conditional regulation of dorsal raphe nucleus 5-HT neuronal activity has also been observed following intravenous injection of 5-HT₄R ligands. When the basal firing rate of raphe neurons was high, 5-HT₄R agonists and antagonists enhanced and reduced the firing rate, respectively [99]. In contrast, when the firing rate of the raphe neurons was low, the same drugs were inactive. These results indicate that 5-HT₄Rs exert both a tonic and a phasic positive, frequency-related control of 5-HT neurons raising the possibility that 5-HT₄R may constitute a possible therapeutic target to treat depression.

All these results favor an intriguing role for 5-HT₄Rs in the fine tuning of neuronal activity. They clearly indicate that their physiological effect may depend on the nature and activity of the neurons in which they are expressed, perhaps explaining why 5-HT₄R ligands are active in very specific situations (morphine-treated animals, aged animals, scopolamine-induced cognitive deficits, for example).

The long-term potentiation (LTP) of the Schaffer collateral-CA1 synaptic transmission, as well as the population spike amplitude in the CA1 field evoked by Schaffer collateral

stimulation is enhanced by the 5-HT₄R agonist, SC 53116 [35]. The 5-HT₄R augmented LTP effect was blocked both by a 5-HT₄R antagonist as well as by the muscarinic antagonist, scopolamine. These findings suggest functional interactions between the serotonergic system mediated via 5-HT₄Rs and cholinergic systems associated with cognitive processes.

A very interesting effect of 5-HT₄Rs on a subpopulation of DRG (dorsal root ganglion) neurons has been described [101]. These DRG are sensitive to capsaicin and express tetrodotoxin (TTX)-insensitive Na⁺ channels. They represent subclasses of A δ - and C-type afferent nociceptors. 5-HT via 5-HT₄Rs and prostaglandins increases the activity of these particular Na⁺ channels. The mechanisms by which 5-HT₄Rs act are unclear. The 5-HT effect was delayed by 20-30s and was not mimicked by cAMP. This suggests that 5-HT₄Rs may be involved in the generation of hyperesthesia.

VI. Control of neurotransmitter release

VI-1. Acetylcholine release

In line with the effects of 5-HT₄Rs on K⁺ channels and neuronal excitability, there are numerous reports demonstrating the ability of 5-HT₄Rs to modulate the release of various neurotransmitters. Because of earlier suggestions that these receptors may be implicated in cognitive functions, and the fact that they facilitate ACh release in gastro-intestinal tract, central ACh release has been particularly studied. Consolo *et al.* were the first to report that i.c.v. injections of the 5-HT₄R agonist / 5-HT₃R antagonist, BIMU-1 caused ACh release in the frontal cortex [39]. Yamaguchi *et al.* raised extracellular 5-HT with the 5-HT re-uptake blockers (citalopram or indeloxazine) or a releaser (p-chloroamphetamine) and found that in the frontal cortex, the 5-HT₄R antagonists, RS 23597 and GR 113808, blocked the associated release in ACh [104]. In the hippocampus, one report found no ACh release following i.c.v. injection of BIMU-1 [39], whereas another group found an increase in ACh release by a local application of the agonist, SC 53116 [35]. As already discussed, the same authors found that the SC 53116-induced enhancement of LTP was mediated by a cholinergic mechanism. In guinea pig brain slices, BIMU-8 increases the electrically-stimulated ACh outflow in cerebral cortex, hippocampus and nucleus basalis magnocellularis slices [105]. In the hippocampus, 5-HT₄Rs-induce ACh release and stimulate and inhibit GABA release via muscarinic M1 and M4 receptors, respectively [106]. 5-HT₄R antagonists did not decrease ACh release under

basal conditions. This indicates that 5-HT₄R do not exert a tonic effect on cholinergic neurons.

VI-2. Dopamine release

There are numerous studies that have reported an effect of 5-HT₄R agonists on DA release. However, a simple picture of the results is difficult to make due to the various experimental conditions used in the studies and also some contradictory results. In general, *in vitro* and *in vivo* studies have reported that when 5-HT or 5-HT₄R agonists are locally applied to the striatum or substantia nigra, a 5-HT₄R-mediated increase in DA release is observed (see [25] [99, 107]). Systemic administration of 5-HT₄R agonists and antagonists is usually ineffective. Striatal DA release induced by haloperidol or morphine but not that induced by cocaine or amphetamine, is reduced by intraperitoneal injection of 5-HT₄R antagonists and increased by intraperitoneal injection of a 5-HT₄R agonist [107, 108]. Using identical protocols, mesoaccumbal dopaminergic function was not regulated by 5-HT₄R ligands. To be able to specifically activate either the nigro-striatal or the mesoaccumbal pathways may be of great therapeutic interest. However, note that systemic injection or local perfusion of the 5-HT₄R antagonist, SDZ 205557 into the nucleus accumbens shell inhibits cocaine-induced hyperactivity. This result could be explained by an effect of SDZ 205557 on 5-HT₃R or by an unknown indirect effect [109].

The cellular mechanisms by which the state-dependent facilitatory control of the nigro-striatal pathway occurs are still unclear. The finding that 5-HT₄R are able to influence DA release in striatal slice preparations indicates that they may control local circuitry within the striatum (cholinergic or GABAergic neurons) (see [25]). Indeed, 5-HT₄R are present on cell bodies of striatal neurons. However, the finding that 5-HT₄R modulate the firing rate of nigral DA neurons raises the possibility that striatal 5-HT₄ receptors also affect DA release through an indirect control of DA neurons [107] via modulation of the activity of striatal efferent GABAergic neurons (striato-pallidal or striato-nigral).

VI-3. Serotonin release

The activation of 5-HT₄R increases 5-HT release in the hippocampus while the 5-HT₄R antagonists, GR 113808 and GR 125487, reduced basal 5-HT release. This indicates the presence of an endogenous tone on 5-HT₄R in this preparation [110], perhaps consistent with

the finding that 5-HT₄ binding sites increase in number after the innervation by serotonin neurons has been abolished [29].

VII. 5-HT₄Rs: potential therapeutic CNS drugs targets

VII-1. Cognition

We proposed several years ago that 5-HT₄Rs may have pro-cognitive properties [113, 114]. More recent data support this possibility [35, 100]. 5-HT₄Rs are localized in numerous structures, such as hippocampus and cortex, that are considered important for mnemonic processes. In the hippocampus, localization and functional studies indicate that they play a modulatory role on LTP formation. The latter effect suggests the involvement of a cholinergic relay. 5-HT₄Rs stimulate ACh release in frontal cortex and hippocampus. There is evidence for a loss of 5-HT₄Rs in Alzheimer's disease in the cortex (-23%) and, particularly, in the hippocampus (-66%) [31]. Furthermore, in transfected CHO cells, 5-HT₄Rs were found to stimulate the secretion of non-amyloidogenic soluble amyloid precursor protein (sAPP α), thought to have neuroprotective and memory enhancing effects [115]. Behavioral experiments have generally confirmed a pro-cognitive effect of 5-HT₄R ligands with several aspects of memory performance being modulated.

1. Memory acquisition

- BIMU-1 (5-HT₄ agonist / 5-HT₃ antagonist) increased social olfactory memory in rats when administered before training. This effect was reversed by GR 125487, a specific 5-HT₄R antagonist [116].
- BIMU-1 and BIMU-8 enhanced acquisition of an auto-shaping task when injected before training. This effect was blocked by GR 125487 [117].
- BIMU-1 and BIMU-8 as well as the specific 5-HT₄R partial agonist, RS 67333, induce a substantial improvement of acquisition of an olfactory associative memory when injected before training [118, 119] (Fig. 2).
- RS 67333 improved the acquisition of place and object recognition memory in young and old rats. This effect was blocked by GR 125487 [120].
- RS 67333 accelerated learning acquisition in the Morris water maze task when the inter-trial intervals were high (2 h), but not low (30sec). This indicates that

5-HT₄R agonists can improve memory processes in a high demand memory task [121].

- RS 17017 (a relatively hydrophilic 5-HT₄R agonist) administered orally and chronically enhanced delayed matching performance in young as well as in old macaques [122].

2. Memory deficit

- RS 67333 reversed the performance deficit induced by atropine in the Morris water maze test, an effect eliminated by the 5-HT₄R antagonist, RS 67532 [73].
- RS 67333 and, to a lesser extent, RS 17017, reversed the deficit in the acquisition of olfactory associative memory induced by the 5-HT₄ antagonist, RS 67532 [119] (Fig. 2).
- The 5-HT₄R agonist, SC 53116 improved the scopolamine-induced deficit in the passive avoidance test [35].
- BIMU1 and RS 67333 reversed scopolamine-induced impairments in spontaneous working memory, confirming a role for 5-HT₄R in cholinergically-mediated memory processes.

Taken together, the results suggest that there could be a potential therapeutic role for 5-HT₄ agonists in restoring memory deficits related to short-term working memory disorders [123].

3. Memory consolidation

The effect of 5-HT₄R ligands on memory consolidation is less clear. Meneses and Hong [117] reported that BIMU-1 and -8 have a negative effect on an auto-shaping learning task when injected during the consolidation phase. In contrast, Lamirault and Simon [120] found an increased consolidation of object recognition in old rats with RS 67333.

Sanofi-Synthelabo is currently testing SL 65.0155 (Phase II) in an attempt to treat dementia of the Alzheimer type. The compound does not induce any change in either rat gastrointestinal peristalsis or in rat and dog cardiac rhythm [48]. In particular, SL 65.0155 has been found to alleviate cognitive deficits in aged rats using a linear maze test, as well as scopolamine-induced deficits in Morris water maze performance [48]. Moreover, SL 65.0155 and the cholinesterase inhibitor rivastigmine have a synergic effect in the object recognition test (Fig.

2) and in linear maze performances in aged rats [48]. Similarly, combined treatment with galanthaminium, a new cholinesterase inhibitor and RS 67333, enhanced place and object recognition in young and aged rats [124]. These synergisms are particularly interesting as they suggest a possible co-treatment in Alzheimer's disease and offer the possibility of reducing the therapeutic dose of cholinesterase inhibitors and, consequently, their side effects.

VII-2. Pain

A centrally-mediated antinociceptive effect of both the 5-HT₄R agonists and the 5-HT₃R antagonists BIMU-1 and -8 has been reported following noxious thermal (hot-plate test), chemical, (abdominal constriction test) and mechanical (paw pressure test) stimuli [125]. However, the effects of BIMU-1 and -8 may be related to their inactivation of 5-HT₃ receptors; although the 5-HT₃R / 5-HT₄R antagonist, SDZ 205557, and a new selective 5-HT₄R antagonist, ATBI 221, have been found to reduce visceral pain [94, 126]. An increase in 5-HT₄R mRNA has been found in dorsal root ganglia following hind paw inflammation. The partial 5-HT₄R agonist, tegaserod (ZelnormTM), which could be acting as an antagonist, is reported to reduce the symptoms of abdominal pain, bloating and constipation [58, 127]. Tegaserod may produce abdominal analgesia *via* a reduction in the activity of extrinsic spinal afferents to the colon. Overall, the results suggest that an inactivation of 5-HT₄Rs may decrease some types of clinical pain.

VII-3. Anxiety and Stress

The selective 5-HT₄R antagonists SB 204070, SB 207266 and GR 113808 display anxiolytic-like effects in the elevated plus-maze test, albeit at high doses [128, 129]. In contrast, both SDZ 205-557 and SB 204070 have been reported to attenuate the effects of diazepam and did not modify behavior when administered alone [130]. Similarly, stress-induced anxiety-like behavior, was enhanced in the 5-HT₄R knockout male mice, again using the elevated plus-maze test ([131] and in preparation). Complex and bi-directional modulation of GABAergic systems by 5-HT₄ receptors, as described by Cai *et al.*, may account for such discrepancies [37].

VIII-4. Reward and motor behavior

The high density of 5-HT₄Rs in the striatum and nucleus accumbens suggests an involvement in the control of reward and motor behavior. However, the supporting data is not clear and is often contradictory. Under basal conditions, no effect on locomotion has been detected

following the administration of the partial 5-HT₄ receptor agonists, RS 67333 or RS 67506 [73, 109]. Similarly, the 5-HT₄R antagonist, SB 204070 did not alter amphetamine-induced changes in rat locomotor activity or rotational behavior induced by the unilateral lesion of the nigrostriatal dopaminergic projection [132]. Conversely, it has been shown that hyperlocomotion following 5-MeOT administration was abolished or attenuated by local injection into the hippocampus of the 5-HT₄R antagonists GR 125487, SB 204070 or RS 23597 [133]. We have further found that 5-HT₄R knockout mice do not exhibit a change in locomotion in their home cage but show a marked hyposensitivity to novelty-induced locomotion in the open-field, elevated plus-maze and alley tests. Given the high levels of 5-HT₄ receptors in the shell of the nucleus accumbens [29] and taking into account that 5-HT₄R null mice have been found to be less sensitive to stress-induced anorexia [131], it is hypothesized that 5-HT₄R null mice will be less sensitive to the motor, rewarding and/or addictive properties of cocaine.

Conclusion

Although 5-HT₄Rs are the targets for earlier (metoclopramide, cisapride) or recent (tegaserod) therapeutic drugs to treat gastrointestinal diseases, as yet no 5-HT₄R ligands are used to treat CNS disorders. This is probably due to the lack of availability of specific and stable drugs able to cross the blood brain barrier (Table 3). To our knowledge, SL 65.0155, which is being tested for dementia of the Alzheimer types [48], is the only 5-HT₄ receptor ligand in development (phase II) for a CNS disorder. However, in the light of the emerging profile of the behavioral pharmacology of 5-HT₄ receptor ligands, the possibility that they may also be of therapeutic value in pain, especially of intestinal origin, anxiety and other stress-related disorders cannot be dismissed.

Legends to Tables

TABLE 1

Exon-intron boundaries of the human 5-HT₄ receptor gene.

From [18, 19]. Completed using NCBI Human Genome Resources:

(<http://www.ncbi.nlm.nih.gov/genome/guide/human/>) and Model Maker for HTR4.

This table presents all the identified splicing sites and the corresponding exons in the human 5-HT₄R gene. Exons 1 to 25 correspond to the 5'-untranslated region (5'UTR) and promoter region of the gene. Exons 26, 27, 28, 29, and 31 are present in all the splice variants cloned and constitute the common part of the receptor. Exon 30 and exons 31' to 38 are alternatively spliced to produce the 8 human splice variants cloned to date.

Splice donor and acceptor sequences between exons (uppercase) and introns (lowercase) are given in bold. The size of exons and introns are indicated.

ND: not determined.

TABLE 2

5-HT₄Rs signaling in excitable cells and cell lines. For details see the text

TABLE 3

5-HT₄Rs ligands that could be of interest in CNS research

A range of affinities taken from the literature (pK_i and pEC₅₀) is indicated for several ligands.

(F): full agonist; (P): partial agonist; (S): specific; (HS): highly specific; (1): commercially available; CNS: useful in CNS research.

Legend to figures

FIGURE 1

Human 5-HT₄ receptor splice variants and structure of the gene

A. C-terminal amino acid sequences and schematic representation of the human splice variants. The cloned 5-HT₄Rs have identical sequences up to Leu³⁵⁸ and differ by the length and composition of their C-terminal domain. The 5-HT_{4h} variant, which has an insertion of 14 residues in the second extracellular loop, has only been isolated in combination with the b isoform. This variant is called 5-HT_{4hb}.

B. Organization of the human 5-HT₄R gene. From [18] and [19]. Completed using NCBI Human Genome Resources: (<http://www.ncbi.nlm.nih.gov/genome/guide/human/>) and Model Maker for HTR4.

Upper panel: The locations of exons are indicated as filled bars with numbers denoted over or under the bars. Letters indicate C-terminal splice variant exons. The discontinuous part of the thick line indicates large gaps in intron 17 (>10kb) and 20 (>100kb). ATG indicates the position of the translation start site. The location of the β₂-adrenergic receptor gene (β₂ AdR) is shown over the solid bar with an arrow indicating the transcriptional direction.

Middle panel: Schematic representation of the exons (boxes) present in the coding sequences of 5-HT₄R variants. The exons in gray are always present and constitute the common part of all the receptor splice variants. Letters indicate which exon is conserved to generate the corresponding isoform.

Left insert: Variant n is generated by the use of an alternative 5'-donor site for intron 31. An in-frame stop codon leads to the shorter C-terminal domain. Exons are in uppercase, introns are in lower case.

Right insert: The use of different 3'-acceptor sites for intron 34 generates three different exon possibilities (35, 36 and 37) and three corresponding splice variants (g, e and f). Exons are in uppercase, introns are in lower case.

FIGURE 2

5-HT₄Rs and cognition

This figure illustrates two experiments showing the positive effects of 5-HT₄Rs in cognition.

Olfactory associative task: In this task, rats, deprived of water for 48 hours, were trained to make two odor-reward associations. Each odor had to be associated with a specific reward, one arbitrary designed as positive (S+) and the other as negative (S-). Rats had to approach

the odor and water ports to interrupt the light beam only when S+ is delivered. Response to odor designated as negative resulted in presentation of non-aversive light, and water was not delivered. Individual trials were presented in a quasi-random fashion. A daily session was made up of 60 trials and five sessions were made with one session per day. The latency to give a response was analyzed and given as mean latencies in seconds for the positive and negative odors. In controls a significant latency difference between S+ and S- was only observed from session 4. After injection of the partial agonist RS 67333 the difference was observed after session 3. After injection of the antagonist RS 67532 the difference was only observed after session 5 whereas a situation similar to the control was observed when both the partial agonist and the antagonist were co- injected (modified from [119]).

Synergy between subliminal doses of a 5-HT₄ partial agonist (SL 65.0155) and an inhibitor of cholinesterase (rivastigmine) in the object recognition test: Each experiment consisted of 3 sessions. During the first session the animals were allowed to become familiar with the box. Twenty-four hours later, the animals were again placed in the enclosure in the presence of two identical objects for the amount of time necessary to spend 20s exploring these objects. After another interval of 24 hours, the rats were again placed in the box with a previously presented object and a novel object. Time spent exploring the familiar object and the novel object was measured. Drugs (SL 65.0155: 0.1 μ g/kg po and rivastigmine: 0.03mg/kg ip) were administered separately or together before each session (modified from [48]).

References

- [1] Clarke, D.E.; Craig, D.A.; Fozard, J.R. *Trends Pharmacol. Sci.*, **1989**, *10*, 385.
- [2] Hoyer, D.; Fozard, J.R.; Saxena, P.R.; Mylecharane, E.J.; Clarke, D.E.; Martin, G.R.Humphrey, P.P.A. *Pharmacol. Rev.*, **1993**, volume?, page?
- [3] Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. *Mol. Pharmacol.*, **1988**, *34*, 880.
- [4] Dumuis, A.; Sebben, M.; Bockaert, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1989**, *340*, 403.
- [5] Ford, A.P.D.W.; Clarke, D.E. *Med. Res. Rev.*, **1993**, *13*, 633.
- [6] Bockaert, J.; Fagni, L.; Dumuis, A. In *Handbook of experimental pharmacology: Serotonergic neurons and 5-HT receptors in the CNS*; Baumgarten, H.G.Göthert, M., Berlin Heidelberg New York, **1997**; Vol. *129*, pp. 439.
- [7] Eglen, R.M.; Wong, E.H.F.; Dumuis, A.; Bockaert, J. *Trends Pharmacol. Sci.*, **1995**, *16*, 391.
- [8] Gerald, C.; Adham, N.; Kao, H.T.; Olsen, M.A.; Laz, T.M.; Schechter, L.E.; Bard, J.A.; Vaysse, P.J.; Hartig, P.R.; Branchek, T.A. *et al. Embo J*, **1995**, *14*, 2806.
- [9] Claeyens, S.; Sebben, M.; Journot, L.; Bockaert, J.; Dumuis, A. *FEBS Lett.*, **1996**, *398*, 19.
- [10] Claeyens, S.; Sebben, M.; Becamel, C.; Bockaert, J.; Dumuis, A. *Mol. Pharmacol.*, **1999**, *55*, 910.
- [11] Blondel, O.; Vandecasteele, G.; Gastineau, M.; Leclerc, S.; Dahmoune, Y.; Langlois, M.; Fischmeister, R. *FEBS Lett.*, **1997**, *412*, 465.
- [12] Claeyens, S.; Faye, P.; Sebben, M.; Lemaire, S.; Bockaert, J.; Dumuis, A. *NeuroReport*, **1997**, *8*, 3189.
- [13] Van den Wyngaert, I.; Gommeren, W.; Verhasselt, P.; Jurzak, M.; Leysen, J.; Luyten, W.; Bender, E. *J. Neurochem.*, **1997**, *69*, 1810.
- [14] Blondel, O.; Gastineau, M.; Dahmoune, Y.; Langlois, M.; Fischmeister, R. *J. Neurochem.*, **1998**, *70*, 2252.
- [15] Mialet, J.; Dahmoune, Y.; Lezoualc'h, F.; Berque-Bestel, I.; Eftekhari, P.; Hoebeke, J.; Sicsic, S.; Langlois, M.; Fischmeister, R. *Br J Pharmacol*, **2000**, *130*, 527.
- [16] Joubert, L.; Claeyens, S.; Sebben, M.; Bessis, A.S.; Clark, R.D.; Martin, R.S.; Bockaert, J.; Dumuis, A. *J Biol Chem*, **2002**, *277*, 25502.
- [17] Claeyens, S.; Faye, P.; Sebben, M.; Lemaire, S.; Bockaert, J.; Dumuis, A.; Taviaux, S. *Cytogenet. Cell Genet.*, **1997**, *78*, 133.
- [18] Bender, E.; Pindon, A.; van Oers, I.; Zhang, Y.B.; Gommeren, W.; Verhasselt, P.; Jurzak, M.; Leysen, J.; Luyten, W. *J Neurochem*, **2000**, *74*, 478.
- [19] Hiroi, T.; Hayashi-Kobayashi, N.; Nagumo, S.; Ino, M.; Okawa, Y.; Aoba, A.; Matsui, H. *Biochem Biophys Res Commun*, **2001**, *289*, 337.
- [20] Mialet, J.; Berque-Bestel, I.; Eftekhari, P.; Gastineau, M.; Giner, M.; Dahmoune, Y.; Donzeau-Gouge, P.; Hoebeke, J.; Langlois, M.; Sicsic, S.; Fischmeister, R.; Lezoualc'h, F. *Br J Pharmacol*, **2000**, *129*, 771.
- [21] Mialet, J.; Berque-Bestel, I.; Sicsic, S.; Langlois, M.; Fischmeister, R.; Lezoualc'h, F. *Br J Pharmacol*, **2000**, *131*, 827.
- [22] Vilaro, M.T.; Domenech, T.; Palacios, J.M.; Mengod, G. *Neuropharmacology*, **2002**, *42*, 60.
- [23] Bach, T.; Syversveen, T.; Kvingedal, A.M.; Krobert, K.A.; Brattelid, T.; Kaumann, A.J.; Levy, F.O. *Naunyn Schmiedeberg's Arch Pharmacol*, **2001**, *363*, 146.
- [24] Hoyer, D.; Martin, G. *Neuropharmacology*, **1997**, *36*, 419.
- [25] Barnes, N.M.; Sharp, T. *Neuropharmacology*, **1999**, *38*, 1083.
- [26] Chen, K.; Yang, W.; Grimsby, J.; Shih, J.C. *Brain Res Mol Brain Res*, **1992**, *14*, 20.

- [27] Xie, E.; Zhu, L.; Zhao, L.; Chang, L.S. *Genomics*, **1996**, *35*, 551.
- [28] Bockaert, J.; Dumuis, A. In *5-HT₄ Receptor in the Brain and Periphery*; Bioscience, L., Austin, TX USA, **1997**; Vol. pp. 63.
- [29] Compan, V.; Daszuta, A.; Salin, P.; Sebben, M.; Bockaert, J.; Dumuis, A. *Eur. J. Neurosci.*, **1996**, *8*, 2591.
- [30] Waeber, C.; Sebben, M.; Nieoullon, A.; Bockaert, J.; Dumuis, A. *Neuropharmacology*, **1994**, *33*, 527.
- [31] Patel, S.; Roberts, J.; Moorman, J.; Reavill, C. *Neuroscience*, **1995**, *69*, 1159.
- [32] Reynolds, G.P.; Mason, S.L.; Meldrum, A.; De Keizer, S.; Parnes, H.; Eglen, R.M.; Wong, E.H. *Br J Pharmacol*, **1995**, *114*, 993.
- [33] Torres, G.E.; Arfken, C.L.; Andrade, R. *Mol. Pharmacol.*, **1996**, *50*, 1316.
- [34] Bickmeyer, U.; Heine, M.; Manzke, T.; Richter, D.W. *Eur J Neurosci*, **2002**, *16*, 209.
- [35] Matsumoto, M.; Togashi, H.; Mori, K.; Ueno, K.; Ohashi, S.; Kojima, T.; Yoshioka, M. *J Pharmacol Exp Ther*, **2001**, *296*, 676.
- [36] Bijak, M.; Misgeld, U. *Neuroscience*, **1997**, *78*, 1017.
- [37] Cai, X.; Flores-Hernandez, J.; Feng, J.; Yan, Z. *J Physiol*, **2002**, *540*, 743.
- [38] Feng, J.; Cai, X.; Zhao, J.; Yan, Z. *J Neurosci*, **2001**, *21*, 6502.
- [39] Consolo, S.; Arnaboldi, S.; Giorgi, S.; Russi, G.; Ladinsky, H. *NeuroReport*, **1994**, *5*, 1230.
- [40] Medhurst, A.D.; Lezoualc'h, F.; Fischmeister, R.; Middlemiss, D.N.; Sanger, G.J. *Brain Res Mol Brain Res*, **2001**, *90*, 125.
- [41] Ponimaskin, E.G.; Heine, M.; Joubert, L.; Sebben, M.; Bickmeyer, U.; Richter, D.W.; Dumuis, A. *J Biol Chem*, **2002**, *277*, 2534.
- [42] Claeyens, S.; Sebben, M.; Becamel, C.; Parmentier, M.L.; Dumuis, A.; Bockaert, J. *EMBO Rep*, **2001**, *2*, 61.
- [43] Claeyens, S.; Sebben, M.; Bécamel, C.; Eglen, R.; Clark, R.D.; Bockaert, J.; Dumuis, A. *Mol. Pharmacol.*, **2000**, *58*, 136.
- [44] Claeyens, S.; Joubert, L.; Sebben, M.; Bockaert, J.; Dumuis, A. *J Biol Chem*, **2003**, *278*, 699.
- [45] Coward, P.; Wada, H.G.; Falk, M.S.; Chan, S.D.; Meng, F.; Akil, H.; Conklin, B.R. *Proc Natl Acad Sci U S A*, **1998**, *95*, 352.
- [46] Langlois, M.; Fischmeister, R. *J Med Chem*, **2003**, *46*, 319.
- [47] Pindon, A.; van Hecke, G.; van Gompel, P.; Lesage, A.S.; Leysen, J.E.; Jurzak, M. *Mol Pharmacol*, **2002**, *61*, 85.
- [48] Moser, P.C.; Bergis, O.E.; Jegham, S.; Lochead, A.; Duconseille, E.; Terranova, J.P.; Caille, D.; Berque-Bestel, I.; Lezoualc'h, F.; Fischmeister, R.; Dumuis, A.; Bockaert, J.; George, P.; Soubrie, P.; Scatton, B. *J Pharmacol Exp Ther*, **2002**, *302*, 731.
- [49] Mialet, J.; Fischmeister, R.; Lezoualc'h, F. *Br J Pharmacol*, **2003**, *138*, 445.
- [50] Ansanay, H.; Sebben, M.; Bockaert, J.; Dumuis, A. *Mol. Pharmacol.*, **1992**, *42*, 808.
- [51] Norum, J.H.; Hart, K.; Levy, F.O. *J Biol Chem*, **2002**, *277*, 21.
- [52] Ponimaskin, E.G.; Profirovic, J.; Vaiskunaite, R.; Richter, D.W.; Voynoyasenetskaya, T.A. *J Biol Chem*, **2002**, *277*, 28.
- [53] Sheng, M.; Sala, C. *Annu Rev Neurosci*, **2001**, *24*, 1.
- [54] Clark, R.D. In *5-HT₄ receptors in the brain and periphery*; Eglen, R.M., **1998**; Vol. pp. 1.
- [55] Craig, D.A.; Eglen, R.M.; Walsh, L.K.M.; Perkins, L.A.; Whiting, R.L.; Clarke, D.E. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1990**, *342*, 9.
- [56] Buchheit, K.H.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Klöppner, E.; Pfannküche (Pfannfücke in Ref 57), H.J.; Mattes, H. *J. Med. Chem.*, **1995**, *38*, 2326.

- [57] Buchheit, K.; Gamse, R.; Giger, R.; Hoyer, D.; Klein, F.; Klöppner, E.; Pfannfücke, H.; Mattes, H. *J. Med. Chem.*, **1995**, *38*, 2331.
- [58] Muller-Lissner, S.A.; Fumagalli, I.; Bardhan, K.D.; Pace, F.; Pecher, E.; Nault, B.; Ruegg, P. *Aliment Pharmacol Ther*, **2001**, *15*, 1655.
- [59] Briejer, M.R.; Akkermans, L.M.A.; Meulemans, A.L.; Lefevre, R.A.; Schuurkes, J.A.J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1993**, *347*, 464.
- [60] Baxter, G.S.; Boyland, P.; Gaster, L.M.; King, F.D. *Biorg. Med. Chem. Lett.*, **1993**, *3*,
- [61] Flynn, D.L.; Zabrowski, D.L.; Becker, D.P.; Nosal, R.; Villamil, C.I.; Gullikson, G.W.; Moumami, C.; Yang, D.C. *J. Med. Chem.*, **1992**, *35*, 1486.
- [62] Briejer, M.R.; Prins, N.H.; Schuurkes, J.A. *Neurogastroenterol Motil*, **2001**, *13*, 465.
- [63] Kato, S.; Morie, T.; Yoshida, N. *Chem Pharm Bull (Tokyo)*, **1996**, *44*, 1484.
- [64] Itoh, K.; Kanzaki, K.; Ikebe, T.; Kuroita, T.; Tomozane, H.; Sonda, S.; Sato, N.; Haga, K.; Kawakita, T. *Eur J Med Chem*, **1999**, *34*, 977.
- [65] Langlois, M.; Zhang, L.; Yang, D.; Brémont, B.; Shen, S.; Manara, L.; Croci, T. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 1433.
- [66] Dumuis, A.; Sebben, M.; Monferini, E.; Nicola, M.; Ladinsky, H.; Bockaert, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1991**, *343*, 245.
- [67] Rizzi, C.A.; Coccini, T.; Onori, L.; Manzo, L.; Tonini, M. *J. Pharmacol. Exp. Ther.*, **1992**, *261*, 412.
- [68] Turconi, M.; Schiantarelli, P.; Borsini, F.; Rizzi, C.A.; Ladinsky, H.; Donetti, A. *Drugs of the Future*, **1991**, *16*, 1011.
- [69] Bonhaus, D.W.; Loury, D.N.; Jakeman, L.B.; To, Z.; DeSouza, A.; Eglen, R.M.; Wong, E.H.F. *J. Pharmacol. Exp. Ther.*, **1993**, *267*, 921.
- [70] Clark, R.D.; Jahangir, A.; Langston, J.A.; Weinhardt, K.K.; Miller, A.B.; Leung, E.; Eglen, R.M. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 2481.
- [71] Eglen, R.M.; Bonhaus, D.W.; Johnson, L.G.; Leung, E.; Clark, R.D. *Br J Pharmacol*, **1995**, *115*, 1387.
- [72] Eglen, R.M.; Bonhaus, D.; Clark, R.D.; Daniels, S.; Leung, E.; Wong, E.H.F.; Fontana, D.J. *Br. J. Pharmacol.*, **1995**, *116*, 235p.
- [73] Fontana, D.J.; Daniels, S.E.; Wong, E.H.F.; Clark, R.D.; Eglen, R.M. *Neuropharmacology*, **1997**, *36*, 689.
- [74] Gale, J.D.; Grossman, C.J.; Whitehead, J.W.F.; Oxford, A.W.; Bunce, K.T.; Humphrey, P.P.A. *Br. J. Pharmacol.*, **1994**, *111*, 332.
- [75] Grossman, C.J.; Kilpatrick, G.J.; Bunce, K.T. *Br. J. Pharmacol.*, **1993**, *109*, 618.
- [76] Gale, J.D.; Green, A.; Darton, J.; Sargant, R.S.; Clayton, N.M.; Bunce, K.T. *Br. J. Pharmacol.*, **1994**, *113*, 119P.
- [77] Parker, S.G.; HHamburger, S.; Taylor, E.M.; Kaumann, A.J. *Br. J. Pharmacol.*, **1993**, *108*, 68p.
- [78] Gaster, L.M.; Joiner, G.F.; King, F.D.; Wyman, P.A.; Sutton, J.M.; Bingham, S.; Ellis, E.S.; Sanger, G.J.; Wardle, K.A. *J Med Chem*, **1995**, *38*, 4760.
- [79] Wardle, K.A.; Bingham, S.; Ellis, E.S.; Gaster, L.M.; Rushant, B.; Smith, M.I.; Sanger, G.J. *Br J Pharmacol*, **1996**, *118*, 665.
- [80] Gaster, L.M.; King, F.D. *Med Res Rev*, **1997**, *17*, 163.
- [81] Cohen, M.L.; Bloomquist, W.; Schaus, J.M.; Thompson, D.C.; Susemichel, A.D.; Calligaro, D.A.; Cohen, I. *J Pharmacol Exp Ther*, **1996**, *277*, 97.
- [82] Schaus, J.M.; Thompson, D.C.; Bloomquist, W.E.; Susemichel, A.D.; Calligaro, D.O.; Cohen, M.L. *J Med Chem*, **1998**, *41*, 1943.
- [83] Buchheit, K.H.; Gamse, R.; Pfannkuche, H.J. *Naunyn Schmiedebergs Arch Pharmacol*, **1992**, *345*, 387.

- [84] Eglen, R.M.; Bley, K.; Bonhaus, D.W.; Clark, R.D.; Hegde, S.S.; Johnson, L.G.; Leung, E.; Wong, E.H. *Br J Pharmacol*, **1993**, *110*, 119.
- [85] Yang, D.; Soulier, J.L.; Sicsic, S.; Mathe-Allainmat, M.; Bremont, B.; Croci, T.; Cardamone, R.; Aureggi, G.; Langlois, M. *J Med Chem*, **1997**, *40*, 608.
- [86] Gaster, L.M.; Sanger, G.J. *Drugs of the Future*, **1994**, *19*, 1109.
- [87] Brown, A.M.; Young, T.L.; Patch, T.L.; Cheung, C.W.; Kauman, A.J.; Gaster, L.; King, F.D. *Br. J. Pharmacol.*, **1993**, *110*, 10P.
- [88] Dumuis, A.; Gozlan, H.; Sebben, M.; Ansanay, H.; Rizzi, C.; Turconi, M.; Monferini, E.; Giraldo, E.; Schiantarelli, P.; Ladinsky, H.; Bockaert, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1992**, *345*, 264.
- [89] Baxter, G.S.; Clarke, D.E. *Eur. J. Pharmacol.*, **1992**, *212*, 225.
- [90] Lopez-Rodriguez, M.L.; Benhamu, B.; Viso, A.; Morcillo, M.J.; Murcia, M.; Orensanz, L.; Alfaro, M.J.; Martin, M.I. *Bioorg Med Chem*, **1999**, *7*, 2271.
- [91] Lopez-Rodriguez, M.L.; Murcia, M.; Benhamu, B.; Viso, A.; Campillo, M.; Pardo, L. *Bioorg Med Chem Lett*, **2001**, *11*, 2807.
- [92] Hedge, S.S.; Bonhaus, D.W.; Johnson, L.G.; Leung, E.; Clark, R.D.; Eglen, R.M. *Br. J. Pharmacol.*, **1995**, *115*, 1087.
- [93] Clark, R.D.; Jahangir, A.; Flippin, L.A.; Langston, J.A.; Leung, E. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2119.
- [94] Hirschberger, A.; Butt, S.; Lelong, V.; Boulouard, M.; Dumuis, A.; Dauphin, F.; Bureau, R.; Pfeiffer, B.; Renard, P.; Rault, S. *J Med Chem*, **2003**, *46*, 138.
- [95] Blondel, O.; Gastineau, M.; Langlois, M.; Fischmeister, R. *Brit.J. Pharmacol.*, **1998**, *125*, 595.
- [96] Andrade, R.; Chaput, Y. *J. Pharmacol. Exp. Ther.*, **1991**, *257*, 930.
- [97] Torres, G.E.; Holt, I.L.; Andrade, R. *J. Pharmacol. Exp. Ther.*, **1994**, *271*, 255.
- [98] Ansanay, H.; Dumuis, A.; Sebben, M.; Bockaert, J.; Fagni, L. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 6635.
- [99] Lucas, G.; Di Matteo, V.; De Deurwaerdere, P.; Porras, G.; Martin-Ruiz, R.; Artigas, F.; Esposito, E.; Spampinato, U. *Eur J Neurosci*, **2001**, *13*, 889.
- [100] Kulla, A.; Manahan-Vaughan, D. *Cereb Cortex*, **2002**, *12*, 150.
- [101] Cardenas, C.G.; Del Mar, L.P.; Cooper, B.Y.; Scroggs, R.S. *J Neurosci*, **1997**, *17*, 7181.
- [102] Janel, U.; Rupp, J.; Ertl, R.; Nawrath, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1992**, *346*, 346.
- [103] Ouadid, H.; Seguin, J.; Dumuis, A.; Bockaert, J.; Nargeot, J. *Mol. Pharmacol.*, **1992**, *41*, 346.
- [104] Yamaguchi, T.; Suzuki, M.; Yamamoto, M. *Brain Res*, **1997**, *772*, 95.
- [105] Siniscalchi, A.; Rodi, D.; Morari, M.; Marti, M.; Cavallini, S.; Marino, S.; Beani, L.; Bianchi, C. *Br J Pharmacol*, **2002**, *136*, 1178.
- [106] Bianchi, C.; Rodi, D.; Marino, S.; Beani, L.; Siniscalchi, A. *Neuroreport*, **2002**, *13*, 2177.
- [107] Porras, G.; Di Matteo, V.; De Deurwaerdere, P.; Esposito, E.; Spampinato, U. *Neuropharmacology*, **2002**, *43*, 1099.
- [108] Pozzi, L.; Trabace, L.; Invernizzi, R.; Samanin, R. *Brain Res*, **1995**, *692*, 265.
- [109] McMahan, L.R.; Cunningham, K.A. *J Pharmacol Exp Ther*, **1999**, *291*, 300.
- [110] Ge, J.; Barnes, N.M. *Br J Pharmacol*, **1996**, *117*, 1475.
- [111] Kandel, E.R. *Science*, **2001**, *294*, 1030.
- [112] Bockaert, J.; Ansanay, H.; Letty, S.; Marchetti-Gauthier, E.; Roman, F.; Rondouin, G.; Fagni, L.; Soumireu-Mourat, B.; Dumuis, A. *C R Acad Sci III*, **1998**, *321*, 217.

- [113] Bockaert, J.; Fozard, J.; Dumuis, A.; Clarke, D. *Trends Pharmacol. Sci.*, **1992**, *13*, 141.
- [114] Bockaert, J.; Ansanay, H.; Waeber, C.; Sebben, M.; Fagni, L.; Dumuis, A. *CNS Drugs*, **1994**, *1*, 6.
- [115] Robert, S.J.; Zugaza, J.L.; Fischmeister, R.; Gardier, A.M.; Lezoualc'h, F. *J Biol Chem*, **2001**, *276*, 44881.
- [116] Letty, S.; Child, R.; Dumuis, A.; Pantaloni, A.; Bockaert, J.; Rondouin, G. *Neuropharmacology*, **1997**, *36*, 681.
- [117] Meneses, A.; Hong, E. *Pharmacol Biochem Behav*, **1997**, *56*, 347.
- [118] Marchetti-Gauthier, E.; Roman, F.S.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B. *Neuropharmacology*, **1997**, *36*, 697.
- [119] Marchetti, E.; Dumuis, A.; Bockaert, J.; Soumireu-Mourat, B.; Roman, F.S. *Neuropharmacology*, **2000**, *39*, 2017.
- [120] Lamirault, L.; Simon, H. *Neuropharmacology*, **2001**, *41*, 844.
- [121] Lelong, V.; Dauphin, F.; Boulouard, M. *Neuropharmacology*, **2001**, *41*, 517.
- [122] Terry, A.V., Jr.; Buccafusco, J.J.; Jackson, W.J.; Prendergast, M.A.; Fontana, D.J.; Wong, E.H.; Bonhaus, D.W.; Weller, P.; Eglen, R.M. *Psychopharmacology (Berl)*, **1998**, *135*, 407.
- [123] Le Long, V.; Lhonneur, L.; Dauphin, F.; Boulouard, M. *Naunyn Schmiedebergs Arch Pharmacol*, **in press**.
- [124] Lamirault, L.; Guillou, C.; Thal, C.; Simon, H. *Prog Neuropsychopharmacol Biol Psychiatry*, **2003**, *27*, 185.
- [125] Ghelardini, C.; Galéotti, N.; Casamenti, F.; Malmberg-Aiello, P.; Pepeu, G.; Gualtieri, F.; Bartolini, A. *Life Sci.*, **1996**, *58*, 2297.
- [126] Espejo, E.F.; Gil, E. *Brain Res*, **1998**, *788*, 20.
- [127] Callahan, M.J. *J Clin Gastroenterol*, **2002**, *35*, S58.
- [128] Silvestre, J.S.; Fernandez, A.G.; Palacios, J.M. *Eur J Pharmacol*, **1996**, *309*, 219.
- [129] Kennett, G.A.; Bright, F.; Trail, B.; Blackburn, T.P.; Sanger, G.J. *Neuropharmacology*, **1997**, *36*, 707.
- [130] Costall, B.; Naylor, R.J. *Br J Pharmacol*, **1997**, *122*, 1105.
- [131] Compan, V.; Zhou, M.; Grailhe, R.; Gazzara, R.A.; Martin, R.; Gingrich, J.; Dumuis, A.; Brunner, D.; Bockaert, J.; Hen, R. *J Neurosci*, **2003**, *23*,
- [132] Reavill, C.; Hatcher, J.P.; Lewis, V.A.; Sanger, G.J.; Hagan, J. *Eur J Pharmacol*, **1998**, *357*, 115.
- [133] Takahashi, H.; Takada, Y.; Urano, T.; Takada, A. *Hippocampus*, **2002**, *12*, 304.
- [134] Bijak, M.; Tokarski, K.; Maj, J. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1997**, *355*, 14.
- [135] Lucas, G.; Debonnel, G. *Eur J Neurosci*, **2002**, *16*, 817.

Exon number	Positions in cDNA	Exon size (bp)	3'-Acceptor site	5'-Donnor site	Intron size (kpb)
1	1-193	193		GGGCCCAG gt gagt	10
2	194-292	99	ttctccagGTAAAGAA	TAATCCAG gt atccaa	4.3
3	293-420	128	tctttaagACCTGACA	TGTTTAAG gt gagcat	3.4
4	421-526	106	ttacgtagGACCTCTC	TAATTTAG gt atggta	1.7
5	527-670	144	cttcttagGCTACGTA	ACAGGAAG gt gagtgt	1.1
6	671-872	202	atccacagGCCACTTC	TTCCATCAG gt gagtag	0.8
7	873-946	74	attggcagGTGGTTCC	TCAGATTG gt atggct	2.1
8	947-1142	196	ttccacagGCAGAGGA	TCCCCAAT gt gagtat	6.6
9	1143-1276	134	cgatgcagGAGCACGA	CCGGCTCAG gt gagtgt	2.9
10	1277-1318	42	ctacttagGTGGGTTT	TCTGAGTG gt gagtag	0.1
11	1319-3013	1695	cettacagCATCCCAG	TCTAAAG gt aagtat	1.8
12	3014-3194	181	gtgttcagGTCAGCTT	ACCGCCAG gt gagtca	15
13	3195-3344	150	gtccccagGTCCCTCA	GCCTGCAG gt aggcct	2.2
14	3345-3467	123	ccacctagGCAGCCAT	ACTACCG gt aagtcc	1.3
15	3468-3618	151	gttcttagGCTGGAGC	AGTCACAG gt cacagg	1.8
16	3619-3819	201	attcttagGAGATCAG	AGCTGAAG gt aagagc	3.0
17	3820-3938	119	tgagacagTTTCTCCT	AACTACAG gc aggtgc	>50
18	3939-4151	213	atgttagGGCAGTTG	AAGGCAAG gt aagtaa	30.5
19	4152-4351	200	tctgatagATGGCTTC	GGTAAAG gt gaatat	0.5
20	4352-4418	67	tcacacagCAAAAGAC	CCAGA ACTgt aagaaa	>240
21	4419-4672	254	tatttcagTTCCCATG	TAAGTGAG gt gatgag	2.5
22	4673-4716	44	tctcatagGTCCATAG	CATTCAAG gt aacata	7.5
23	4717-4758	42	aactacagAGGGAGAG	GATTGAAG gt aagt	1.8
24	4759-5006	248	tcttcaagGTACTTTA	GCCTGGAG gt gagttg	2.9
25	5007-5088	82	tcctctagAGACCTCT	TCCAAAAG gt agatct	25
26	5089-5161	73	catttttagGACCCTGT	AATGTGAG gt atgtta	86
27	5162-5287	126	tctttcagTTCTGAGG	CAGCTCAG gt gagcag	1.3
28	5288-5488	201	actcacagGAAAATAA	CTGGATAG gt aaggac	25
29	5489-5642	154	ctctccagGTATTACG	TTGATTTG gt gagtat	2.2
30	5643-h	42	tttcaagGAAAGGAG	TTCATGCG gt atgtca	11
31	5643-6211	569	actctcagATAGAAAA	GTACTAAG gt gagtat	26
31'	5643-n	708	actctcagATAGAAAA	TACTATCT ggt catgg	-
32	6212-b	234	ctctctagGGATGCAG	TCGCTGGG ct tttct	1.4
33	6212-c	≥160	tacttttagTTCTGGAA	ND	≤4.7
34	6212-d	110	cctaccagATTTTGAG	TGGATGT Att gtttg	11
35	6212-g	76	actaaaagTGGCTGTT	AGTGCTCT gt aagtct	-
36	6212-e	55	GTCTCCAGCTTCCTCC	AGTGCTCT gt aagtct	-
37	6212-f	31	TGCAATAGACCAGTTC	AGTGCTCT gt aagtct	14
38	6212-a	157	ctttctagGTACACCG	CCTGGCAT cact tctg	

Table 1

Receptor variants (when known)	G protein	Second messenger	Ionic current Small G protein	Type of cell
NEURONAL CELLS				
	G _s	↗ cAMP	⊖ AHP	CA ₁ , colliculi neurons
	G _s	↗ cAMP	⊖ K ⁺ channels	CA ₁ , colliculi neurons
	G _s	↗ cAMP	⊖ GABA _A current (PKA-high)	Pyramidal cortical neurons
	G _s	↗ cAMP	⊕ GABA _A current (PKA-low)	Pyramidal cortical neurons
	G _s	↗ cAMP	⊕ I _h (see text for discussion)	CA ₁ neurons
	?	?	⊕ TTX-insensitive Na ⁺ channels	DRG neurons (Aδ and C types)
CARDIAC CELLS				
	G _s	↗ cAMP	L-Type Ca ⁺⁺ channels	Atrial myocytes
TRANSFECTED CELLS				
5-HT _{4b}	G _i / G _o	↘ cAMP	—	HEK-293
5-HT _{4a}	?	?	Ca ⁺⁺ channels	HEK-293
5-HT _{4a}	G ₁₃	?	rhoA	Sf9, NIE-115, NIH 3T3
5-HT _{4b}	G _s	↗ cAMP	ras (ERK1/2), rap1	COS-cells

Table 2

	pK _i (binding)	pEC ₅₀	Efficacy	Selectivity	Bio-availability	Status
AGONISTS						
5-HT	7.1 - 7.5	7.4 - 8	(F)	None		(1)
5-MeOT	6.2	7 - 7.7	(F)	5-HT ₄ >>5-HT ₃		(1)
HTF919	8	8.8	(P)	S		Zelnorm™ (USA)
Cisapride	7.1	7.25	(F/P)	5-HT ₂ , α ₁ AR		
SC 53 116	7.5 - 8.1	7.64	(F)	S		
SB 205 149		8	(F/P)	S		
Prucalopride	8 - 8.6	7.5 - 7.8	(P)	HS		
Mosapride	6.8 - 7	7.1	(P)	S		
Y-34959	8.5	8.5	(F)	HS	Long duration	
ML10302	7.9 - 9	7.7 - 8.6	(P)	HS	Short duration	
LS-65 0155	8.78	6.7 - 8.3		HS	Long duration	Phase II, CNS
RS 67333	8.7	8.4	(P)	HS	Long duration	(1), CNS
RS 67506	8.8	8.6	(P)	HS	Long duration	(1), CNS
BIMU 1	6.4 - 7	6.5 - 8.4	(P/F)	LS	Long duration	
BIMU 8	7 - 7.5	7.1 - 8.1	(F)	LS	Long duration	
ANTAGONISTS						
GR 113308	9.2 - 9.8	8.8 to 9.5		S	Short duration	(1) [³ H], CNS
GF 125487	10 - 10.4	9.75 - 10		HL	long duration	CNS
SB 203186	9	8.3 to 8.7		HS		(1)
LY 353433				HS	Long duration	CNS
SB 207 266	9.5 - 10	10.6		HS	Long duration	Phase II, CNS
SB 204070	9.2	10.5		HS	Short duration	(1) [¹²⁵ I], CNS
RS 23597	7.8	8.4		LS		(1)
ML 10375	8.5 - 9	9		HS	Short duration	
ATBI 221	8.54	8.3		HS	Long duration	CNS
RS 39606	9.1	9.3		HS	Long duration	(1), CNS
RS 67532	8.7	8.8		HS	Long duration	(1), CNS
RS 100235	10	11.2		HS	Long duration	
RS 100302	10.5	11		HS		
INVERSE AGONISTS						
RO 116-2617	9.6	10.5		HS		
RO 116-0086	9.5	9.5		HS		
RO 116-1148	9.32	9.5		HS		

Table 3

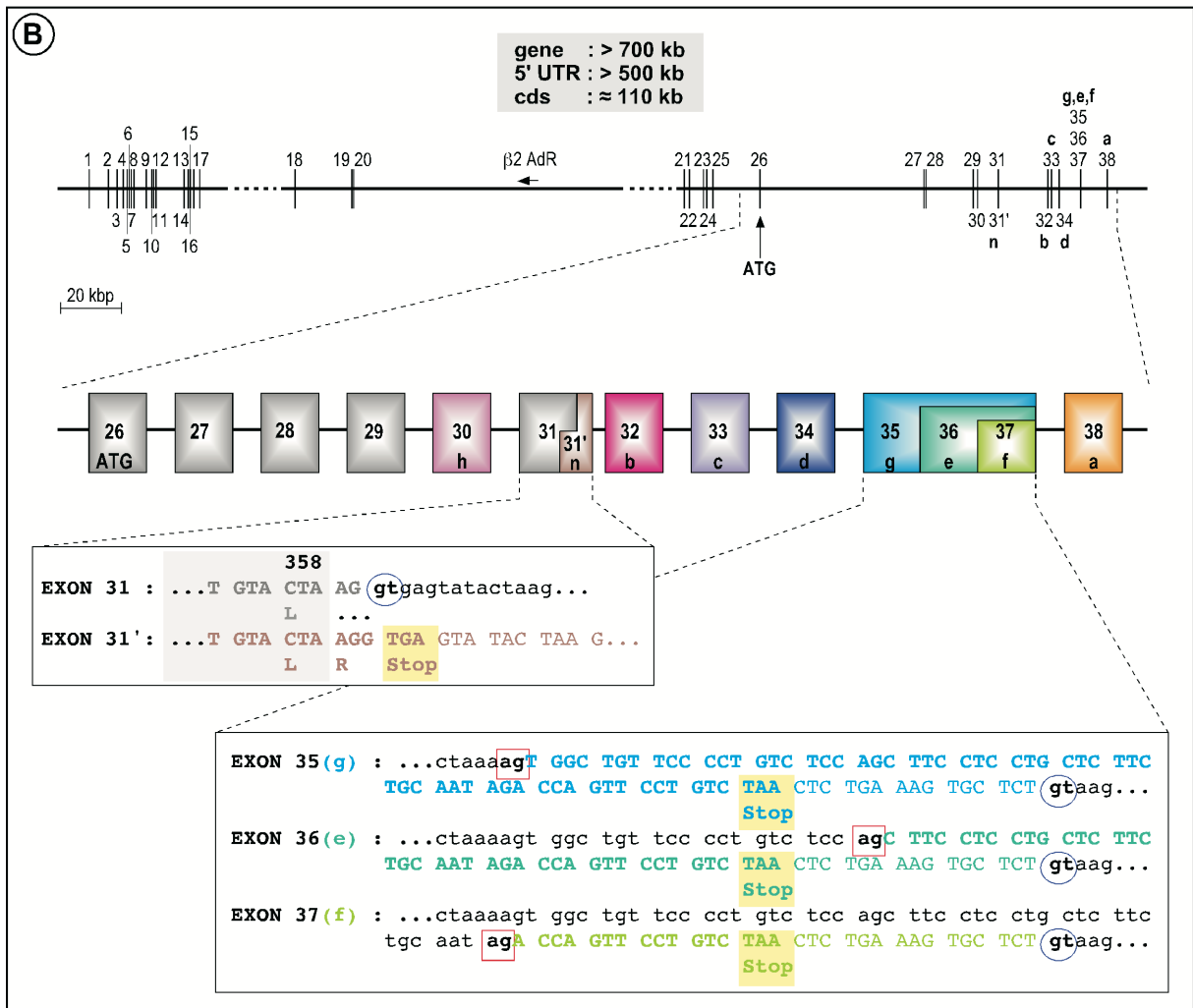
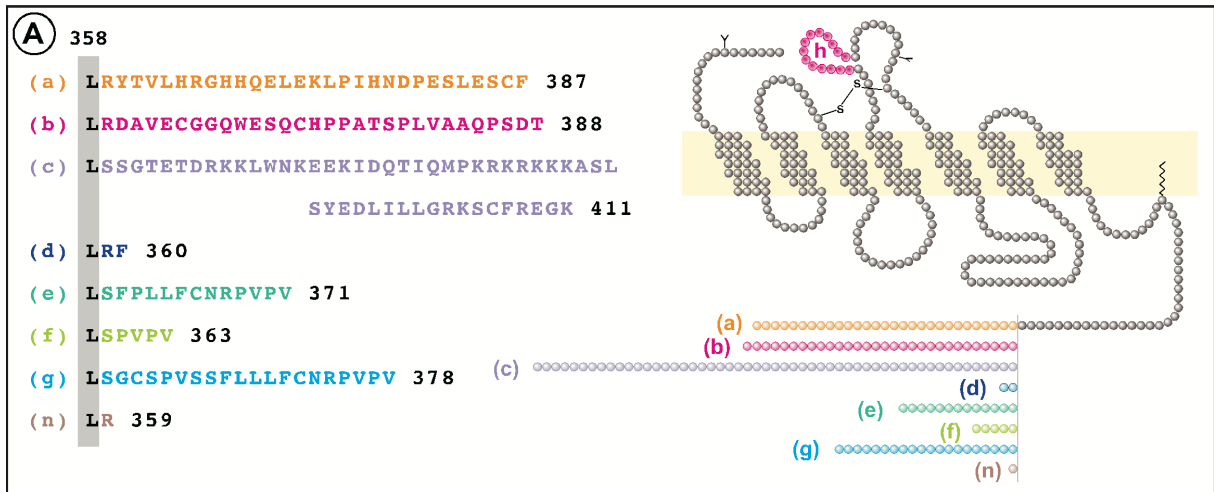


Figure 1

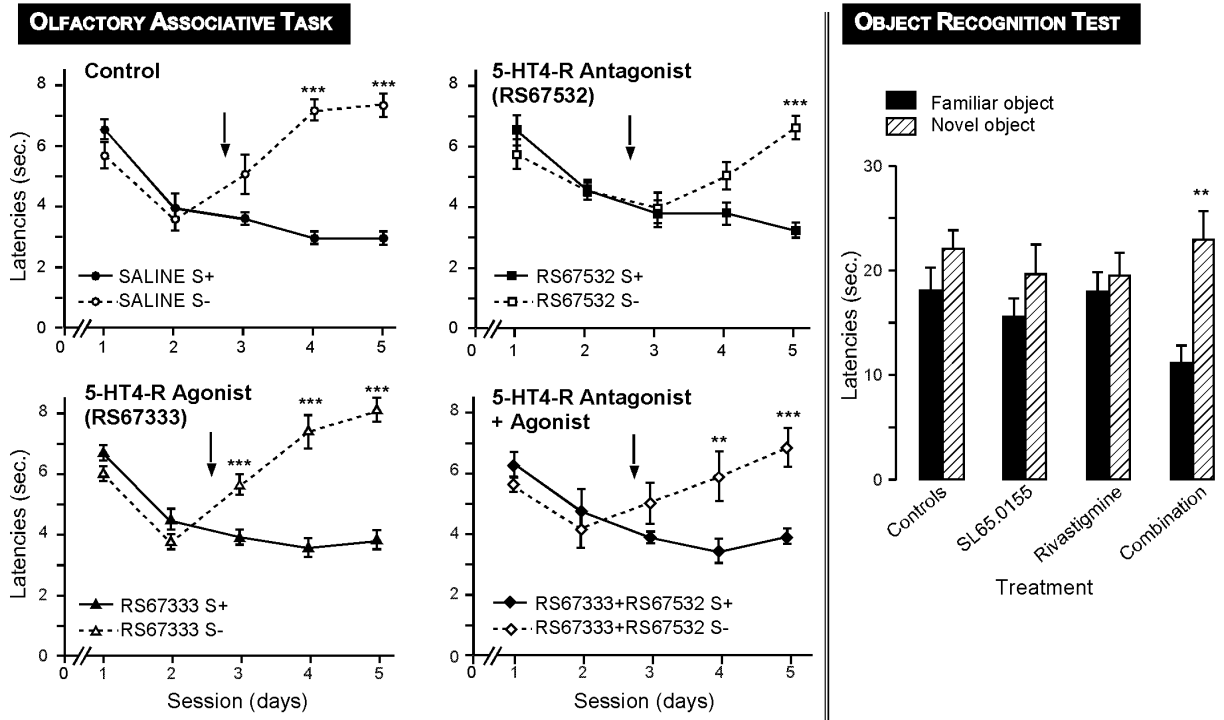


Figure 2