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Perspective

5-HT_4 Receptor Ligands: Applications and New Prospects

Michel Langlois*,† and Rodolphe Fischmeister‡

CNRS-BIOCIS (UPRES A 8076) and Laboratoire de Cardiologie Cellulaire et Moléculaire, INSERM U-446, Institut de Signalisation et Innovation Thérapeutique (IFR-ISIT), Faculté de Pharmacie, Université de Paris-Sud, 92296 Châtenay-Malabry, France

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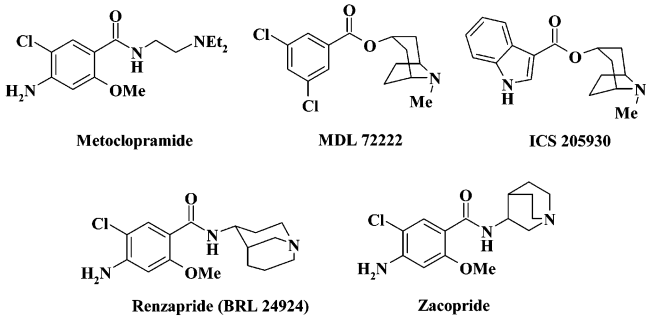
Introduction

The physiological effect of serotonin (5-HT) is mediated in the central and periphery systems by seven subtypes of receptors. All but one are members of the G protein coupled receptor (GPCRs) family (Table 1). Thirteen genes coding for the 5-HT GPCRs have been characterized, and only three 5-HT receptors (5-HT_4R, 5-HT_6R, and 5-HT_7R) are coupled to G_s proteins and stimulate adenylyl cyclase activity, giving rise to an increase of the intracellular cAMP.[1] Considerable interest has been devoted to the characterization of 5-HT_4 receptors since their discovery[2,3] in mouse colliculi neuronal cells and in the guinea pig ileum. Their presence in the gut allowed the proposal of a mechanism responsible for the pharmacological activity of several gastrokinetic benzamide drugs, such as metoclopramide, zacopride, and renzapride (Chart 1). Several splice variants of the 5-HT_4 receptors have been cloned,[4] and they differ in the length of their C-terminal ends. When studied in heterologous expression systems, these receptors present some functional and pharmacological differences. In particular, the length of the C-terminal sequence of the receptor was shown to be implicated in its constitutive activity.[5] 5-HT_4 receptors are localized in the central nervous system,[6] heart,[7] intestine,[8] adrenal cortex,[9] and the bladder,[10] and several important physiological processes such as the release of acetylcholine in the hippocampus,[11] the increase of the Ca^{2+} and pacemaker currents in the atrium,[12] the initiation of the intestinal peristaltic reflex,[8] and the increase of the release of corticosterol in the adrenal gland[9] are mediated through the activation of these receptors. Consequently, 5-HT_4 receptors have been implicated in a variety pathological disorders and constitute a valuable target for the design of new drugs. To date, several advances have been made to develop new molecules, in particular to cure the irritable bowel syndrome (IBS),[13] which is characterized by an altered bowel function with an alternation of constipation and diarrhea due to the dysfunction of the intestinal serotoninergic system. But several other promising routes are currently under study, such as the prevention of atrial fibrillation[14] with 5-HT_4 receptor antagonists, the improvement of the cognitive functions[15] by the enhancement of the cholinergic transmission in the hippocampus with the 5-HT_4 receptor agonists, and the treatment of the voiding disorders associated with detrusor hypocontractility.[10]

Chart 1. 5-HT_3 Receptor Antagonists of the First Generation



* To whom correspondence should be addressed. Address: CNRS-BIOCIS (UPRES A 8076), Faculté de Pharmacie, F-92296 Châtenay-Malabry Cedex, France. E-mail: zacopride@aol.com. Phone: 33 1 46835736. Fax: 33 1 46835740.
† CNRS-BIOCIS.
‡ Laboratoire de Cardiologie Cellulaire et Moléculaire.

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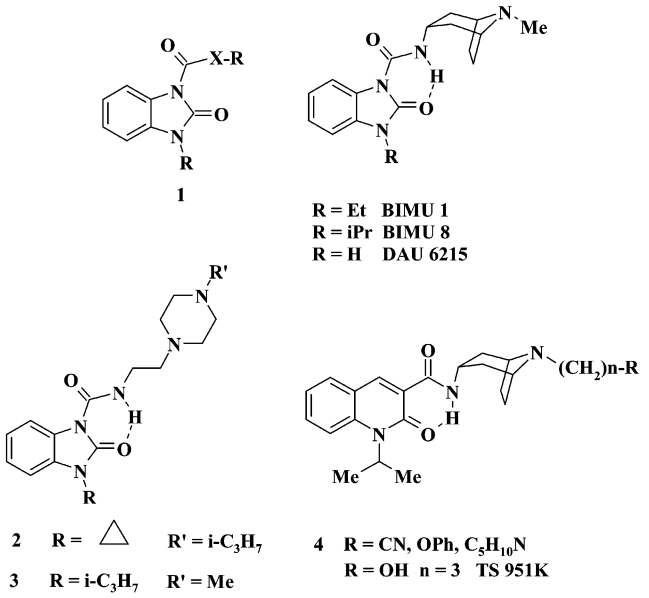
Table 1. Different 5-HT Receptor Subtypes

	5-HT$_1$	5-HT$_2$	5-HT$_3$	5-HT$_4$	5-HT$_5$	5-HT$_6$	5-HT$_7$
subtypes	5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$	5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$			5-HT$_{5A}$, 5-HT$_{5B}$		
signaling pathways	cAMP ↓ [a]	IP$_3$ ↑ [b]	ion channel ↑ [c]	cAMP ↑ [d]	cAMP [e]	cAMP ↑ [d]	cAMP ↑ [d]

[a] Decrease of cAMP synthesis. [b] Stimulation of the phospholipase C. [c] Increase of ion channel. [d] Increase of cAMP synthesis. [e] A possible negative coupling with adenylyl cyclase.

Chart 2. 2-Benzimidazolone and Quinoline Carboxamide Derivatives as 5-HT$_4$ Agonists and Antagonists



The first generation of the 5-HT$_3$ receptor antagonists benzamides, which turned out to be ligands for both 5-HT$_3$ and 5-HT$_4$ receptors (Chart 1), played a major role in the discovery of the potent and selective 5-HT$_4$ receptor ligands available to date. Previous recent reviews [16,17] have covered the different aspects of the SARs (structure−activity relationships) of this field. The present paper describes the recent advances made in medicinal chemistry and in the mechanisms proposed to explain the physiological role of 5-HT$_4$ receptors in the different tissues where they are present and the putative clinical applications of the agonists and antagonists.

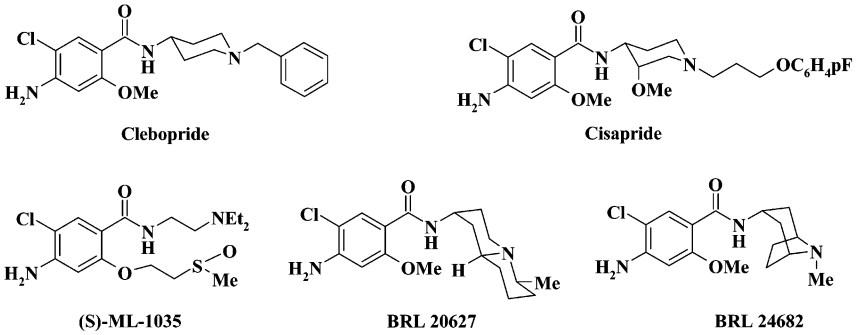
Chemistry

The 5-HT$_4$ receptor ligands are related to the following chemical groups: benzimidazolones, benzamides, benzoic esters, aryl ketones, indole carboxylates or carboxamides and serotonin analogues.

Benzimidalone Derivatives. Initially, benzimidazole-1-carboxylic acid ester and amides **1** (Chart 2, X = O or NH) with the basic framework *endo* tropane or quinuclidine were described as potent 5-HT$_3$ receptor antagonists.[18] In contrast with the reference compounds in the field such as MDL 72222[19] and ICS 205-930,[20] the amidic derivatives were more potent than the corresponding esters. On the other hand, like benzamides such as zacopride[21] or renzapride,[22] the hydrogen bond between the NH amidic and the carbonyl group of benzimidazolone ring stabilized the coplanar conformation. In contrast with ondansetron, these compounds were capable of enhancing the electric-field-stimulated contraction of the intestine and possessed gastrokinetic properties.[23] It was demonstrated[24] that these properties were related to their ability to stimulate the 5-HT$_4$ receptors. Then, in the primary culture of mouse *Colliculi neurons*, compounds BIMU 1 and BIMU 8 were found to be potent agonists (EC_{50} = 360 and 72 nM, respectively) for stimulating the production of cAMP mediated by this receptor in a range of concentrations similar to that of 5-HT (EC_{50} = 360 nM). The potency and the pharmacological profile of benzimidazolones were dependent on the N-substitution of the benzimidazole ring because the NH compound, DAU 6215, was inactive in this bioassay and was a competitive antagonist (K_i = 220 nM) of cAMP production by BIMU 8 and 5-HT.[25] These pharmacological properties were confirmed in the gastrointestinal (GI) system and, in particular, in the relaxation of the carbachol contracted rat oesophagus,[26] an efficient model for the characterization of the 5-HT$_4$ ligands. BIMU 1 and BIMU 8 were equipotent to 5-HT (pEC_{50} = 8 and 7.9, respectively) but with a weaker intrinsic activity. DAU 6215 inhibited the effects of 5-HT and BIMU 8 (pA_2 = 6.9−7.2) in this model, confirming the identity between the central and peripheral receptors.[27]

Although a combination of 5-HT$_3$ receptor antagonist and 5-HT$_4$ receptor agonist properties could have some advantage in clinical use, the search for selective compounds was mandatory to characterize correctly the 5-HT$_4$ receptors. In this respect, it was only recently[28] that selective compounds were designed in this benzimidazolone family. The tropane moiety was substituted by the piperazine ring linked to the benzimidazole group with the flexible ethyl chain (compounds **2** and **3**, Chart 2). This structural modification brought about a decrease of affinity for 5-HT$_3$ receptors while the affinity for 5-HT$_4$ receptors was maintained. As in the parent series, the pharmacological profile depended on the nature of the substitution on the nitrogen atom of the benzimidazole ring: compound **2** (R = *c*-C_3H_5; R′ = *i*-C_3H_7) was a selective ligand with 5-HT$_4$ receptor antagonist activity (K_i = 6.7 nM; pK_i = 7.78), and compound **3** (R = *i*-C_3H_7; R′ = Me) was a selective high partial agonist with moderate affinity (intrinsic activity = 0.94; K_i = 91.1 nM). More recently,[29] derivatives of 3-quinolocarboxamide **4**, a bioisosteric group of benzimidazolone, were synthesized as structural analogues of BIMU 8. Several potent and selective 5-HT$_4$ receptor agonists were obtained by introducing various substituents on the nitrogen atom of the tropane ring. This work demonstrated the ability of the binding site of the 5-HT$_4$ receptor to accept voluminous groups around the ionic anchorage site. The alkyl chains with different groups such as piperidine, OH, phenoxy, and CN fitted correctly to give rise to agonists more potent than

Chart 3. Gastrokinetic Benzamides of the First Generation



cisapride. TS-951K (**4**, R = OH, n = 3) was selected as a promising agent to alleviate symptoms of the gastrointestinal dysfunctions.[30]

Benzamide Derivatives. The parent compound of this class was metoclopramide[31] (Chart 3), and the amides of 4-amino-5-chloro-2-methoxybenzoic acid were the key compounds in the discovery of 5-HT_4 receptors ligands. Metoclopramide was characterized as a gastric prokinetic agent possessing dopamine receptor antagonist properties. Clebopride,[32] a potent gastrokinetic compound in the guinea pig ileum bioassay, was designed by the modification of the amino chain of metoclopramide that was introduced into the piperidine framework. However, the presence of the *N*-benzyl substitution strengthened the affinity for the D_2 dopamine receptors and provided a highly potent D_2 receptor antagonist. Nevertheless, by introduction of other substitutions such as the aryloxypropyl chain, new compounds were discovered that turned out to be potent gastrokinetic compounds devoid of antidopaminergic properties. Cisapride[33] was the first molecule of this family largely used in a human clinic to treat gastrointestinal disorders.

The structural modifications of the 2-alkoxy chain of metoclopramide were also claimed to give compounds with potent gastrokinetic properties and weak affinity for the D_2 receptors in the guinea pig and rat. In particular, (*S*)-ML-1035, a sulfoxide derivative, was described as equipotent to metoclopramide in the enhancement of guinea pig ileum contraction and rat gastric emptying.[34,35]

But more promising results were obtained with the derivatives of metoclopramide, such as BRL 20627,[36] BRL 24682,[37] renzapride,[22] and zacopride,[21,38] where the amino flexible chain was introduced into a rigid framework such as the quinolizidine, tropane, and quinuclidine moieties. These compounds were potent 5-HT_3 receptor antagonists of the 5-HT-evoked Bezold−Jarisch reflex and more potent stimulants of the gastrointestinal motility than metoclopramide. On the other hand, and in contrast to metoclopramide, they were inactive on the dopaminergic receptors. Since 5-HT_3 receptors are present in the gastrointestinal system, blockade of these receptors might account for the gastrokinetic properties of these compounds. However, this putative mechanism was discarded[39] because several potent 5-HT_3 antagonists structurally different from benzamides had no effect on gastrointestinal motility. It was demonstrated[3] that renzapride and 5-HT induced relaxation of the rat oesophagus and facilitated the peristaltic reflex in guinea pig ileum. Simultaneously,[2] these compounds were shown to be full agonists of the newly characterized 5-HT_4 receptor in mouse embryo colliculi neurons, which was positively coupled to adenylyl cyclase. In this bioassay, cisapride, renzapride, and zacopride behaved as agonists with pEC_{50} values equal or inferior to that of 5-HT (7.14, 6.90, and 5.95, respectively) but with superior efficacy (142%, 133%, and 144%, respectively) while they were partial agonists in a different assay using 5-HT_4 receptor mediated cAMP response in guinea pig hippocampal membranes.[40]

The structural requirements implicated in the recognition of the benzamides by the D_2 and 5-HT_4 receptors were studied by comparing the pharmacological properties of BRL 20627 and the phenyl-substituted indolizidine analogues **5** and **6** (Chart 4) mimicking the restricted conformers of clebopride.[41] Only the β-phenyl stereoisomer **5** retained the gastric prokinetic activity, while the central dopamine antagonist activity was present in the α-phenyl stereoisomer **6**. Preliminary structural parameters implicated in the recognition by the 5-HT_4 and 5-HT_3 receptors were determined by King[22] with the synthesis of benzamide analogues of the high-energy conformations of the cis junction of the quinolizidine ring of BRL 20627 (Chart 4). The compounds possessed an aza bicycle in such a way that the amino group of the second ring was "tied back". This induced a drop of the steric hindrance around the basic nitrogen atom.[17] The most representative of this class of compounds was renzapride, which can be viewed as an analogue of zacopride and which was characterized as a potent stimulant of the electrically evoked guinea pig ileum contraction and of rat intragastric pressure. However, almost all of these compounds were also potent 5-HT_3 receptor antagonists in the 5-HT evoked Bezold−Jarisch reflex, indicating that 5-HT_3 and 5-HT_4 receptors possessed common steric requirements around the anchorage point of the ammonium ion.

The first breakthrough for obtaining more selective benzamides for 5-HT_4 versus 5-HT_3 receptors was reported by Flynn[42] who used the pyrrolizidine ring as the basic constrained framework for the synthesis of SC-53116. This compound was characterized as a potent and efficient 5-HT_4 agonist in the rat tunica muscularis mucosae. With an EC_{50} of 23.7 nM when tested in this preparation, SC-53116 was more potent than cisapride, renzapride, (*S*)- and (*R*)-zacopride, and BIMU 8 (EC_{50} = 55, 44, 173, 505, and 40.3 nM, respectively). Similar to cisapride, SC-53116 had a moderate affinity for 5-HT_3

Chart 4. Influence of the Stereochemistry of the Basic Framework for the Recognition by the 5-HT_4 and the D_2 Receptors: Structural Intermediates between BRL 20627 and Renzapride

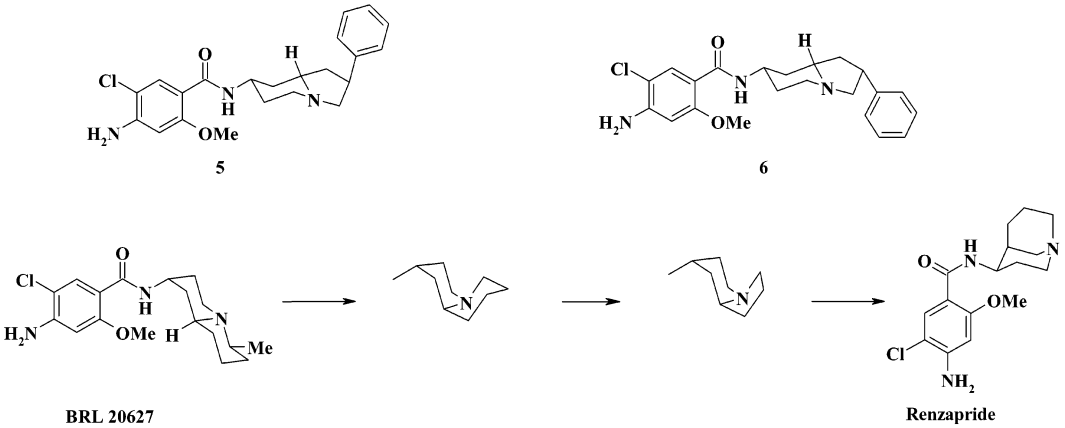
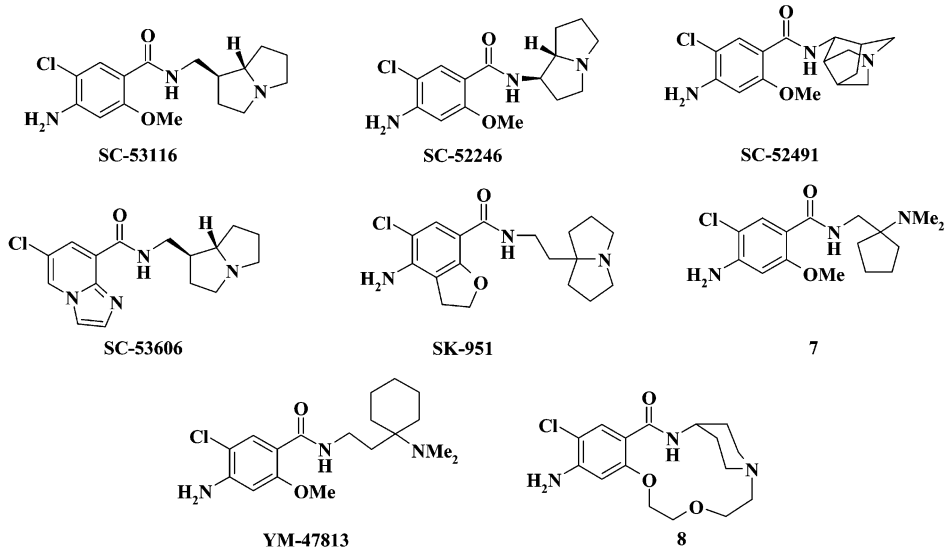


Chart 5. Second Generation of Benzamide Derivatives with Structural Modification of the Basic Chain

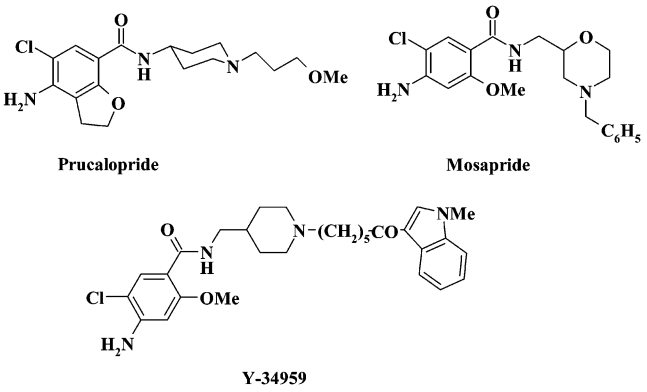


receptors (K_i = 152 nM) but was more selective because of the lack of affinity for the dopamine and adrenergic receptors. An enantioselectivity was observed because the exo (1*S*,8*S*) enantiomer was 10-fold more potent than the corresponding antipode. The SAR developed in this series highlighted the close structural relationships between the 5-HT_3 and 5-HT_4 ligands, since a modest structural variation led to large effect on the activity. Hence, shortening the amino chain gave rise to a potent 5-HT_3 receptor antagonist (SC-52246) with very weak affinity for the 5-HT_4 receptor. A comparison of SC-53116 with renzapride and zacopride indicated that the 5-HT_3 receptor binding site preferred more compact aza bicycles as the basic moiety. This point was clearly demonstrated with a series of azanoradamentane derivatives[43] that were 5-HT_4 receptor agonists but with a potent affinity for the 5-HT_3 receptor (SC-52491). The structural modification of the benzamide ring of SC-53116 led to a dramatic change of the pharmacological profile, since the imidazopyridine carboxamide SC-53606 (Chart 5) was a relatively selective potent antagonist of 5-HT_4 receptors.[44] Benzamides,[45] such as SK-951,[46] with an achiral pyrrolizidine were more recently described. The essential structural modification with regard to SC-53116 resided in the substitution of the classical 4-amino-5-chloro-2-methoxybenzoic acid by the 4-amino-2,3-dihydro-2-methylbenzo[*b*]-furan-7-carboxylic. SK-951 was claimed as a potent 5-HT_4 agonist (EC_{50} = 14 nM) with a selectivity versus 5-HT_3 receptor of K_i = 420 nM in in vitro and in vivo models.[46,47]

Structural constraints were also introduced into the basic ethyl side chain of metoclopramide in which the vicinal carbon atom of the basic nitrogen atom of the ethyl chain was introduced into a ring. Compound **7**[48] (Chart 5) was described as a 5-HT_3 receptor antagonist (K_i = 9 nM) devoid of any affinity for the 5-HT_4 receptor, while compound YM-47813[49] was shown to enhance the gastric motility and gastric emptying in dog when administered orally. On the other hand, the inclusion of the cyclopropane ring[50] in the ethyl chain gave rise to compounds with trans or cis configuration that turned out to be inactive on the 5-HT_4 receptors.

Pertinent information on the role of the steric requirements and of the orientation of the nitrogen atom lone pair of the basic framework was obtained from the macrocyclic benzamide **8**,[51] which was compared to the flexible analogue piperidine derivative. In contrast with the derivatives in which the nitrogen substituent was not tied back, **8** was a more potent 5-HT_4 receptor agonist (K_i(rat brain) = 29.7 nM, EC_{50}(electrically stimulated myenteric plexus) = 69 nM) than 5-HT_3 receptor antagonist (K_i = 53.2 nM).

Chart 6. Benzamides with a Large Subtituent on the Basic Nitrogen Atom as 5-HT_4 Receptor Agonists



However, the lack of bulk around the basic nitrogen was not an essential structural requirement for obtaining 5-HT_4 receptor agonists. As already reported, and in contrast to the 5-HT_3 receptor, the 5-HT_4 receptor binding site can fit the linear flexible substituent on the basic nitrogen atom. By use of this property, several potent and selective agonists of the 5-HT_4 receptors were designed in the benzamide series. Thus, mosapride[52] (Chart 6), a benzylmorpholine derivative, was shown to possess potent gastrokinetic properties mediated by the 5-HT_4 receptors with an affinity value of 84 nM measured in the myenteric plexus of the guinea pig ileum.[53] Preliminary SAR[54] studies on this compound showed that the 5-HT_4 receptor agonist properties were always present with the phenylbutyl chain, confirming the existence of the large hydrophobic pocket around the cationic anchorage point in the binding site of the 5-HT_4 receptor. This point had been already emphasized with cisapride, but it might be implicated in the lack of selectivity of this compound that interacted with the 5-HT_1, 5-HT_2, and adrenergic receptors. The role of this secondary binding site in the functionality of the 5-HT_4 receptor and the selectivity versus that of the other receptors were clearly demonstrated with new benzamides such as prucalopride,[55] prepared from the metoclopramide-like benzofuran acid and *N*-methoxypropylpiperidine. This compound was particularly selective with pK_i values of 8.6 and 8.1 for the human 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors, respectively. In different in vitro preparations of 5-HT_4 receptors, prucalopride was a potent agonist with pEC_{50} values around 7.50. Similarly, Y-34959,[56] a benzamide of the aminomethylpiperidine framework, was characterized by the presence of a (1-methylindol-3-ylcarbonylamino)pentyl chain on the basic nitrogen atom. It was a potent and selective ligand for the 5-HT_4 receptor (K_i (nM) values of D_2, 5-HT_{1A}, 5-HT_2, 5-HT_4 receptors were ≥1000, 110, >1000, and 0.3, respectively).

Benzoates. The interest in the ester derivatives for the characterization of the 5-HT_4 receptors was shown with ICS 205-930, a potent 5-HT_3 antagonist that was the first surmountable antagonist of 5-HT_4 receptors[2] in the stimulation by 5-HT of cAMP formation in the primary culture of the mouse Colliculi neurons with moderate activity (pK_i = 6.2). The first potent antagonist was described by Buchheit[57] who prepared the ester analogue of metoclopramide SDZ 205-557. In contrast with the parent compound, the affinity for the 5-HT_4 receptor was dramatically increased and the compound antagonized the effect of 5-HT in the isolated guinea pig ileum longitudinal muscle preparation, with a pA_2 value of 7.4 while it was 5.6 in the contraction induced by the 5-HT_3 agonist 2-methylserotonin.[58] However, the good selectivity of this compound was not confirmed in binding studies using mouse neuroblastoma NG 108-15 cells, which express the 5-HT_3 but not the 5-HT_4 receptor, since the affinity of these cells was similar to that observed for the 5-HT_4 receptors in the striatum.[59] With the goal of obtaining more potent and selective compounds, several SAR studies on the esters derived from benzamides were designed.[60−62] They confirmed the preliminary results on the increase in affinity for the 5-HT_4 receptor with regard to the corresponding benzamide. In particular, ML 10302[63] (Chart 7) was characterized as a partial agonist equipotent to serotonin in the isolated guinea pig ileum longitudinal muscle preparation (EC_{50} = 4 nM (80%)) and in the carbachol-contracted rat oesophageal muscularis mucosae (IC_{50} = 2.4 nM (80%)). This compound was found to be selective on a large set of receptors with a weak affinity for the 5-HT_3 receptors (K_i = 782 nM). The introduction on the piperidine ring of various substituents modified the efficacy of the compounds, and a complete drop in efficacy was observed with the 3,5-dimethyl derivative. The cis compound ML 10375 was characterized as a potent antagonist of 5-HT in the rat oesophageal muscularis mucosae preparation.[62] An examination of the X-ray crystal structures of ML 10302 and ML 10375 showed a similar folded conformation of the ethyl chain, giving an orientation of the hydrogen atom of the quaternary nitrogen atom that is similar to that of aza bicycle benzamides such as zacopride and renzapride. The data on the X-ray crystal structures were confirmed by structural analysis and showed a limited number of permissible conformations. The calculation of the minimum energy conformer indicated the putative equilibrium between the extended and the different folded conformations. An attractive hypothesis is that activation of the receptor is triggered by the propensity of a molecule such as ML 10302 to adopt the folded conformation in the binding site, mimicking conformational restricted molecules such as zacopride and renzapride.[62] On the other hand, ML 10375 might bind the receptor site in the extended conformation and be unable to give the active folded form because of the steric interactions of both methyl groups with the binding site. A superior homologue of ML 10302 was characterized as a potent antagonist of the 5-HT_4 receptor (RS 23597),[64] confirming the implication of the steric properties in the pharmacological profile of the molecules.

For various larger groups on the basis nitrogen atom, the possibility of binding with the secondary binding site of the receptor was explored with the synthesis of the derivatives of ML 10302, where the piperidine ring was substituted by the different aryl- and heteroarylpiperazines.[65] In contrast with ML 10302, these compounds were antagonists of the 5-HT stimulated cAMP synthesis induced by activation of cloned human 5-HT_4 receptors isoforms and **9** was shown to antagonize the stimulatory effect of 5-HT on the L-type calcium current (I_{Ca}) in human cardiac myocytes (K_D = 0.7 nM).

Chart 7. Benzoate and Carbamate Derivatives as Selective 5-HT_4 Receptor Agonists and Antagonists

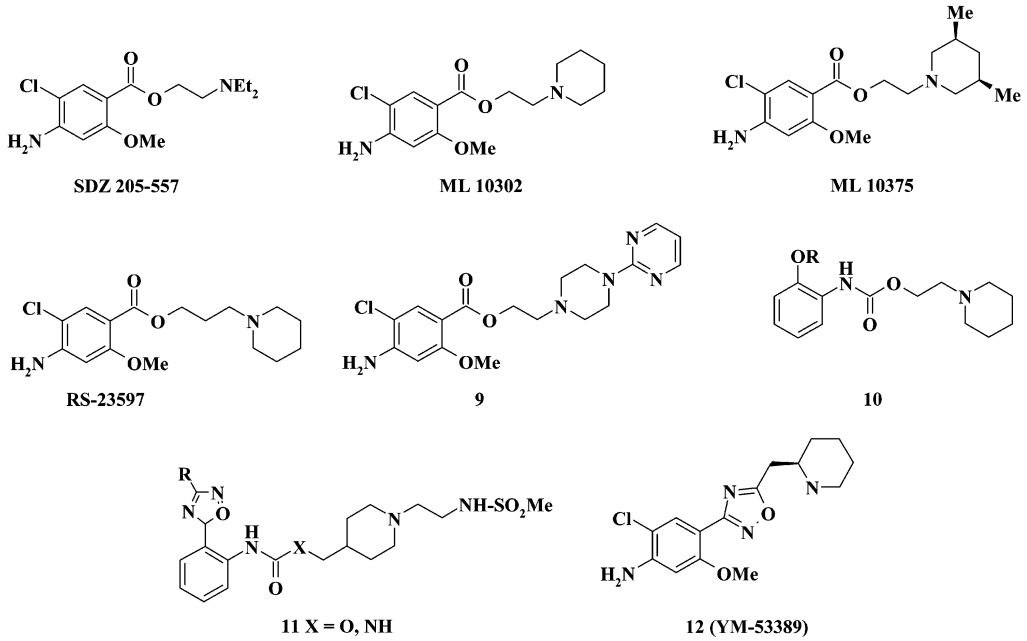
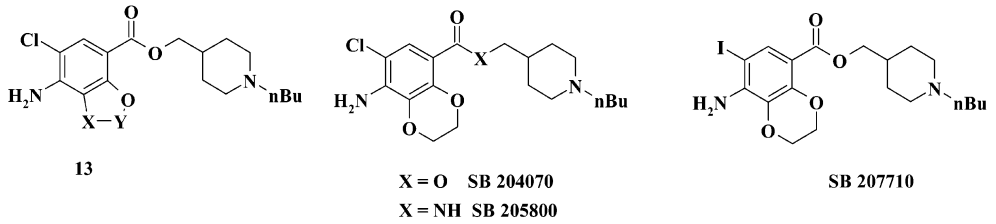


Chart 8. Benzoate Derivatives as Selective and Potent 5-HT_4 Receptor Antagonists



The presence of the ester function is a significant drawback for the administration in vivo of the compounds because of their putative short half-lives. A series of carbamates[66] derived from the previous esters were prepared (Chart 7). In contrast to the esters where the presence of the 4-amino-5-chloro substituents was mandatory for obtaining active compounds, the *o*-alkoxyphenyl carbamates **10** were the most potent and were characterized as a 5-HT_4 receptor antagonist in the guinea pig ileum. Similarly, aryl carbamates and ureas **11** in which the *o*-methoxy group was substituted by a heterocycle such as 1,2,4-oxadiazole were claimed as potent 5-HT_4 receptor antagonists.[67]

Another attempt to overcome the problems related to the bioavailability of the esters was presented recently with the preparation of the 1,2,4-oxadiazol derivatives[68] as the bioisosteric moiety of the carbonyl function. Compound **12** (YM-53389) was a highly selective 5-HT_4 agonist equipotent to cisapride. It was shown to possess significant activity that shortens the total gut transit time but without effect on the upper gastrointestinal propulsion.[69]

An important series[70] of benzoate derivatives (Chart 8) were obtained by introduction of the ortho oxygen atom of benzoic acid within a five- or six-membered ring, **13** (X−Y = O−CH_2 or O−$(CH_2)_2$−), and coupling with (1-butyl-4-piperidinyl)methylamine. Benzodioxan derivative SB 204070 was the most potent and highly selective antagonist of the 5-HT_4 receptors in the guinea pig distal colon longitudinal muscle myenteric plexus preparation (LMMP).[71] In this assay, this compound was characterized as an insurmountable antagonist with a pK_a value of 10.4, although its effect could be reversed with washout of the drug. The antagonist effect of derivative SB 204070 was confirmed in the in vivo model of the conscious dog Heidenhain pouch where the drug inhibited the 5-HT evoked response in a dose-dependent manner.[72] As previously reported for the other series, the corresponding amidic derivative of SB 204070 (SB 205800) was less potent although still active in the nanomolar range (pK_a = 9). The high affinity and the selectivity of SB 204070 led to the development of the iodo derivative SB 207710 as a radioligand that binds the 5-HT_4 receptors with a high affinity (pK_i = 9.2).[73]

Aromatic Ketone Derivatives. The design of aromatic ketones structurally related to the benzoates opened another way to overcome the problem of the metabolic hydrolysis of the esters and of their weak bioavailability. From the 5-HT_4 receptor antagonist RS-23597,[64] the corresponding ketone RS 17017[74] (Chart 9) was shown to be a partial agonist about 1 order of magnitude less potent than 5-HT in the relaxation of carbachol contracted rat oesophageal muscularis mucosae (pEC_{50} = 7.4). However, more potent compounds[75] were obtained with the basic framework of GR 113808 substituted by *n*-Bu (RS 67333) or $(CH_2)_2NHSO_2Me$ (RS 67506). These compounds are highly selective for the 5-HT_4 receptor, although they also bind σ_1 and σ_2 receptors, and they behave as potent partial agonists in the model of relaxation of rat oesophagus (pEC_{50} = 8.4 and 8.6, respectively). The 5-HT_4 receptor agonist

Chart 9. Ketone Derivatives as 5-HT_4 Receptor Agonists and Antagonists

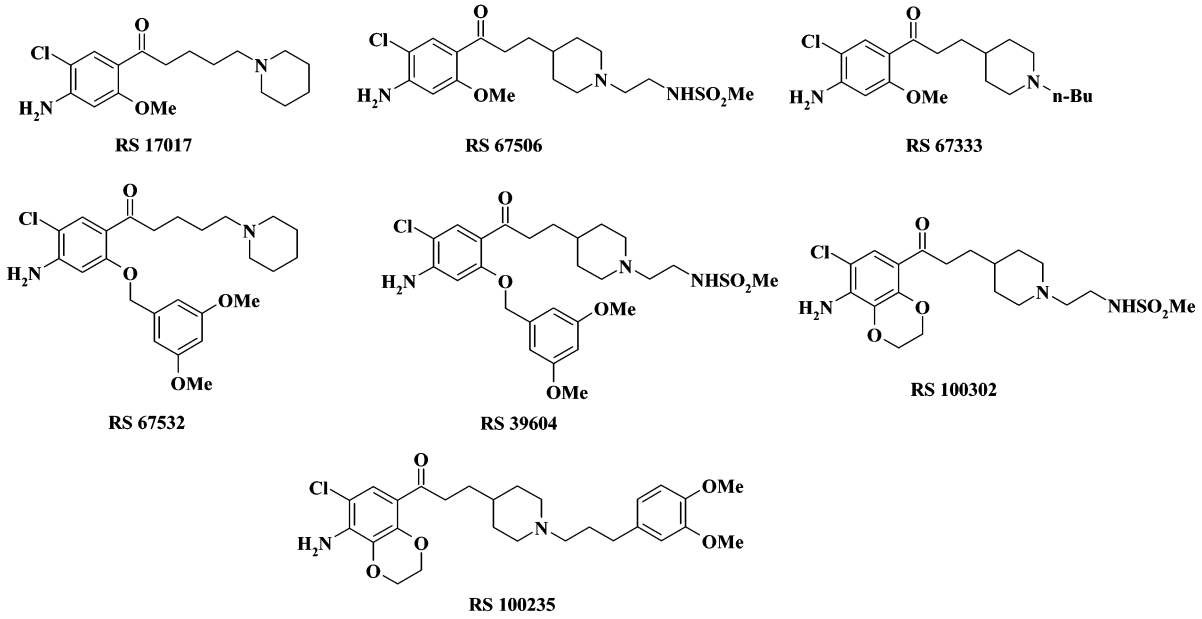
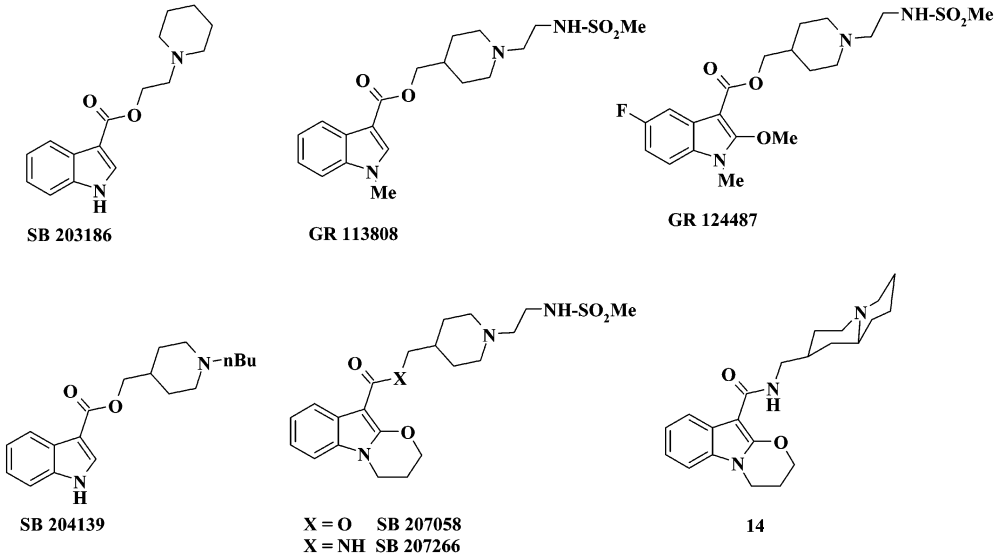


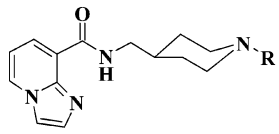
Chart 10. Indole Amides and Esters as 5-HT_4 Receptor Antagonists



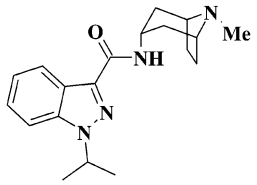
activity was demonstrated in vivo in the piglet model of tachycardia.[76] In contrast to the 5-HT_3 receptor antagonists and 5-HT_4 receptor agonists renzapride and zacopride, RS 67506 enhanced the lower intestinal propulsion in mice, suggesting that blocking of 5-HT_3 receptors might not be suitable for the treatment of propulsion impairment in the colon.[77] Modification of the *o*-methoxy group of the 5-HT_4 receptor agonists RS 17017 and RS 67506, in particular by the substitution with the 3,5-dimethoxybenzyl group, led to a complete loss of agonist efficacy and to the highly potent 5-HT_4 receptor antagonists RS 67532 and RS 39604, respectively. These compounds inhibited the 5-HT mediated relaxation of carbachol contracted rat oesophageal muscularis (pK_i = 8.5 and pK_i = 9.2).[75] Moreover, the combination of the favorable effects of the benzodioxane moiety in the benzoic esters and of the previous results obtained with the ketones led to new antagonist derivatives, such as RS 100302 and RS 100235.[78]

Indole Carboxylic Acid Ester and Amide Derivatives. As reported previously, this class of compounds was derived from ICS 205-930 or tropisetron, a potent 5-HT_3 antagonist, which binds to 5-HT_4 receptors with a medium affinity (1 μM) and has an antagonist profile.[2] The substitution of the tropane moiety (Chart 10) by the more flexible ethylamido chain and the more bulky piperidine ring gave rise to SB 203186,[79] a potent 5-HT_4 receptor antagonist in the piglet right atrium. However, an important advance was made with the synthesis of GR 113808[80] where the 4-piperidinylmethyl chain was introduced for the first time in the field of 5-HT_4 receptor ligands. This compound had a subnanomolar affinity and was very selective compared with other receptors. GR 113808 was a very useful pharmacological tool for the characterization of 5-HT_4 receptors, particularly with the tritiated molecule, which was used for the localization of 5-HT_4 receptors in various tissues and for receptor binding studies.[81] A more elaborated compound, GR 124487, was claimed as a more potent

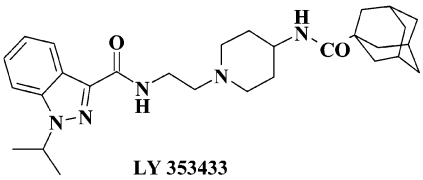
Chart 11. Imidazopyridine, Indazole, and Benzimidazole Derivatives as 5-HT_4 Receptor Antagonists



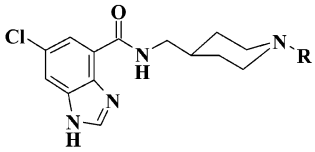
15



16



LY 353433



17

R = nBu
R = nPent
R = $(CH_2)_2NHSO_2Me$
R = $CH_2CH(CH_3)_2$

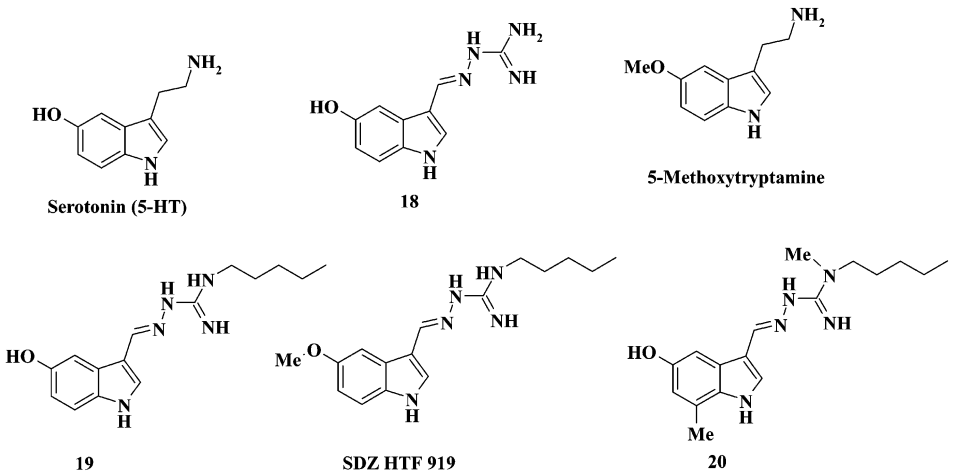
antagonist than GR 113808. Recently,[82] naphthalene bioisosteric analogues were prepared, but they were clearly less potent. In search of new compounds that would be effective orally so that they could be used as therapeutic drugs, SmithKlineBeecham synthesized a series of indole derivatives using the favorable results obtained with SB 204070. The idea was to mimic the benzodioxane ring on the indole by introduction of an oxygen atom in position 2 of the ring. The oxazolo, oxazino, and oxazepino indoles hence obtained were potent antagonists of the 5-HT_4 receptors in the guinea pig distal colon (LMMP preparation), and the derivative with the six-membered ring, SB 207058, was the most potent (pIC_{50} = 10.6).[83] As previously observed, the conversion to the analogous amide was detrimental for the activity, although the effect on these cyclic compounds was less marked and SB 207266 (piboserod) retained a good level of activity (pIC_{50} = 9.2). In contrast to SB 204070, the 5-HT response recovered its control level after washout of this drug, indicating a reversible antagonism. SB 207266 was highly potent in the model of the dog Heidenhain pouch when it was administered orally with a lasting time superior to 2 h, indicating good orally bioavailability.[84]

The influence of the constraints on the basic 4-piperidinylmethyl moiety chain was investigated with the synthesis of indolic esters of quinolizidine derivatives[85] that can mimic this basic group in the folded conformation. The best of these compounds were those obtained with a one methylene linker in the para position of the basic nitrogen atom between the acid and the aza bicycle group in the equatorial conformation. The favorable influence of the oxazino[3,2-*a*]indole group was also confirmed with the synthesis of **14**, which was highly potent in the guinea pig distal colon LMMP preparation (pIC_{50} = 9.5) and inhibited the binding of [^{125}I] SB 207710 to piglet hippocampal membranes.

Several studies of the structural analogues of the indole ring were undertaken to design compounds with potential good bioavailability (Chart 11), and consequently, the search for the amidic derivatives was privileged. Imidazopyridine carboxamides or carboxylic esters **15**, with the basic framework derived from piperidine or quinolizidine rings, were claimed as 5-HT_4 receptors antagonists.[86] An extensive SAR study on the indazole derivatives[87] was realized from a preliminary approach where it was demonstrated that indazole-3-carboxamide of *endo*-3-tropanamine **16** (Chart 10) possessed a greater 5-HT_4 receptor affinity than tropisetron. As reported[18] for the benzimidazolone series, *N*-isopropyl substitution gave the best compounds and the corresponding amides of the monocyclic piperidine linked directly to the ring or through a two-methylene chain were potent 5-HT_4 receptor antagonists. The potency was largely increased by the introduction, on the basic nitrogen atom, of a substituent with a hydrogen donor group associated with a hydrophobic moiety capable of binding to the accessory binding site already characterized in the 5-HT_4 receptor. One such compound, LY 353433, was a selective, potent, and orally active 5-HT_4 receptor antagonist. A similar SAR study was performed on a series of benzimidazole-4-carboxamides by Lopez-Rodriguez[88] who found, in contrast with the results of most previous studies, that amides were more potent 5-HT_4 receptor antagonists than the corresponding esters, particularly the amides **17** of 6-chlorobenzimidazole-4-carboxylic acid.

Serotonin Analogues. Serotonin is a potent agonist of the 5-HT_4 receptors in the different biological preparation in which this receptor is present. It could be a suitable structural model for designing new agonists for this receptor. The activity obtained with carbazimidamide **18** (Chart 12), a structural analogue of 5-HT where the guanidine group provided a stabilized basic function,[89] confirmed the interest in this approach. **18** was 2.5-fold more potent than 5-HT, with an efficacy of 150% and exhibiting a pD_2 value of 8.8 in the electrically stimulated longitudinal muscle preparation of the guinea pig ileum. However, the presence of the guanidine function could be an important drawback for the bioavailability of the compound. A SAR study[90] of the indole carbazimidamide family showed the possibility of introducing lipophilic groups capable of interacting with the secondary accessory hydrophobic site of the 5-HT_4 receptors. As already reported, this region of the receptor can accept a large set of substituents that clearly influence the pharmacological activity. Among a number of potent compounds, **19** was discovered as

Chart 12. Carbazimidamide Derivatives as 5-HT_4 Receptor Agonists or Antagonists



the most potent 5-HT_4 receptor full agonist described so far ($pD_2 = 9.3$). However, SDZ HTF 919 or tegaserod,[91] a moderate partial agonist ($pD_2 = 6.9$), was selected for the clinical investigations in the disorders of the intestinal transit. The presence of the methoxy group in this compound is structurally analogous to 5-methoxytryptamine, a selective 5-HT_4 receptor agonist. The indole carbazimidamide derivatives shared no recognition parameters with ligands of 5-HT_3 receptors, and they were perfectly selective compared with this receptor. However, the structural similarity of these compounds to serotonin might explain why **19** was only 15-fold less potent on the 5-HT_{1D} receptor than on the 5-HT_4 receptor. It is worth noting that, from the SAR study reported, the activity of these compounds resides only in the indole derivatives, since the carbazimidamide derivatives with the isosteric indole groups are either inactive or only weakly active. The introduction of a small substituent, for example, Me or Et, on the aromatic ring of **19** brought about an interesting drop of the intrinsic activity,[92] since the compounds became 5-HT_4 receptor antagonists. **20** was a competitive and selective antagonist in the assay of the inhibition of 5-HT induced contractions of guinea pig ileum ($pA_2 = 8.4$). This last point highlighted the influence of the subtle structural modification of the ligands on their efficacy. Indeed, a small steric hindrance or constraint can hamper the rearrangement of the receptor during the activation step of the receptor, producing a loss of efficacy and an antagonist profile for the molecules. This point has been reported recently with some agonists of 5-HT_{1A} and 5-HT_4 receptors[62,93] that were transformed into the antagonists of the corresponding receptors by the introduction of a methyl group on the agonist structures.

Pharmacophore of the 5-HT_4 Ligands and the Binding Receptor Site

The preliminary data on the structural parameters implicated in the recognition of the 5-HT_4 receptor binding site were influenced by the results obtained in the field of the 5-HT_3 receptors because a number of 5-HT_3 antagonists were also 5-HT_4 receptor agonists or antagonists. Therefore, the presence of an aromatic system, a hydrogen acceptor group such as the carbonyl function, and a basic group was the essential characteristic of the 5-HT_4 receptor pharmacophore. It was structurally closely related to the model generated by Hibert[94] for the 5-HT_3 receptor antagonists. However, several differences were emphasized during the search for more selective ligands and the main ones concerned the structure of the basic chain. The 5-HT_3 receptor site prefers the reduced steric hindrance around the basic nitrogen atom as in zacopride or renzapride, while the 5-HT_4 receptor site can fit very voluminous groups on the basic nitrogen atom. The best illustration of this point is provided by compounds such as cisapride, prucalopride, and LY 353433. The linker between the acceptor hydrogen group and the basic nitrogen atom possesses variable length: six bonds in GR 113808, SB 207266, and RS 67506, five bonds in RS 67532, and fours bonds in ML 10375 and SDZ 205-557.

In contrast with other members of the 5-HT receptor family, few molecular modeling studies have been published on 5-HT_4 receptors. In 1997, Lopez-Rodriguez[95] calculated the geometrical characteristics of a 3-D model of the pharmacophore by the active analogue approach of two sets of 5-HT_3 and 5-HT_4 receptor ligands. It was characterized by the following parameters: a carbonyl function coplanar with the aromatic ring located 3.6 Å from the centroid of the aromatic ring, a basic nitrogen atom separated by 8.0 Å from the centroid and by 5.4 Å from the oxygen atom of the carbonyl function, a hydrophobic accessory pocket capable of binding the voluminous substituent of the nitrogen atom. This study gave quantitative expression of what can be derived from a careful examination of the molecules and provided no information on the exact nature of the nitrogen atom substituent.

Few data were also available from SAR studies concerning the variation of the pharmacological profile of the molecules. For instance, the "tied back" structure of the basic framework of the 5-HT_4 receptor agonists of the first generation, such as zacopride and renzapride, was related to the agonist profile. However, this structural property was not totally relevant to the pharmacological profile because several agonists such as cisapride and prucalopride possess a large substituent on the basic nitrogen atom.

An interesting structural analysis reported by Buchheit[89] compared the 3D structure of serotonin and zacopride and proposed a pharmacophore model where

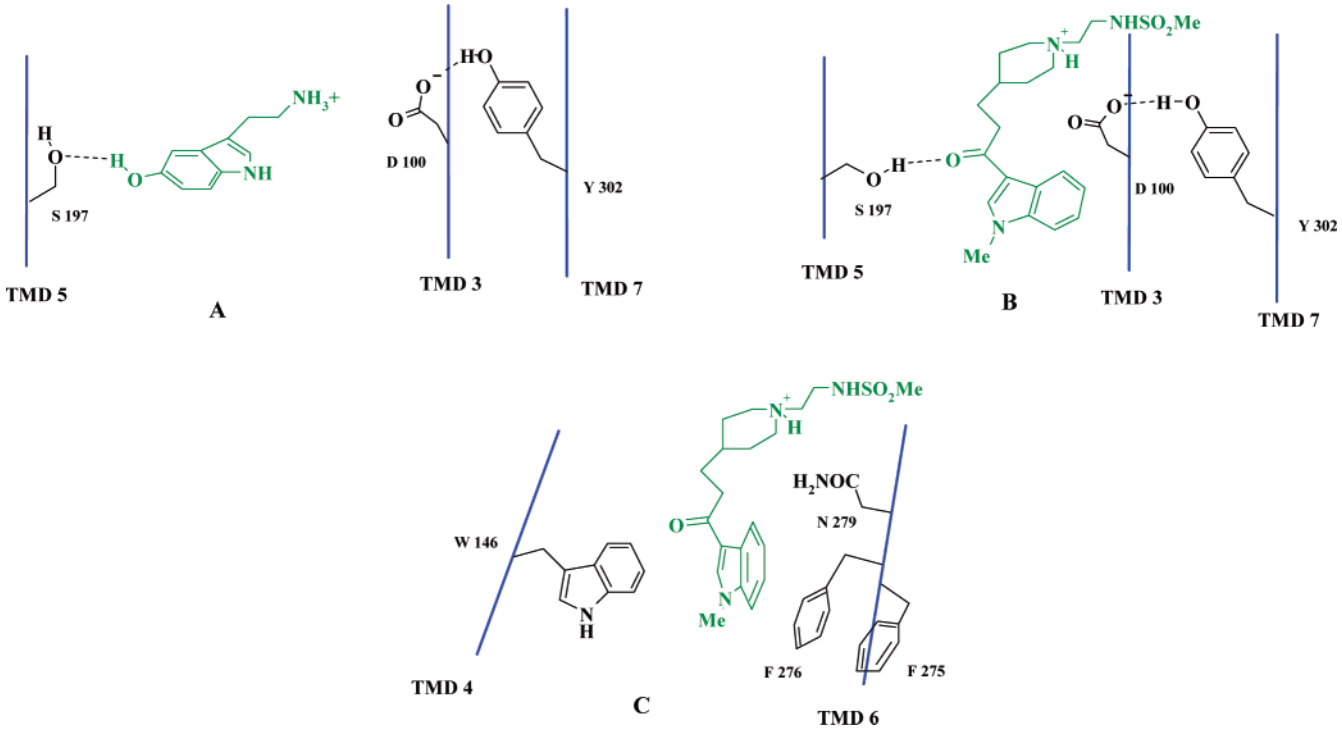


Figure 1. (A) Serotonin−5-HT$_4$ receptor complex. Y 302 (TMD 7) stabilizes the consensus ionic interaction with D 100. (B) GR 113808−5-HT4 receptor complex. S 197 binds through a hydrogen bond to the carbonyl group of the ester function. (C) GR 113808−5-HT4 receptor complex. The indole ring is embedded in a hydrophobic pocket defined by W 146, F 275, F 276, and N 279.

the basic nitrogen can be located either 5.7 or 7.4 Å from the center of the aromatic ring and 0.8 or 0.1 Å above the plane defined by this system. The carbazimidamide **19**, which has an embedded guanidine function, was capable of occupying simultaneously the two positions and of binding the putative hydrogen acceptor group and the charge acceptor located in the receptor site.

Recently,[96] a more direct approach of the nature of the interactions in the binding site and the ligands were reported with a site-directed mutagenesis and molecular modeling study. The new human 5-HT$_{4(a)}$ isoform was expressed in COS-7 cells and several highly conserved amino acids residues in the G protein receptor family were mutated. Docking experiments taking into account the results of the measurements of the affinity and functional activity of serotonin and GR 113808 were realized. Essential results (Figure 1) indicated that, for the binding of serotonin, Y302 in the seventh helix played a major role in the stabilization of the consensus ionic interaction with D100 in the third helix, and S197 on the fifth helix was the anchorage point with the hydroxyl function. For GR 113808, it was proposed that S197 binds the carbonyl group of the function ester. It is worth noting that the substitution D100N had a relative weak effect on GR 113808 binding, which indicates a rather small contribution of the ionic interaction, since it can be substituted by an hydrogen bond. Docking experiments showed that the indole ring was embedded in a pocket defined by the residues W146, F275, F276, and N279 because any mutations of these amino acids decreased dramatically the affinity of the ligand for its site and modified the functional activity of serotonin. These last characteristics were common with other serotoninergic receptors, in particular the 5-HT$_2$ receptor.[97]

Receptors, Localization, Structure, and Functions

The 5-HT$_4$ receptor is a member of the seven transmembrane-spanning G protein coupled family of receptors (GPCR) and constitute an important subtype of the class of serotonin receptors. Initially, the 5-HT$_4$ receptor was characterized in the neuronal cell culture[98] of mouse colliculi and it was shown to be positively coupled to adenylyl cyclase. The effect of serotonin was mimicked by the 5-HT$_4$ receptor agonists BIMU 1 and BIMU 8 and was blocked by the 5-HT$_4$ receptor antagonists DAU 6215.[24,25] This positive effect of 5-HT$_4$ receptors on adenylyl cyclase is shared by the recently discovered 5-HT$_6$ and 5-HT$_7$ receptors.[99,100] 5-HT$_4$ receptors are distributed in the guinea pig and rat central nervous system (CNS) in two anatomical and functional structures: the extrapyramidal motor system (striatum, globus pallidus, and substantia nigra) and the mesolimbic system (nucleus accumbens and olfactory tubercle).[6,101] They were characterized by using the binding bioassay with [^{3}H] GR 113808 as radioligand.[81] In human, their presence was shown in basal ganglia and in the caudate−putamen nuclei where the density is the highest (B_{max} = 225 fmol/mg protein). Several areas with a substantial density of receptors were found: lenticular nucleus, substantia nigra, hippocampus, and frontal cortex.[102,103] Recently,[104] the mapping of the 5-HT$_4$ receptor in the postmortem human brain was determined by using the localization of the 5-HT$_4$ receptor mRNA and the 5-HT receptor protein. The highest levels of 5-HT$_4$ receptors were found in caudate nucleus, putamen, nucleus accumbens, and hippocampal formation. 5-HT$_4$ receptors are also present in the periphery,[105−107] particularly in the overall gastrointestinal tract where they are implicated in the contraction or

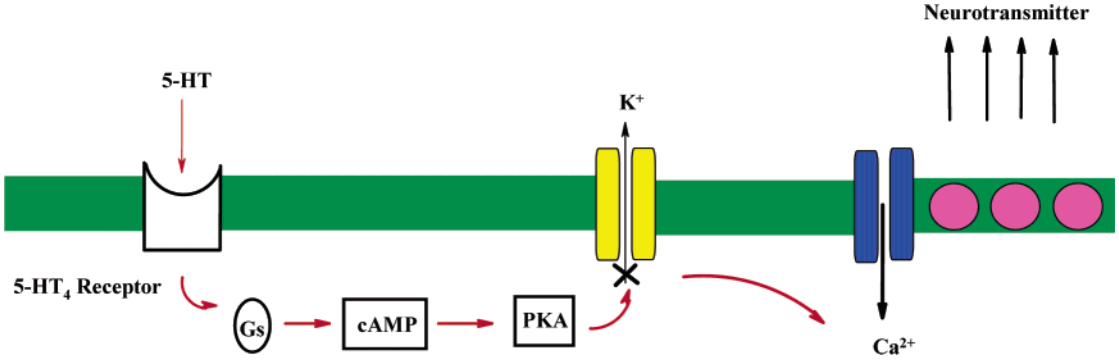


Figure 2. Activation of the 5-HT_4 receptor leads to activation of cAMP-dependent protein kinase (PKA) and inhibition of a voltage-activated K^+ channel. The resulting depolarization activates Ca^{2+} influx, which triggers neurotransmitter release.

the relaxation of the smooth muscle. Saturable binding of [^{3}H] GR 113808 was determined in the longitudinal muscle and myenteric plexus of the guinea pig, with a larger number of sites in the upper part of the intestine: duodenum > jejunum > ileum ≫ colon > rectum. In all cases, the number of binding sites in the intestine is inferior to that in brain. The presence of 5-HT_4 receptors in the intestine allowed for the understanding of the mechanism of several benzamide gastrokinetic drugs, such as cisapride and metoclopramide.[108,109] 5-HT_4 receptors are also present in the pig and human heart,[7] but they are exclusively located in the atrium where they are responsible for the tachycardia and the positive inotropic effect observed after injection of 5-HT. Kaumann[14] has suggested that 5-HT liberated from platelets could induce atrial arrhythmia by activation of these receptors. In the vascular system, 5-HT_4 receptors were characterized on the pulmonary vein and the mesenteric lymphatic system where they cause relaxation, but too few data have been reported to date to give a clear characterization of this receptor in these tissues.[110]

It has been demonstrated that the 5-HT_4 receptor is present in the human detrusor muscle and mediates the facilitation via a cholinergic mechanism of the contraction of the human bladder.[10] 5-HT_4 receptors could be implicated in urinary incontinence because this syndrome has been found to occur in patients treated with cisapride.

5-HT mediates the release of corticosterone and aldosterone via 5-HT_4 receptors on the human adrenal cortex.[9] Zacopride caused the release of cortisol in the human adrenocortical slices,[111] which was blocked only by high doses of tropisetron. To date, it is the only endocrine pathway that is regulated by this receptor subtype.

In almost all tissues where the 5-HT_4 receptors are present, 5-HT or the corresponding agonists are capable of increasing cAMP synthesis.[112] This was the case in the hippocampus, the atrium, the oesophagus, the gut, and the adrenal cortex. One of the characteristics of the 5-HT_4 receptors is their ability to be desensitized after their stimulation. It was shown[113,114] that 5-HT elicited the depolarization of the pyramidal cells of the CA1 region of the hippocampus, and a concentration-dependent reduction in the amplitude of the after-hyperpolarization (AHP) was emphasized. This effect was competitively inhibited by selective 5-HT_4 receptor antagonists such as GR 113808 and SDZ 205-507.[115] Similarly in colliculi neurons,[116] the exposure of the receptors to 5-methoxytryptamine or 5-HT_4 receptor agonists was followed by a rapid and long inactivation that was related to the potency and the efficacy of the agonists. The desensitization was not dependent on the cAMP formed but on the mean occupancy time of the receptor by the agonist, and it was due to phosphorylation of the occupied receptor by βARK or another specific receptor-dependent protein kinase.

A number of physiological processes can be triggered by an increase in intracellular cAMP. For instance, in mouse colliculi neurons,[117] the activation of the 5-HT_4 receptors is followed by the inhibition of the voltage-activated K^+ channel current caused by a phosphorylation reaction through cAMP-dependent protein kinase (PKA) (Figure 2). Consequently, the action potentials are significantly prolonged, resulting in a loss of after-hyperpolarization (AHP). Prolongation of the cell membrane depolarization would result in the activation of the voltage-dependent Ca^{2+} channels, leading to an increase of Ca^{2+} influx that contributes to neurotransmitter release. This phenomenon might explain the positive effect of a 5-HT_4 receptor activation on the release of acetylcholine (ACh) in the CNS. In the atria, the increased production of cAMP induces activation via PKA of the L-type Ca^{2+} channel current (I_{Ca}).[12] In the intestine, the direct effect of an increase in cAMP concentration, via activation of 5-HT_4 receptors, is the relaxation of the smooth muscle. However, 5-HT_4 receptor activation can also induce contraction of the smooth muscle through the indirect release of ACh that results from activation of 5-HT_4 receptors located on the sensory neurons of the myenteric plexus.[105]

The cloning and the structural characterization of the rat 5-HT_4 receptors were described by Gerald[118,119] who first isolated cDNA and expressed it in COS-7 cells. Two isoforms were first characterized. They were identical between residues 1 and 359 and differed only in the C-terminal region. They were generated by the alternative splicing of a unique gene and were named 5-HT_{4L} and 5-HT_{4S} for the long and short isoforms, respectively. More recently, a third shorter isoform was characterized[120] and the official nomenclature for these receptors was r5-$HT_{4(b)}$, r5-$HT_{4(a)}$, and r5-$HT_{4(e)}$. A similar diversity of isoforms was found for the mouse, and four isoforms were described: 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(e)}$, and 5-$HT_{4(f)}$.[120,121] In 1997, Blondel[122] followed by Clayesen[123] and Van den Wyngaert[124] described the first human 5-HT_4 receptor isoform. Blondel[122] isolated the receptor

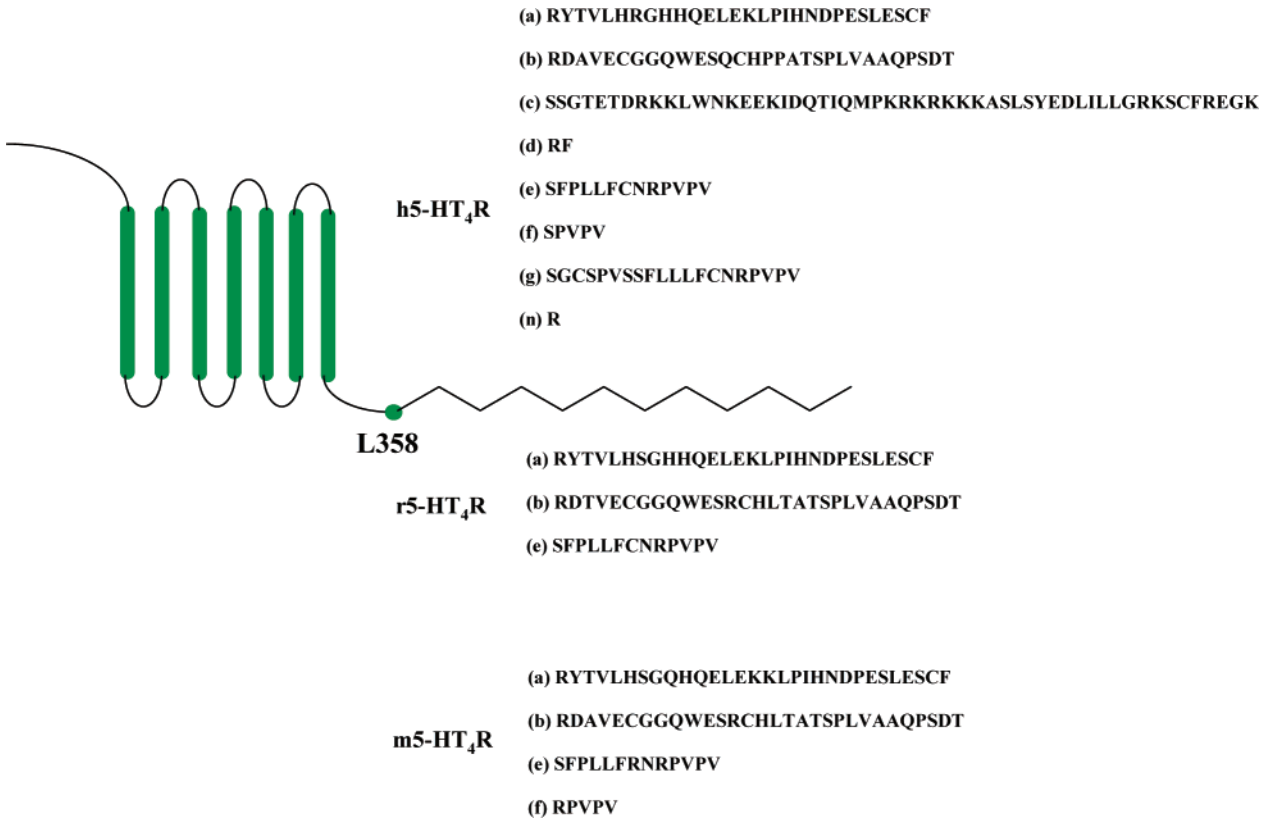


Figure 3. C-terminal amino acid sequences of the 5-HT_4 receptor splice variants in human, mouse, and rat. The cloned 5-HT_4 receptors have identical sequences up to Leu 358 and differ by the length and by the nature of the amino acids. It is worth noting the repeat sequence PV in the variants e, f, and g.

from human atrium and named it h5-$HT_{4(a)}$ because it was considered to be homologous to the short isoform described in rat. This receptor was transiently expressed in COS-7, and the response to 5-HT and some 5-HT_4 receptor agonists was identical to the stimulation pattern of I_{Ca} obtained in response to these compounds in human atrial myocytes.[122] Since then, several other splice variants were cloned: h5-$HT_{4(b)}$,[4] two different h5-$HT_{4(c)}$ isoforms,[4,126] h5-$HT_{4(d)}$,[4,126] h5-$HT_{4(e)}$[127] (which will be called h5-$HT_{4(e/g)}$ in the following, since this isoform was later renamed h5-$HT_{4(g)}$[125,128] and replaced by another h5-$HT_{4(e)}$ isoform[125] found to be more homologous to the mouse 5-$HT_{4(e)}$ receptor[120]), h5-$HT_{4(f)}$,[125] and h5-$HT_{4(n)}$.[129] Whatever the animal species (mouse, rat or human), the different splice variants have an identical sequence up to Leu 358 whereas the length and the composition of the rest of the C-terminus tail is specific for each variant (Figure 3). For instance, in human, the C-terminal end is very short for the 5-$HT_{4(d)}$[4] and 5-$HT_{4(n)}$[129] isoforms (respectively, only 2 and 1 amino acids after Leu 358) and the 5-$HT_{4(d)}$ isoform (only 2 after Leu 358) and is 31 amino acids long for the 5-$HT_{4(b)}$ variant.[4] One of the characterized isoforms, 5-$HT_{4(c)}$, might be the target of different protein kinases because several phosphorylation sites are present on its C-terminus tail.[4] The tissue distribution revealed some degree of specificity. For instance, 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(c)}$, and 5-$HT_{4(e/g)}$ receptors are all expressed in the atrium, brain, and intestine while the 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptor subtypes are the only receptors present in the bladder and kidney, respectively.[4] On the other hand, the 5-$HT_{4(d)}$ isoform was only characterized in the intestine. The different isoforms expressed in COS-7 cells displayed the classical profile of the 5-HT_4 receptors previously observed in the native tissues and showed an identical ability to stimulate adenylyl cyclase activity. No major difference could be found between the different isoforms as far as the K_i values of the agonists or the antagonist were considered. On the other hand, some significant differences in the functionality were observed with the agonists ML 10302 and renzapride. ML 10302, described as a potent agonist in the GI tractus in rat, was a weak partial agonist of each human isoform, whereas renzapride was 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-$HT_{4(e/g)}$ receptors.[127] These functional differences may explain the tissue-dependent specificities observed with benzamides, which act as full or superagonists[2] in the mouse colliculi neurons and as only partial agonists[12] in other systems such as human myocytes. An attractive hypothesis is that a given receptor subtype might convey a specific functional response in a particular tissue. However, more information is needed to give clear conclusions on the exact repartition of the isoforms in the different tissues and the influence of the expression system on the efficacy of the ligands. Recently,[128] by use of a real time quantitative reverse transcription polymerase chain reaction (RT-PCR) method, the distribution of the different variants mRNA was investigated in the CNS, heart, and gut in human. The data obtained suggested the predominant presence of the h5-$HT_{4(b)}$ receptor over the h5-$HT_{4(a)}$ receptor in the different tissues studied. Interestingly, the existence of a new variant h was recently reported,[125] which possesses a 14 amino acid sequence inserted into the second extracellular loop and

combined with the C-terminal sequence of the b variant, which led to its appellation 5-$HT_{4(hb)}$. This receptor has a pharmacological profile similar to those previously described in the competition binding assays for the other isoforms but differs in its response to the reference antagonist GR 113808, which displays a partial agonistic activity on this new isoform.

The influence of the length of the C-terminal part in the activation of the receptor was shown by Claeysen[5] who studied the constitutive activity of the different mouse receptor isoforms. This activity was inversely correlated to the length of the terminal sequences. Thus, it was increased up to the level of the activity of the m5-$HT_{(4f)}$ isoform by the progressive deletion of the C-terminal part of the m5-$HT_{(4a)}$ receptor up to residue 359. Moreover, a dramatic increase of the activity was obtained with deletion of an additional 13 residues. Therefore, it was proposed that the common part of m5-HT_4 receptors up to residue 346 confers to the receptor a constitutive activity (state R*) and that the subsequent residues on the terminal end modulate this constitutive activity, modifying the equilibrium between the inactive state R and the active state R*. The propensity of the receptor to possess a high constitutive activity was characterized by an increase of the thermal denaturation rate.[5] The studies made on several C-terminal mutants modifying the R*/R ratio demonstrated that the receptor in its active state R* denatures much more quickly than the protein in its resting state R and that the denaturation rate depended on the *J* value of the equilibrium constant between the R and R* states. These studies on the constitutive activity of the different isoforms of 5-HT_4 receptors are important for a better understanding of the regulation mechanism of the signal transduction in the cell. As for other GPCRs,[130] constitutive activity of the 5-HT_4 receptors may be implicated in a number of pharmacological and pathological processes and may point to the potential role for an inverse agonist, such as GR 125487 and ML 10375,[131] which reduce the constitutive activity of these receptors.

Recently, it was also shown that 5-HT_4 receptor agonists such as BIMU 8 were capable of stimulating the incorporation of palmitic acid in the 5-$HT_{4(a)}$ receptor expressed in Sf 9 cells.[132] These data emphasized the role of the membrane in the mechanism regulating the molecular events of the receptor and the signaling processes.

5-HT_4 Receptors and the Gastrointestinal System

5-Hydroxytryptamine has variable effects on the gastrointestinal tract. It induces both the contraction and relaxation of smooth muscle and stimulates the intramural nerve plexus. In mammalian small intestine, 5-HT is stored in the enteric neurons and in the mucosal enterochromaffin cells and it is secreted into the lumen and the submucosal tissue. Once liberated, 5-HT can act as a local hormone or a neurotransmitter through the different receptor subtypes present in the gastrointestinal tract: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, and 5-HT_7.[133−137]

5-HT_{1A} receptors have been demonstrated to be responsible for the hyperpolarizing response of the enteric neurons associated with the presynaptic inhibition of the release of ACh at the nicotinic receptors. It was also clearly demonstrated that 5-HT_{1D} receptor agonists, such as 5-CT and sumatriptan, are capable of stimulating the peristaltic reflex in the isolated guinea pig ileum.[133] 5-HT_2 receptors, previously known as D receptors, are localized on smooth muscle and their activation mediates contraction of the guinea pig longitudinal muscle. 5-HT_3 receptors,[138] similar to the 5-HT_4 receptors, are present at the presynaptic level on the enteric neurons and regulate positively the release of ACh. More recently, 5-HT_7 receptors have been characterized in the large intestine.[137]

Activation of 5-HT_4 receptors in the GI system can cause various effects depending on the region studied and the animal species. 5-HT_4 receptor stimulation leads to the contraction of the guinea pig ileum and colon[8] and increases the reflex sensitivity of the isolated and intact guinea pig ileum.[139] 5-HT_4 receptors were also implicated in the gastric emptying in rats[140] and in the 5-HTP (5-hydroxytryptophane) induced defecation and diarrhea in mice.[141] Conversely, stimulation of 5-HT_4 receptors may lead to relaxation of rat oesophagus and terminal ileum[3] and of human intestinal smooth muscle[142] and to a decrease in the spontaneous activity of the human colon.[143] These apparently opposite effects can be explained by the different localization of the 5-HT_4 receptors. They are present on the interneurons of the myenteric plexus and mediate indirectly the release of ACh.[144] The relaxation is induced directly by stimulation of the 5-HT_4 receptors present on the smooth muscle, the intensity of the effect depending upon the region studied.[145]

Initially, the role of the 5-HT_4 receptors in the gut was demonstrated by Clarke[3] who showed an activation of the peristaltic reflex of guinea pig ileum by 5-HT_4 receptor agonists. 5-HT had a biphasic effect depending on the concentration, the first phase (10^{-9}−10^{-7} M) involving activation of 5-HT_4 receptors and the second phase (>1 μM) involving activation of 5-HT_3 receptors. The initial phase was inhibited by atropine and was insensitive to the blockade by ICS 205-930 (<1 μM), granisetron, and ondansetron excluding the contribution of 5-HT_3 receptors. Moreover, agonist-induced desensitization protocols allowed a clear separation of the two response; thus, long exposure of guinea pig ileum to 5-methoxytryptamine, a 5-HT_4 agonist, inhibited completely the first phase of the response to 5-HT but had no effect on the second phase. Likewise, exposure of the preparation to the 5-HT_3 receptor agonist, 2-methylserotonin, inhibited only the second phase of the response to 5-HT but left unchanged the first phase.[146] The benzamides of first generation and the benzimidazolones BIMU 1 and BIMU 8, which possess both 5-HT_3 receptor antagonistic and 5-HT_4 receptor agonist properties, were found to mimic the effect of 5-HT on the first phase and to evoke the peristaltic reflex.[3] These experiments were the first demonstration of the mechanism of action of benzamides that possess gastrokinetic properties and are used as drugs to increase the gastric emptying and the intestinal transit.[108]

The presence of 5-HT_3 receptors in the guinea pig ileum could be a drawback in the study of nonselective molecules. The characterization of 5-HT_4 receptors in

the rat oesophagus and particularly in the tunica muscularis mucosae constituted a clear improvement to the selection of 5-HT_4 receptor agonists.[3] In contrast to guinea pig ileum, the 5-HT_3 agonist 2-methyl-5-HT possessed a very weak activity in this preparation. The assay evaluated the relaxation potency of the carbachol-contracted muscle preparation in the presence of cumulative doses of 5-HT (ED_{50} = 15.6 nM). Benzamides, such as renzapride and cisapride, were agonists equipotent to serotonin, and ICS 205-930 caused a rightward shift of the agonist effect curves. As demonstrated in the CNS, the 5-HT_4 receptors present in oesophagus mediated an increase of cAMP synthesis.[147] 5-HT and 5-HT_4 agonists caused a concentration-dependent increase of intracellular cAMP in the rank order of their efficacy, indicating that the 5-HT_4 receptors were most likely localized postsynaptically on the smooth muscle cells. As reported in colliculi neurons, a rapid desensitization of these nonneuronal 5-HT_4 receptors was observed independently of the level of cAMP production, indicating a homologous rather than heterologous desensitization process. This assay provided clear evidence for marked heterogeneity among animal species in the response to 5-HT. Indeed, different responses to serotonin were found when it was tested on carbachol-contracted oesophagus of rat, guinea-pig, rabbit, and dog. No effect was observed in dog, contractions were observed in guinea pig and rabbit, and a relaxation was found in rat only.[148−150]

5-HT_4 receptors are also localized in the guinea pig stomach where 5-HT enhances the electrically induced contraction in the circular muscle strips from the fundus and corpus.[151,152] However, the effect of 5-HT is rather complex because of the presence of different 5-HT receptors in this preparation. Stimulation of 5-HT_{1A} and 5-HT_2 receptors by a specific agonist (8-OH-DPAT and α-methyl-5-HT, respectively) inhibits contractions, while 5-HT_4 and 5-HT_3 receptors agonists (renzapride, metoclopramide, and 2-methyl-5-HT) increase the electrically induced contraction. Nevertheless, under physiological conditions, it is likely that the response to 5-HT is only mediated by 5-HT_4 receptors, unless large quantities of 5-HT are present. In rat, gastric 5-HT_4 receptors were implicated in the stimulation of the gastric emptying, a test largely used to select gastrokinetic drugs. However, several other data demonstrated an inhibitor role of 5-HT_3 receptors in rat stomach because several selective 5-HT_3 receptor antagonists possess an ability to stimulate the gastric emptying in this animal species.[153]

5-HT_4 receptors were identified in the distal colon longitudinal muscle myenteric plexus (LMMP) of guinea pig, another very useful preparation for the characterization of the ligands.[154] LMMP is a very sensitive model in which the pEC_{50} value of serotonin is particularly high (9.2) compared to other tissues. As in the ileum, the effect of 5-HT on LMMP is blocked by atropine. This is consistent with the putative localization of the 5-HT_4 receptors on the cholinergic neurons. Renzapride, cisapride, and zacopride, when tested in guinea pig ileum, have an intrinsic activity inferior to 1, while all benzamides tested in the distal colon behave as full agonists. In particular, (*S*)-zacopride was a full agonist distinct from the (*R*) enantiomer. The high intrinsic activity in this model may be attributed to the high density of the 5-HT receptors or to a more efficient coupling mechanism. However, the situation is complicated by the observation that mosapride, a benzamide 5-HT_4 receptor agonist, was shown to be active only in the upper gastrointestinal system and not in the colon.[155] On the other hand, not only 5-HT_4 receptors but also 5-HT_3 receptors contribute to the propulsion of the colon, as evidenced in the guinea pig where 5-HT_4 and 5-HT_3 receptor antagonists decreased with an additive effect of the velocity of the propulsion while the agonists HTF 919 and prucalopride increased the propulsion.[156]

In recent years, the interest in drugs effective in relieving the different types of constipation, a major disorder of the gastrointestinal system in human, was considerably increased. In particular, the role of 5-HT_4 receptors in the regulation of the human intestine motility was investigated. While the 5-HT_4 receptors had been well characterized in guinea pig large intestine,[157] the knowledge of their exact physiological role in the human gut was limited. Their implication[158] was shown in the 5-HT induced relaxation of the circular muscle of the human intestine. These receptors are localized in the myenteric plexus and the muscle,[159] and subsequently, the positive effect of 5-HT on cAMP synthesis was demonstrated.[160] Although 5-HT_7 receptors have been implicated in the relaxant effect of 5-HT,[137] Prins[161,162] showed that the selective 5-HT_4 receptor agonists prucalopride and RO76186 elicited a clear dose-dependent relaxation of the KCl-precontracted circular muscle and that the potent selective 5-HT_4 receptor antagonists GR 113808, GR124487, and RS 39604 gave a concentration-dependent rightward shift of the 5-HT dose−effect curve. A similar relaxant effect was observed in the canine isolated rectum circular smooth muscle, an assay that can constitute a predictive 5-HT_4 receptor model for the human colon.[163]

The role of 5-HT_4 receptors on the induction of the contractility was clarified by the recent data that proposed more information about the mechanism regulating the initiation of the peristaltic reflex in the intestine. It was supposed that serotonin was released from the enterochromaffin cells of the epithelium of the mucosa in response to chemical or mechanical stimuli induced by the progression of the faecal bolus.[164] 5-HT acts on the CGRP (calcitonin-gene-related peptide) containing sensory nerve terminal (Figure 4) and liberates CGRP, which brings about the release of excitatory (ACh, substance P, neurokinin A) and inhibitory (vasoactive intestinal peptide (VIP), pituitary adenylyl cyclase activating peptide (PACAP), and NO) neurotransmitters.[165] In contrast with previously published data, it was proposed that 5-HT_4 receptors are located on the sensory CGRP neurons and not on the intermediate cholinergic neurons. Consequently the activation of the 5-HT_4 receptor located on the primary afferent neurons would stimulate an excitatory neuron, producing contraction on the orad side and an inhibitory neuron on the caudad side. This results in a peristaltic reflex with contraction above and relaxation below the site of the stimulus.

The release of CGRP was found to be inhibited by 5-HT_4 antagonists in human intestine and rat colon,[166]

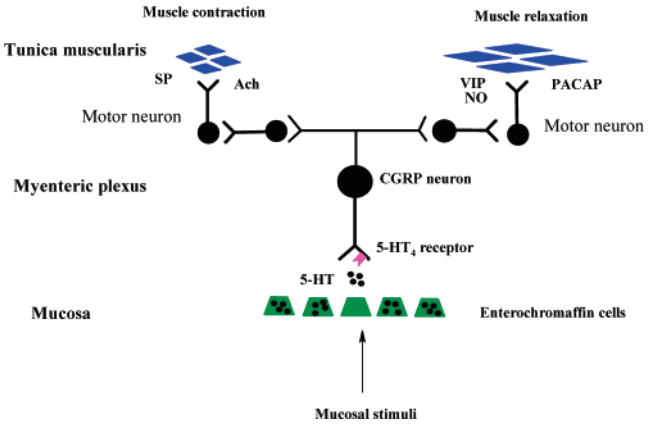


Figure 4. Intrinsic sensory pathways mediating the peristaltic reflex. The activation is brought about by mucosal stimuli, which release serotonin (5-HT) from enterochromaffin cells located in mucosa. 5-HT acts on the 5-HT_4 receptors located on the afferent projection of CGRP neurons. CGRP release is activated via the stimulation of the interneurons, the efferent projections of the excitatory (ACh, SP) and the inhibitory (VIP, PACAP, and NO) motor neurons. This results in the peristaltic reflex with contraction above and relaxation below the site of origination of the stimulus.

although a similar inhibitory effect was obtained in guinea pig colon with either 5-HT_3 or 5-HT_4 antagonists.

The additional relaxation induced by activation of the 5-HT_4 receptors present on the smooth muscle of the intestine or the colon in the different species studied can induce some misleading conclusions about the pharmacological profile of the 5-HT_4 receptors agonists or antagonists. Indeed, the overall effects of 5-HT are a balance between the excitatory and the inhibitory effects. Moreover, 5-HT_4 receptors were found to play a minor role in the normal gut function because administration of a potent 5-HT_4 receptor antagonist, such as SB-207266, had no effect on the level of defecation in mice.[167] However, when the intestinal function was disturbed by an abnormal level of 5-HT produced by stress or administration of 5-HTP, SB-207266 was capable of inhibiting the intestinal disfunction.[167]

To date, gastrokinetic drugs with 5-HT_4 serotoninergic properties are used in a human clinic for the treatment of gastro-oesophageal reflux disease and different gastric stasis. Cisapride, which is particularly efficient in those different disorders, was also demonstrated to be capable of stimulating the peristalsis at the colonic level and of inducing defecation in constipated patients.[168,169] Recently, the interest in drugs acting on gut motility and particularly on the disorders of the small bowel and the colon has largely increased. Irritable bowel syndrome (IBS) is a disorder that has been clinically well characterized and that represents a prevalence of 10% in the Western countries and 40% of the consultations in gastroenterologists.[170,171] It is a complex dysfunction of the intestine with three components: the hypersensitivity of the gut, the altered motility, and the psychosocial disorder.[172] In general, the patients may present either diarrhea or constipation or an alternation of both. Consequently, 5-HT_4 receptor agonists or partial agonists seem to be suitable for the treatment of IBS in which the intestinal propulsion impairment dominates while 5-HT_4 receptor antagonists can be effective in patients with significant diarrhea. Much data[173] have been recently reported on the clinical activity of 5-HT_4 ligands in patients with IBS. SDZ HTF 919 or tegaserod, a partial 5-HT_4 receptor agonist, showed in a healthy subject a clear effect on the total colonic transit time (5 mg twice a day)[174] and a significant improvement in phase III clinical trials in patients with constipation-predominant IBS.[92,175] Prucalopride was shown to be effective in healthy subjects for accelerating the colonic transit without modification of the gastric emptying and the small bowel transit.[176] However in patients with severe constipation, a dose-dependent effect of acceleration of the overall transit time was observed[177] and several double-blind clinical studies demonstrated the significant efficacy of the compound in the mean weekly stool frequency.[178] Diarrhea may be the common side effect observed with the 5-HT_4 receptor agonists, which precludes their use in patients with diarrhea-predominant IBS. The visceral hypersensitivity observed with these patient may be mediated partially through activation of 5-HT_4 receptors, and it can be treated with 5-HT_4 receptor antagonists. SB-207266 or piboserod was effective in the preliminary clinical studies by increasing the orocaecal transit time toward normal values with improvement of the symptoms.[179,180] A recent study in healthy volunteers demonstrated the relatively weak effect of the 5-HT_4 antagonism on the colonic transit in normal physiological conditions. However, a marked effect was observed on the colonic transit in the presence of the 5-HT_4 agonist cisapride, indicating the interest in this compound for the pathophysiological conditions.[181]

The role of 5-HT is not limited to the motor function of the intestine. Indeed, 5-HT was shown to be a potent intestinal stimulant secretagogue for intestinal chloride ion and is implicated in normal physiological conditions for the secretion of water in the digestion process. 5-HT is also a mediator of diarrhea in the carcinoid syndrome where the large release of 5-HT was emphasized. The luminal membrane of the epithelial cells of the small intestine contains an ion channel: a cAMP-dependent Cl^- channel known as cystic fibrosis transmembrane regulator (CFTR). The increase of the intracellular concentrations cAMP results in the secretion of the Cl^- ions in the lumen and subsequently of Na^+ ions, which lead to water secretion. Abnormal activation of the cAMP-dependent Cl^- channel can be triggered by cholera toxin, which maintains the channel in the opened state, causing a massive secretion of water that is manifested as severe diarrhea.[182] It was found that 5-HT stimulates the short-circuit current (SCC), a method for the evaluation of the index of the mucosal electrolyte secretion.[183,184] However, the results depended on the animal species. In the guinea pig, the 5-HT effect was both tetrodotoxin-sensitive and -insensitive and was mediated by 5-HT_4 receptors, since the effect of 5-HT was mimicked by 5-methoxytryptamine and SC 53116 and inhibited by GR 113808 or high concentration of ICS 205-930. This indicates both a neuronal and nonneuronal localization of 5-HT_4 receptors mediating the secretion. Similar experiments performed in rat[185,186] and in human[187,188] led to the conclusion that 5-HT_4 receptors were not located on neurons. Recently, it was demonstrated that activation of the human jejunal mucosa preparation by stroking

induced 5-HT release and Cl^- secretion. The increase of the 5-HT concentration by the release of 5-HT was associated with a positive change of the short circuit, which was blocked by inhibitors of the chloride secretion. The change of the SCC was inhibited by micromolar concentrations of tropisetron and SDZ 205-557 but not by the antagonists of the 5-HT_{1P}, 5-HT_{2A}, or 5-HT_3 receptors.[189] The most likely mechanism that explains these results is that the mechanical deformation induces a 5-HT release from the enterochromaffin cells, which activates the 5-HT_4 receptors present on the enteric sensory neurons. Consequently, some evidence indicates that 5-HT is implicated in the gastrointestinal disorders consequent to a release of water in the intestine, like in diarrhea. The animal models, such as 5-HTP induced diarrhea in mice, demonstrated clearly the role of 5-HT_4 receptors.[141] Hence, 5-HT_4 receptor antagonists can be valuable drugs for treating these disorders by acting simultaneously on the control of the intestinal motricity and the excessive release of water.

Finally, 5-HT present in enterochromaffin cells can be released during treatment with cytotoxic drugs, and this results in episodes of emesis, which is a significant problem in the treatment of patients with cancer. The role of the 5-HT_3 receptors in the initiation of emesis and its inhibition by 5-HT_3 receptor antagonists have been largely documented.[190] However, emesis is only reduced by about 60% with selective 5-HT_3 antagonists used during chemotherapy treatment, which led to the proposal that 5-HT_4 receptors could also be implicated in this phenomenon. Thus, molecules combining 5-HT_3 and 5-HT_4 receptor antagonist properties could be more beneficial for this type of treatment.[191]

5-HT_4 Receptors and the Cardiovascular System

There is a large species-dependent heterogeneity in the number and subtypes of 5-HT receptors expressed in the heart. Activation of the 5-HT_3 receptors was shown to produce bradycardia through the activation of the Bezold−Jarisch reflex,[192] while the activation of 5-HT_1 and 5-HT_2 receptors in cat, rabbit, rat, and dog[193] produces tachychardia. Selective activation of 5-HT_4 receptors also produces tachycardia but only in human and pig atria.[7,194,195] In human and pig atrial strips, 5-HT induces a rapid increase in the contractile force, mimicking the positive inotropic effect of (−)-isoprenaline, a β-adrenergic receptor agonist. The pEC_{50} value for 5-HT is 6.6, and its effect is not antagonized by any of the 5-HT_1, 5-HT_2, 5-HT_3, and β-adrenergic receptor antagonists except ICS 205-930. However, the maximum inotropic effect of 5-HT is smaller than that of (−)-isoprenaline, suggesting a weaker coupling efficiency between receptor and effector or a lower density of receptors.[196] Binding studies have shown that the density of 5-HT_4 receptors in the human atrium was 10 and 5 times lower than the density of β_1 and β_2 receptors, respectively.[197]

The stimulation of 5-HT_4 receptors in piglet isolated right atrium is a useful method for evaluating the potency and the pharmacological profile of 5-HT_4 receptor ligands. For instance, renzapride and cisapride behave as partial agonists in this model, while (−)-zacopride is a full agonist.[198] Similarly, pharmacological differences were observed with benzimidazolones; BIMU 1 is full agonist, and BIMU 8 is only a partial agonist. The selective 5-HT_4 antagonists SB 203186, DAU 6215, and SDZ 205-557 caused a parallel rightward shift of the 5-HT concentration−effect curves, and the slopes of the Schild plots were not significantly different from 1, indicating a competitive antagonism.[199]

An important aspect of cardiac response to 5-HT is the absence of effect of 5-HT or 5-HT_4 receptor agonists in the ventricular tissues of pig and human,[200−202] although mRNA[203] coding for 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors has recently been found in the human ventricle. Thus, the lack of effect of 5-HT_4 agonists in the human ventricle is attributed either to the absence of 5-HT_4 receptors in this tissue or to the lack of functional coupling, therefore making it unlikely that 5-HT_4 receptor agonists will ever be used in the therapy of heart failure.

A mechanism was proposed to explain the effect of 5-HT via 5-HT_4 receptors on human cardiac cells.[12,204] Exposure of human isolated atrial myocytes to 5-HT induced an increase of both whole-cell[4,12] and single-channel Ca^{2+} current (I_{Ca}) through voltage-gated L-type Ca^{2+} channels.[204] This effect is due to 5-HT_4 receptor mediated activation of adenylyl cyclase, cAMP synthesis, and phosphorylation of the Ca^{2+} channels by cAMP-dependent protein kinase A (PKA), increasing their opening probability. The mechanism proposed is similar to that described for the β-adrenoreceptor, suggesting that 5-HT and (−)-isoprenaline share a common signaling pathway. In addition to activating the L-type Ca^{2+} current I_{Ca}, there is also evidence that 5-HT stimulates the so-called pacemaker current I_f in atrial myocytes isolated from human patients.[205,206] I_f is an inward current activated by hyperpolarization and carried via channels with mixed permeability for K^+ and Na^+. In control human myocytes, this current is activated at potentials too negative to play a significant role in pacing. However, when 5-HT is applied, cAMP generation via activation of 5-HT_4 receptors leads to a progressive shift to more depolarized potentials of the activation curve of I_f. This allows a significant contribution of the I_f current to the diastolic depolarization, leading to a positive chronotropic effect. It was demonstrated that the effect of 5-HT on the I_f current was completely inhibited by the 5-HT_4 receptor antagonists DAU 6285 and GR 124487.

In 1994, a hypothetical role for 5-HT in the triggering of atrial arrhythmia through the activation of 5-HT_4 receptors was proposed.[14] By use of a model of arrhythmic contractions in human isolated atrial strips, 5-HT was found to induce arrhythmic contractions with a higher incidence in tissues obtained from patients treated with β-blockers.[207] Arrhythmias were totally blocked by SB 203186, a potent and selective 5-HT_4 receptor antagonist, and the blockade was surmounted by increasing 5-HT concentration. It was supposed that treatment with β-blockers induced a sensitization of 5-HT_4 receptors as well as any other step involved in the cAMP cascade. Subsequent experiments testing the effect of 5-HT on isolated right atrial strips from patients chronically treated or not treated with β-blockers demonstrated significantly lower EC_{50} values for 5-HT in treated patients ($pEC_{50} = 7.9$) than in control patients ($pEC_{50} = 7.3$).[208] Through activation of Ca^{2+}

channels, 5-HT stimulation of the 5-HT_4 receptor might lead to intracellular Ca^{2+} overload, a condition generally associated with the generation of arrhythmia. These experiments suggested that, under pathological conditions such as the enlarged atria of the elderly, endocardial lesions or carcinoid tumors, 5-HT released from platelets may cause atrial arrhythmias.

The 5-HT evoked positive chronotropic effect was characterized in vivo in piglets and minipigs.[209] The effect was clearly antagonized by SB 203186 when it was administered iv or intraduodenum, indicating a good bioavailability of the 5-HT_4 antagonist despite the presence of the ester function. However, it was not possible to induce in vivo arrhythmias in piglets and minipigs by bolus injection of 5-HT. This lack of effect was most likely due to the very low density of 5-HT_4 receptors in pig compared to that found in human.

The presence of 5-HT_4 receptors in the human atrium and their potential role in the generation of arrhythmia or tachycardia is a serious concern when using 5-HT_4 receptor agonists as gastrokinetic agents. For instance, metoclopramide, renzapride, and cisapride, which are used clinically, all behave as partial agonists of the human atrial 5-HT_4 receptors.[199] However, the undesirable cardiac side effects of the 5-HT_4 receptor agonists are not necessarily related to their stimulant effect on cardiac 5-HT_4 receptors. For instance, when administered in humans, cisapride, a nonselective 5-HT_4 receptor agonist, produces arrhythmogenic effects that are not related to activation of cardiac 5-HT_4 receptors. Indeed, cisapride behaves as a blocker of the delayed rectifying K^+ current (I_{KR}), an effect that leads to a lengthening of the action potential,[210] a prolongation of the electrocardiographic QT, and polymorphous ventricular tachycardia such as torsades de pointes.[211] However, this deleterious effect of cisapride on the QT interval is not encountered by the structurally related drug mosapride[212] or by other 5-HT_4 receptor agonists such as ML 10302,[213] TS-951,[214] and TKS159 (4-amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide).[215]

The recent characterization of several human 5-HT_4 receptor isoforms with some degree of specificity in their tissue distribution may indicate a different potency and/or efficacy for a given 5-HT_4 receptor ligand when tested in different tissues.[4,122] For instance, ML 10302 behaves as a potent stimulant of intestine in vitro and in vivo and as a partial and weak stimulant of I_{Ca} in isolated human atrial myocytes (1 μM ML 10302 and 1 μM 5-HT increased I_{Ca} by 17.4% and 157%, respectively, with regard to the basal level).[122] Similarly, cisapride and renzapride behave as partial agonists on the 5-$HT_{4(e/g)}$ isoform, while they behave as full agonists on the 5-$HT_{4(c)}$ and 5-$HT_{4(d)}$ isoforms.[126,127] However, in the case of ML 10302, a similar pharmacological profile was obtained on 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, and 5-$HT_{4(e/g)}$ variants,[122,127] which are all expressed in the heart,[128] indicating that another mechanism must account for the tissue specificity of the drug. A difference in the level of expression of a given receptor in different tissues might provide a possible explanation for the change in the profile of the molecules.[123]

As shown above, 5-HT_4 receptors are connected to the electrical activity of the human atrium via activation of two inward currents I_{Ca} and I_f, making these receptors potential candidates for the generation of atrial arrhythmia. However, down regulation of 5-HT_4 receptors was observed in atrial tissues of patients with atrial fibrillation (AF), which may provide a feedback mechanism for the stabilization of the arrhythmia.[216] This is accompanied by a general reduction of the transcription of L-type Ca^{2+} channel subunits. The overall reduction of the 5-HT_4 receptor and the Ca^{2+} channel transcription may contribute to the shortening of the action potential, providing a general protective effect against excessive intracellular Ca^{2+} accumulation and for stabilization of AF.[216]

Altogether, these data highlighted the interest in 5-HT_4 receptor antagonists as potential antiarrhythmic drugs for humans for the prevention or treatment of atrial fibrillation. A recent study by Rahme[217] confirmed this hypothesis. RS-100302, a new 5-HT_4 receptor antagonist, inhibited the atrial flutter and fibrillation induced by rapid right pacing in juvenile pigs, and the effect was reversed by cisapride, a partial 5-HT_4 receptor agonist. It is worth noting the absence of ventricular electrophysiological effects, confirming that the only target of the 5-HT_4 receptor ligands in the heart was located on atria, and consequently minimum ventricular side effects can be expected with such a drug. Consequently the 5-HT_4 receptor antagonists have the potential to become a valuable alternative to the β-adrenergic and Ca^{2+} channel blockers for the treatment of AF, without depressive effects on the ventricle.[218]

5-HT_4 Receptors and the Central Nervous System

As reported previously, 5-HT_4 receptors are expressed in the region of the limbic system and the nigro-striatal pathways. They were shown to be implicated in the long-term blockade of K^+ channels,[117] resulting in the opening of Ca^{2+} channels and the release of neurotransmitters such as ACh,[219,220] serotonin,[221] and dopamine.[222] Consequently, 5-HT_4 receptors are potentially involved in a number of physiological processes of the central nervous system, such as learning, memory, depression, and anxiety, and agonists or antagonist of these receptors can be valuable pharmacological tools to explain the mechanisms of these processes.

The increase of cAMP mediated by the stimulation of the 5-HT_4 receptors in the hippocampus is supposed to induce long-term potentiation of the neurons, which plays an essential role in the cellular mechanism of learning and memory. Several behavioral studies have demonstrated that the 5-HT_4 receptor agonists can be considered as cognitive and learning function enhancers. For instance, metoclopramide, cisapride, and SR-17 (*endo*-8-methyl-8-azabibyclo[3.2.1]octan-3-yl benzofuran-3-carboxylate), a bioisosteric analogue of ICS 205-930, were shown to be active in the mouse passive avoidance test in the range of the doses of piracetam.[223] However, because of the lack of specificity of the compounds used, it was impossible to conclude that 5-HT_4 receptors were solely involved in the effect of these drugs. More convincing evidence in favor of the role of the 5-HT_4 receptors was given by the effect of BIMU-1, which was active on the social olfactory recognition test and on the olfactory associative task, both effects being inhibited

by the 5-HT_4 receptor antagonist GR 124487 and not inhibited by the 5-HT_3 receptor antagonist ondansetron.[117,224,225] In the rat, the selective hydrophobic 5-HT_4 receptor agonist RS 67333 but not the hydrophilic 5-HT_4 receptor agonist RS 67506 was shown to reverse the atropine-induced deficit performance in the Morris water maze, an effect that was inhibited by GR 113-808.[226] More recently,[227] the role of 5-HT_4 receptors in the neuronal mechanism of memory enhancement and the cognitive process was clearly demonstrated with SC 53116, which increased the spike amplitude in the hippocampal CA1 neurons, the tetanus-induced long-term potentiation, and the concentration-dependent release of ACh. The effects of SC 53116 was blocked by the 5-HT_4 receptor antagonists GR 113808. On the other hand, application of GR 113808 alone had no effect on the release of ACh, indicating that the 5-HT_4 receptors are not constitutively coupled to the cholinergic neuronal system. This latter result indicates that 5-HT_4 receptor agonists may prevent memory deficit but may exert little influence on the memory process itself. The beneficial effect of 5-HT_4 receptor agonists on the memory deficit was confirmed in vivo by a behavioral study in rat where SC 53116 improved the scopolamine-induced deficit in the passive avoidance test.[227] However, these results are in contrast with those reported on the activity of 5-HT_4 receptor ligands RS 17017, RS 67333, and RS 67532 in the olfactive associative discrimination task in rats.[228] In this study, the antagonist RS 67532 induced a marked impairment in the learning performance observed with regard to the control while the deficit induced by the antagonist was reversed by the administration of the agonists RS 17017 and RS 67333. Moreover, the hydrophobic compound RS 67333 administered alone gave a significant improvement in the learning of the task and the memory performance with regard to the control. These results were confirmed with RS 67333 in the Morris water maze where improvement of the learning rate was demonstrated.[229]

The above-mentioned memory-enhancing effects of 5-HT_4 receptors agonists may be relevant for the treatment of memory dysfunction in patients suffering from Alzeihmer's disease. Indeed, a binding study reported a decrease of the density of 5-HT_4 receptors in the hippocampus[230] of patients with Alzeihmer's disease. Recent data[231] indicated another potential promising way to alleviate symptoms of the Alzheimer's disease with 5-HT_4 receptor agonists. In CHO cells expressing the neuronal h5-$HT_{4(e/g)}$ receptor, 5-HT was found to stimulate the release of the non-amyloidgenic-soluble amyloid precursor protein (sAPPα), which exerts neuroprotective and enhancing memory effects. This process was blocked by GR 113808 and SB 204070.

Recently, indeloxazine, an inhibitor of 5-HT and noradrenaline reuptake, was shown to increase the release of ACh in the rat frontal cortex. The effect was mediated through 5-HT_4 receptors because it was blocked by the selective antagonists GR 113808 and RS 23597 and not blocked by 5-$HT_{1A/1B/2A/2C/3}$ antagonists. Thus, 5-HT_4 receptors may also play a role in the effects of antidepressants by increasing ACh release upon an increase of the level of 5-HT in the frontal cortex.[232]

Since modulation of the concentration of 5-HT in the CNS is a common mechanism involved in the action of anxiolytic and antidepressant agents, the role of 5-HT_4 was examined. In 1996, Barnes[221] showed that a systemic administration of the 5-HT_4 receptor agonist renzapride induced a clear and dose-dependent increase of the extracellular levels of 5-HT (200%) in the rat hippocampus. This effect was antagonized by the 5-HT_4 receptor antagonist GR 125487D, which, when used alone, reduced the concentration of 5-HT to 80% below basal level. Although the previous study might suggest the involvement of 5-HT_4 receptors in the effects of antidepressants, a more recent study suggests the opposite. In this study,[233] the potent and selective 5-HT_4 receptor antagonist SB 204070 was inactive on the effect of fluoxetine, a selective uptake inhibitor, on the decrease of the immobility time in the forced swim test. On the other hand, SB 204070 and GR 113808 were shown to possess marked anxiolytic properties in two animal models: the rat social interaction test[234] and the elevated plus-maze.[235] However, the effect was species-dependent because in the BKW mice the 5-HT_4 receptor antagonists SDZ 205-557, GR 113808, and SB 204070 were unable to modify the behavior of the mice in the light/dark test box and caused an inhibition of the disinhibitory behavior of diazepam.[236]

By use of the microdialysis technique,[237] it was shown that 5-methoxytryptamine, a 5-HT_4 receptor agonist, increased the release of dopamine and that this effect could be blocked by a high concentration of tropisetron. This result was confirmed with renzapride and (*S*)-zacopride by in vitro studies in rat striatal slices and in vivo studies using microdialysis.[222,238] The localization of the 5-HT_4 receptors in the extrapyramidal forebrain areas allowed the proposal of a hypothesis that the increase in dopamine release could play a role in the modulation of the reward and motor behavior processes. Preliminary data indicated that the antagonist GR 113808 reduced the ethanol intake in alcohol-preferring rats, suggesting a role of 5-HT_4 receptors in the regulation of the alcohol intake control.[239] On the other hand, no antagonist effect of SB-204070 was found in the pharmacological test, implicating the basal forebrain, such as the stimulation of motor activity by amphetamine or nicotine and the cocaine intracranial self-stimulation reward threshold. As previously reported, such a lack of activity of 5-HT_4 receptor antagonists may reflect the absence of 5-HT tone in the area considered.[240] Nevertheless, the partial 5-HT_4 receptor agonist RS 67333 and the antagonist SDZ 205-557, when injected in the accumbens nucleus in the rat, were found to significantly decrease the hyperactivity induced by cocaine injection.[241]

In summary, the data available in the literature indicate that in the future 5-HT_4 receptor ligands may play an important role in the treatment of several pathological disorders of the CNS. In particular, 5-HT_4 receptor agonists seem to be promising molecules for overcoming deficits of the memory and of the learning tasks. Although no clinical data are available to date, the observed decrease in the density of 5-HT_4 receptors in patients with Alzheimer disease[230] indicates a potential therapeutic use for these new molecules. On the other hand, further work is needed to confirm the promising results obtained with different 5-HT_4 recep-

tors ligands in different animal models on anxiety, mood, and drug dependence.

5-HT$_4$ Receptors and Urinary Tract

Normal bladder functions require the simultaneous occurrence of detrusor relaxation and sphincter contraction during the filling phase and the converse during micturition. The descending bulbospinal pathway is an inhibitory circuit driven by the release of 5-HT, which inhibits bladder contraction through the activation of 5-HT$_{1A}$ receptors. Indeed, it was clearly demonstrated that the application of 5-HT$_{1A}$ antagonists in rat releases the inhibition of contraction and increases the capacity of the bladder through control exerted at the central level.[242]

However, 5-HT receptors are also present on the isolated bladder and may contribute to the peripheral effects of 5-HT. As already observed in several other tissues, the types of 5-HT receptors present in the bladder vary depending on the animal species. In guinea pigs, potentiation of the electrically evoked bladder is mediated by 5-HT$_{2A}$, 5-HT$_3$, and 5-HT$_4$ receptors,[243] in rabbits by 5-HT$_3$ receptors,[244] and in humans by 5-HT$_4$ receptors.[245] Stimulation of 5-HT$_4$ receptors leads to activation of cholinergic neurons, release of ACh, and ACh-mediated contraction of the bladder. Several cases have been reported in clinical studies showing a facilitation of bladder emptying and, occasionally, urinary incontinence in patients treated with metoclopramide[246] or cisapride.[247] However, the nature of the pharmacological response of the activation of 5-HT$_4$ receptors also depends on the animal species, since 5-HT was found to inhibit the electrically evoked contraction of the bladder strip in *Rhesus* and *Cynnomolgus* monkeys. This effect was mimicked by BIMU 8 and renzapride, and it was competitively inhibited by the reference 5-HT$_4$ antagonists such as GR 113808, DAU 6285, and RS 23597−190.[248]

A complete pharmacological characterization[249] of the role of 5-HT$_4$ receptors in the human isolated detrusor muscle was realized by evaluating the activities of reference agonists such as 5-HT, 5-MeOT, BIMU 8, zacopride, and cisapride in the isolated detrusor muscle. All these compounds induced bladder contraction with a rank order of potency comparable to that observed in other tissues (pEC_{50} = 8.02, 6.02, 6.86, 5.67, and 6.61, respectively). In addition, 5-HT and 5-MeOT behaved as full agonists while the other compounds had intermediate intrinsic activity. Unexpectedly, RS 23597, which was described as a 5-HT$_4$ antagonist, behaved as a partial agonist. The 5-HT effect was competitively antagonized by GR 125487 with a pA_2 value of 9.75. Recently, the detrusor muscle of pig was shown to be an efficient model for studying potential drugs to be used in human clinics; 5-HT increases the electrical field stimulated cholinergic response of the pig bladder strips, and the effect is inhibited by RS-100235 and GR-113808.[250]

In conclusion, the pharmacological and clinical data reported with 5-HT$_4$ receptor agonists provide a rationale for the development of new therapeutic drugs for the management of the voiding disorders associated with the detrusor hypocontractility in human.[10]

5-HT$_4$ Receptors and Adrenal Gland

The first evidence for the role of 5-HT$_4$ receptors in the adrenal gland came from the finding that 5-HT stimulated the production of corticosteroides such as corticosterone and aldosterone in the adrenal glands of frog. This effect of 5-HT was demonstrated to be mediated by 5-HT$_4$ receptors, since it was mimicked by zacopride, cisapride, renzapride, BIMU 1, and BIMU 8.[251,252] These drugs behaved as full or partial agonists in the model with the following rank order of potency: (*S*)-zacopride > BIMU 8 = (*R*,*S*)-zacopride > BIMU 1 = (*R*)-zacopride. Only high doses of ICS 205-930 were capable of blocking the production of aldosterone or corticosterone, while the selective 5-HT$_3$ antagonist MDL 72222 was totally inactive to inhibit this effect. As already reported in other tissues, the effect of 5-HT in adrenal glands appears to be mediated by stimulation of cAMP synthesis followed by activation of PKA and stimulation of the Ca^{2+} channels.[253] Similar effects were observed in humans treated with metoclopramide,[254,255] which induced a transient increase of the plasmatic aldosterone concentration. The implication of 5HT$_4$ receptors was demonstrated in the human adrenal cortex where 5-HT and zacopride, as previously observed in frogs, mediated the increase of aldosterone secretion.[256] The administration of zacopride[257] in normal volunteers induced an increase of plasma aldosterone concentration without any modification of renin and ACTH levels. However, the increase of aldosterone level was only transient because the level of the steroid returned to the basal level during chronic administration of zacopride.[258,259] These data explain why there was no change in blood pressure in patients during chronic administration of cisapride.[260] As reported for other tissues, the transient effect of 5-HT$_4$ receptor activation on aldosterone secretion likely reflects the desensitization of the receptors. Hyperaldosteronism can exert deleterious effects on the cardiovascular system under pathological conditions. Since the inhibition of the renin−angiotensin system by inhibitors of the converting enzyme is not sufficient to restore a normal level of aldosterone, the combination of this therapy with a 5-HT$_4$ receptor antagonist could constitute a new approach for treating cardiovascular disorders such as heart failure and hypertension.[261]

Conclusions and Perspectives

Since the pharmacological characterization of the 5-HT$_4$ receptors in brain and intestine, considerable progress has been made in the design of several potent and selective compounds from a relatively limited number of chemical leads and in the understanding of the functions of these receptors. Interest in this class of receptors resides in the existence of several isoforms distributed in various central and peripheral tissues and in the putative role of these different variants in the regulation mechanism of signal transduction and cell function. Preliminary data indicate the presence of 5-HT$_4$ receptors in major organs and tissues, although large qualitative and quantitative differences exist in their distribution. For instance, 5-HT$_{4(a)}$ and 5-HT$_{4(b)}$ receptors are the only isoforms present in the bladder and kidney, respectively, and 5-HT$_{4(d)}$ receptor was only characterized in the intestine. To date, no information

is available on the specific role of the different isoforms and, in pathological disorders, on the possible implication of the variability in tissue distribution. Potent 5-HT_4 receptor agonists are now available. However, from measurements of their binding capacity on expressed receptors, none of these agonists showed any specificity for any given 5-HT_4 receptor isoform, which is understandable because the binding site is structurally identical for all isoforms. Surprisingly, from measurements of their capacity to activate cAMP synthesis or to modulate cellular function, the efficacy of a given 5-HT_4 receptor agonist depends strongly on the variant considered. A partial explanation for this observation may reside in the allosteric influence of the C-terminal end on the rest of the receptor. Indeed, truncation of the C-terminal end of a given 5-HT_4 receptor was shown recently to increase its constitutive activity. This is particularly relevant, since the main differences between the 5-HT_4 receptor variants reside in their C-terminal composition and length. Therefore, a specific pattern of expression of the different 5-HT_4 receptor isoforms in a given tissue may confer to the cell a unique mechanism to finely regulate signal transduction and function. This may also provide a clue for the development of "selective" ligands, since the selectivity for a particular receptor isoform may not be revealed in binding studies but rather in functional studies (e.g., as observed with renzapride). The elucidation of the structural factors in this fascinating phenomenon is a challenge for the medicinal chemist. Also intriguing for the chemist is the structural similarity between some agonists and antagonists that differ in their chemical structures by subtle steric factors. However, to date, it is not realistic to imagine the design of selective ligands for a given isoform.

It is worth also noting that the localization and the functionality of the 5-HT_4 receptors are animal-species-dependent and that potential drugs have to be selected on the basis of the animal models relevant to the clinical activity.

Originally designed to cure gastrointestinal disorders, 5-HT_4 receptor agonists and antagonists available to date are worthwhile pharmacological tools for understanding the physiological and pathophysiological roles of these receptors in the GI. Several drugs are currently in development to treat constipation and the IBS syndrome, a complex disorder of the GI system where the inactivation and the activation of the peristaltic reflex were closely mixed. It will be of interest to observe the effects in humans of partial agonists, such as tegaserod, which is currently under clinical investigation. But several other promising drugs are under study, in particular the use of antagonists in the prevention or treatment of atrial arhythmia, a major pathological disorder of people suffering heart failure or other cardiovascular problems related to platelet aggregation. Development of a new class of antiarrhythmic drugs potentially devoid of ventricular effects constitutes promising progress in cardiovascular therapeutics. Finally, a number of other pharmacological studies allow for prediction of a good future for 5-HT_4 receptor ligands with potential therapeutic applications for treatments of memory disorders, hypertension, and the dysfunction of the urinary tract.

Biographies

Michel Langlois graduated as a chemical engineer from ENSCP (Paris, 1960). In 1964, he received his Ph.D. under the supervision of Professor Marc Julia. From 1976, as head of the Medicinal Chemsitry Department (Delalande Research), he supervised several programs on the design of reversible inhibitors of monoamine oxydase (MAO) A and B and the gastrokinetic benzamides (cimoxatone, zacopride). In 1987, he left industrial research and occupied different academic positions as Director of Research at CNRS. Currently, his research team is focused on the design of ligands of the G protein coupled receptors, particularly in the field of serotonin and melatonin receptors.

Rodolphe Fischmeister is Director of Research at INSERM and head of an INSERM unit devoted to cellular and molecular cardiology. His lab is based at the Faculty of Pharmacy of the University of Paris. After graduating as an electrical engineer from Supélec in 1978, he moved first to theoretical biology for doctoral studies (Ph.D. in 1980) and then to cellular physiology during the 2 $^1/_2$ years of postdoctoral studies at Dalhousie University (Halifax, Canada) and Emory University (Atlanta, GA). He returned to France in 1983 and became a researcher at INSERM. He obtained a Doctorat d'État ès Sciences in 1987. His main research interests are directed toward cardiac electrophysiology, neuroendocrine regulation of heart function, cardiac membrane receptors and their signaling cascades, and cellular control of cardiac ion channels.

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