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Review

Functional characteristics of heterologously expressed 5-HT receptors

Cindy C. Gerhardt [1], Harm van Heerikhuizen [*]

Department of Biochemistry and Molecular Biology, Research Institute Neurosciences, Vrije Universteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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Abstract

Over the past 10 years, molecular cloning has revealed the presence of 15 serotonin (5-hydroxytryptamine; 5-HT) receptor subtypes, which can be subdivided in seven subfamilies. Except for the 5-HT_3 receptors, which are ligand-gated ion channels, all 5-HT receptors belong to the superfamily of G-protein-coupled receptors. The large multiplicity of 5-HT receptor subtypes has been suggested to be a direct result of the evolutionary age of the 5-HT system. Molecular information on G-protein-coupled 5-HT receptors is currently available for several mammalian species as well as for a limited number of invertebrate species (insects, molluscs). The aim of this review is to give an overview of all cloned 5-HT receptor subtypes belonging to the superfamily of G-protein-coupled receptors with specific emphasis on the pharmacological and signaling properties of the receptors upon expression in several heterologous expression systems.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT receptor; G-protein-coupled receptor; Signal transduction

Contents

[*] Corresponding author. Tel.: (31-20) 444-7573; Fax: (31-20) 444-7553; e-mail: vheerik@chem.vu.nl
[1] Present address: Institut Cochin de Genetique Moléculaire, Laboratoire d'Immunopharmacologie Moleculaire, 22 Rue Méchain, 75014 Paris, France.

1. Introduction

Receptors interacting with serotonin (5-hydroxytryptamine; 5-HT) can be classified into seven different subfamilies. All but one, the 5-HT$_3$ receptor, belong to the superfamily of G-protein-coupled receptors. These receptors function by activating a GTP-binding protein (G-protein), that in turn can influence the activity of several membrane-bound effector proteins (second messenger-producing enzymes and ion channels). The six G-protein-coupled 5-HT receptor subfamilies can be distinguished on the basis of their degree of amino acid sequence identity (see Fig. 1) and their coupling to particular signaling pathway(s) (see Table 1). In short, the 5-HT$_1$ receptor subfamily consists of five members, each of which is negatively coupled to adenylyl cyclase. The 5-HT$_2$ receptor subfamily comprises three members, all positively coupled to phospholipase C. The 5-HT$_4$, 5-ht$_6$ and 5-HT$_7$ receptor subfamilies all stimulate adenylyl cyclase, but their pharmacological properties differ considerably. The 5-ht$_5$ receptor subfamily, which contains two members, couples to an as yet unknown signal transduction pathway.

This review presents the functional properties of all members of the 5-HT receptor subfamilies when expressed in a variety of heterologous systems. Emphasis is put on the differences in structure and function between species variants of particular receptor subtypes, or variations in signal transduction pathways activated by the same receptors in different cellular contexts. For the description of 5-HT receptors in endogeneous tissues the reader is referred to a number of extensive reviews (Zifa and Fillion, 1992, Hoyer et al., 1994).

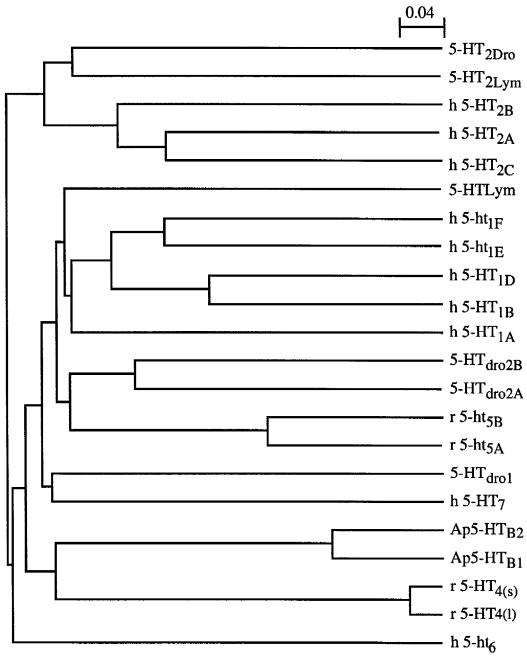

Fig. 1. Sequence dendrogram of all vertebrate and invertebrate 5-HT receptor subtypes. The tree was generated using the multiple alignment program Clustal W (Thompson et al., 1994). From the branches of this dendrogram, the evolutionary age and relationship of individual receptor families can be deduced. The scale bar indicates percentage of divergence (4%) along the branch.

2. 5-HT$_1$ receptor family

5-HT$_1$ receptors form the largest subclass of 5-HT receptor subtypes. Originally, these receptors were grouped together because they share a high affinity for 5-HT and 5-carboxyamidotryptamine (5-CT) and are antagonized by methiothepin and methysergide (Bradley et al., 1986). At present, the defining characteristics of 5-HT$_1$ receptors are their primary sequences, the fact that they do not contain introns in their coding sequence (with the exception of two receptors identified in *Drosophila* (Saudou et al., 1992) and their preferential coupling to a pertussis toxin-sensitive G-protein leading to the inhibition of adenylyl cyclase.

2.1. 5-HT$_{1A}$ receptors

5-HT$_{1A}$ receptors play an important role in feeding behaviour and in the regulation of body temperature, they are thought to control (at least in part) sexual behaviour

and are also implicated in psychiatric disorders, such as anxiety and depression.

When the gene encoding the human 5-HT_{1A} receptor was first cloned it was named G21 and classified as a putative G-protein-coupled receptor-encoding gene (Kobilka et al., 1987). The identification of the protein product of this gene as the 5-HT_{1A} receptor was performed by expressing it transiently in COS-7 (CV1 origin, SV40; transformed monkey kidney) cells and showing that it exhibited all the typical ligand-binding characteristics of a 5-HT_{1A} receptor (Fargin et al., 1988). Later, the homologues have been cloned from rat (Albert et al., 1990; Fujiwara et al., 1993) and mouse (Charest et al., 1993). The DNA sequences of the rat receptor reported by both groups differ at two positions, resulting in a single amino acid difference in the third intracellular loop. The rat 5-HT_{1A} receptor, expressed in mouse Ltk^- fibroblasts (Albert et al., 1990) or HeLa cells (Fujiwara et al., 1993) shows a similar pharmacology as its human homologue: 5-CT > 5-HT = 8-hydroxy-2-(di-1-propylamino)tetralin (8-OH-DPAT) = ipsapirone > buspirone > spiperone > mesulergine (Fargin et al., 1989; Guan et al., 1992; Newman-Tancredi et al., 1992).

In a mutational analysis of the human 5-HT_{1A} receptor, Guan et al. (1992) showed that a single asparagine residue at position 385 (in transmembrane region VII) is very important for the high affinity of the receptor for a class of β-adrenoceptor antagonists (i.e., pindolol and other aryloxyalkylamines). Other classes of ligands were not affected by this Asn^{385} to Val mutation. Subsequently, a number of residues important for agonist binding were pinpointed by Ho et al. (1992) using [^{3}H]5-HT binding, and by Chanda et al. (1993) using [^{3}H]8-OH-DPAT; these were Asp^{82}, Asp^{116}, Ser^{198}, Ser^{393} and Asn^{396}.

The signaling properties of heterologously expressed 5-HT_{1A} receptors have been studied in a number of different mammalian cells and in *E. coli* (see Table 1). Activation of the 5-HT_{1A} receptor induces a decrease in agonist- or forskolin-stimulated cAMP levels, which is antagonized by known antagonists as, e.g., methiothepin and spiperone. This negative coupling to adenylate cyclase is pertussis toxin-sensitive.

5-HT_{1A} receptors can also be linked to activation of the phospholipase C pathway, although less effectively and not in all cell types. The physiological relevance of this coupling is, as yet unclear since no 5-HT_{1A} receptor-mediated phospholipase C stimulation has ever been documented in tissue preparations. 5-HT_{1A} receptor-mediated phospholipase C stimulation has been described in HeLa, Ltk^- and CHO (Chinese hamster ovary) cells, however, in GH4-C1, COS-7 and Swiss 3T3 cells no coupling to phospholipase C could be detected (Fargin et al., 1989; Liu and Albert, 1991; Raymond et al., 1989; Stam et al., 1992). The increase in inositol 1,4,5 trisphosphate production is pertussis toxin-sensitive, independent of cAMP (Fargin et al., 1989) and can furthermore be selectively blocked by acute pretreatment with the protein kinase C activator, TPA (Liu and Albert, 1991). In HeLa cells it was established that phospholipase C stimulation is strongly dependent on receptor density. Compounds as ipsapirone and buspirone were shown to act as agonists in cells expressing high receptor levels, while they acted as antagonists on cells expressing low receptor densities (Boddeke et al., 1992). Fenrick et al. (1996) demonstrated that for 5-HT-induced phospholipase C stimulation a lowering of the number of receptors by means of an alkylating antagonist resulted both in a decreasing maximal effect as well as an increasing EC_{50}; lowering receptor number had no effect on maximal adenylyl cyclase inhibition. At all receptor concentrations tested the EC_{50} for phospholipase C stimulation was 10 to 100-fold higher than the EC_{50} for adenylyl cyclase inhibition (Fenrick et al., 1996). In contrast, the EC_{50} values for phospholipase C stimulation and adenylyl cyclase inhibition were similar in Ltk^- cells (Liu and Albert, 1991). The different modulation of adenylyl cyclase and phospholipase C can, in principle, result from coupling to different G-proteins. However, it has been demonstrated that the human receptor expressed in HeLa cells preferentially couples to G_{i3}, both to inhibit adenylyl cyclase and to stimulate phospholipase C (Fargin et al., 1991). Raymond et al. (1993) in a study that used both HeLa and CHO-K1 cells, showed that functional coupling of the human 5-HT_{1A} receptor to adenylyl cyclase inhibition may be mediated by $G\alpha_{i1}$, $G\alpha_{i2}$ and $G\alpha_{i3}$ and that the relative level of expression of these G-proteins determined their relative importance in the adenylyl cyclase coupling. Since $G\alpha_i$ is known not to activate phospholipase C these data can be taken to indicate that the dual coupling of the 5-HT_{1A} receptor may be explained by $G\alpha_i$ inhibiting adenylyl cyclase and $\beta\gamma$ stimulating phospholipase C.

In HeLa cells expressing the 5-HT_{1A} receptor, protein kinase C-mediated stimulation of sodium-dependent phosphate uptake (Raymond et al., 1991, 1989) and activation of Na^+/K^+-ATPase (Middleton et al., 1990) have also been described. The physiological significance of these 5-HT_{1A} receptor-induced effects are uncertain, as yet. A link between the 5-HT_{1A} receptor and K^+ channels was shown upon heterologous expression of the human 5-HT_{1A} receptor in cardiac atrial cells. In these cells, activation of 5-HT_{1A} receptors leads to the opening of K^+ channels that are normally activated by the endogenous muscarinic acetylcholine receptors (Karschin et al., 1991). Similarly, the human 5-HT_{1A} receptors transfected in CHO cells can mediate the regulation of high-conductance anion channels in these cells (Mangel et al., 1993).

Human 5-HT_{1A} receptors expressed in CHO cells show a dose-dependent decrease in the potency and no effect on the efficacy of 5-HT to inhibit cAMP accumulation when the cells are treated with phorbol esters which stimulate protein kinase C (Raymond, 1991). This phorbol ester treatment also induces a phosphorylation of the receptor

Table 1

Receptor	Expression system	Signal transduction	PTX sensitive?	Ref.
5-HT_1				
h 5-HT_{1A}	COS-7	cAMP↓	NT	Fargin et al. (1989)
	HeLa	cAMP↓&IP↑	+&+	Fargin et al. (1989)
	HeLa	cAMP↓&IP↑& phosphate uptake ↑	?&+ +	Raymond et al. (1989)
	HeLa	Ca^{2+} ↑	NT	Boddeke et al. (1992)
	CHO	AC↓	NT	Newman-Tancredi et al. (1992)
	NIH-3T3	cAMP↓	NT	Varrault et al. (1992)
	E. coli	coupling to αi3	NT	Bertin et al. (1992)
	Swiss 3T3	cAMP↓	NT	Stam et al. (1992)
	Sf9	cAMP↓	+	Parker et al. (1994)
r 5-HT_{1A}	GH4C1	cAMP↓	NT	Albert et al. (1990)
	GH4ZD	cAMP↓	+	Liu and Albert (1991)
	Ltk^-	cAMP↓&IP↑	+&+	Liu and Albert (1991)
h 5-HT_{1B}	HeLa	cAMP↓	NT	Hamblin et al. (1992b)
	Ltk^-	cAMP↓	NT	Weinshank et al. (1992)
	Ltk^-	cAMP↓&Ca^{2+} ↑&IP↑	+&+&+	Zgombick et al. (1993)
	CHO-K1	cAMP↓	NT	Veldman and Bienkowski (1992)
	Ltk^-	cAMP↓	NT	Levy et al. (1992b)
r 5-HT_{1B}	Y-1	cAMP↓	NT	Adham et al. (1992)
m 5HT_{1B}	NIH-3T3	cAMP↓	+	Maroteaux et al. (1992)
opossum 5HT_{1B}	CHO-K1	cAMP↓	NT	Cerutis et al. (1994)
h 5-HT_{1D}	CHO-K1	cAMP↓	+	Hamblin and Metcalf (1991)
	Ltk^-	cAMP↓	NT	Weinshank et al. (1992)
	Ltk^-	cAMP↓&Ca^{2+} ↑&IP↑	+&+&+	Zgombick et al. (1993)
	CHO-K1	cAMP↓&cAMP↑	+&−	van Sande et al. (1993)
r 5-HT_{1D}	HeLa	cAMP↓	NT	Hamblin et al. (1992a)
ca 5-HT_{1D}	Y-1	cAMP↓	NT	Maenhaut et al. (1991)
	Ltk^-	cAMP↓	NT	Zgombick et al. (1991)
	Y-1	cAMP↓&cAMP↑	+&−	van Sande et al. (1993)
h 5-ht_{1E}	HEK293	AC↓	NT	McAllister et al. (1992)
	Ltk^-	AC↓	NT	Zgombick et al. (1992)
	Ltk^-	AC↓	NT	Gudermann et al. (1993)
h 5-ht_{1F}	NIH-3T3	cAMP↓	NT	Adham et al. (1993b)
r 5-ht_{1F}	HeLa	cAMP↓	NT	Lovenberg et al. (1993b)
m 5-ht_{1F}	NIH-3T3	cAMP↓	NT	Amlaiky et al. (1992)
5-HT_{dro2A}	NIH-3T3	cAMP↓&IP↑	+&NT	Saudou et al. (1992)
5-HT_{dro2B}	NIH-3T3	cAMP↓&IP↑	+&NT	Saudou et al. (1992)
	Sf-9	IP↑	—	Obosi et al. (1996)
5-HTLym	COS-7	?	NT	Sugamori et al. (1993)
5-HT2				
h 5-HT_{2A}	Swiss 3T3	IP↑	NT	Stam et al. (1992)
	CHO-K1	arachidonic acid release	NT	Berg et al. (1996)
r 5-HT_{2A}	HEK293	IP↑&Ca^{2+} ↑	NT	Pritchett et al. (1988)
	NIH3T3	Ca^{2+} ↑	NT	Julius et al. (1990)
	Xenopus oocytes	Ca^{2+}-activated Cl^- current	NT	Buck et al. (1991)
	HEK293	IP↑	—	Apud et al. (1992)
	NIH-3T3	$Na^+/K^+/Cl^-$ transport	NT	Mayer and Sanders-Bush (1994)
m 5-HT_{2A}	NIH-3T3	IP↑	NT	Yang et al. (1992)
	AV12	IP↑&cAMP↑&cAMP↓	−&−&+	Lucaites et al. (1996)
hamster 5-HT_{2A}	CCL39	IP↑	NT	Van Obberghen-Schilling et al. (1991)
	Xenopus oocytes	Ca^{2+} release, Cl^- current	NT	Van Obberghen-Schilling et al. (1991)
h 5-HT_{2B}	HEK293	IP↑	NT	Schmuck et al. (1994)
	AV-12	IP↑	NT	Kursar et al. (1994)
r 5-HT_{2B}	*Xenopus* oocytes	Ca^{2+}-activated Cl^- current	NT	Foguet et al. (1992a)
	AV12	IP↑	NT	Kursar et al. (1992)
	AV-12	IP↑&cAMP↑	NT	Lucaites et al. (1996)
	A600K2, 3MTX	IP↑	NT	Wainscott et al. (1993)
m 5-HT_{2B}	Ltk^-	IP↑ via Gαq	—	Loric et al. (1995)
	Ltk^-	ras, MAPK	NT	Launay et al. (1996)

that is closely correlated to the phorbol ester-induced desensitization. Van Huizen et al. (1993), however, found that when the same receptor was expressed in Swiss 3T3 cells 5-HT pretreatment induces a decreased efficacy of 5-HT to inhibit cAMP accumulation rather than a decrease in affinity. Since the human 5-HT_{1A} receptor is not coupled to phospholipase C in Swiss 3T3 cells (Stam et al., 1992) protein kinase C-mediated phosphorylation of the receptor can not be involved in this desensitization. Van Huizen et al. (1993) hypothesize that the desensitization is caused by an as yet unknown mechanism. Agonist-induced desensitization of the 5-HT_{1A} receptor has also been studied by Nebigil et al. (1995) using receptors expressed in *Spodoptera frugiperda* (Sf-9) insect cells. Upon pretreatment with 5-HT the receptor uncouples from G-proteins, shows an increased phosphorylation on serine and threo-

Table 1 (continued)

Receptor	Expression system	Signal transduction	PTX sensitive?	Ref.
h 5-HT_{2C}	NIH-3T3	IP↑	NT	Stam et al. (1994)
	CHO	Ca^{2+} ↑	NT	Akiyoshi et al. (1995)
	CHO-K1	arachidonic acid release	NT	Berg et al. (1996)
r 5-HT_{2C}	NIH-3T3	Ca^{2+} ↑	NT	Julius et al. (1988)
	Xenopus oocytes	Ca^{2+}-activated Cl^- current & closing of K^+ channels	NT	Panicker et al. (1991)
	A9	Ca^{2+}-activated outward current and Ca^{2+} ↑	NT	Boddeke et al. (1993a,b)
	COS-7	PKC-α	NT	Lutz et al. (1993)
	NIH-3T3	IP↑	NT	Westphal and Sanders-Bush (1994)
	NIH-3T3	$Na^+/K^+/Cl^-$ transport	NT	Mayer and Sanders-Bush (1994)
	SF9	IP↑	NT	Labrecque et al. (1995)
	COS-7	IP↑	(1)	Tohda et al. (1995)
m 5-HT_{2C}	*Xenopus* oocytes	Ca^{2+}-activated Cl^- current	NT	Lubbert et al. (1987)
	Xenopus oocytes	Ca^{2+}-activated Cl^- current	NT	Yu et al. (1991)
5-HT_{2Dro}	COS-1	?		Colas et al. (1995)
5-HT_{2Lym}	HEK293	IP↑	NT	Gerhardt et al. (1996)
5-HT_4				
r 5-$HT_{4(s)}$	COS-7	cAMP↑	NT	Gerald et al. (1995)
r 5-$HT_{4(l)}$	COS-7	cAMP↑	NT	(Gerald et al. (1995)
5-ht_5				
h 5-ht_{5A}	COS-M6	?		Rees et al. (1994)
r 5-ht_{5A}	COS-M6	?		Erlander et al. (1993)
	HeLa	?		Erlander et al. (1993)
m 5-ht_{5A}	COS-7	?		Plassat et al. (1992)
	NIH-3T3	?		Plassat et al. (1992)
	P. pastoris	?		Weiss et al. (1995)
	S. cerevisiae	?		Bach et al. (1996)
r 5-ht_{5B}	COS-M6	?		Erlander et al. (1993)
	HeLa	?		Erlander et al. (1993)
	COS-1	?		Wisden et al. (1993)
m 5-ht_{5B}	COS-7	?		Matthes et al. (1993)
5-ht_6				
h 5-ht_6	HeLa	cAMP↑	NT	Kohen et al. (1996)
r 5-ht_6	HEK293	cAMP↑	NT	Monsma et al. (1993)
	COS-7	cAMP↑	NT	Ruat et al. (1993a)
5-HT_7				
h 5-HT_7	COS-7	cAMP↑	NT	Bard et al. (1993)
r 5-HT_7	HEK293	cAMP↑	NT	Shen et al. (1993)
	CHO-K1	cAMP↑	NT	Ruat et al. (1993b)
	HeLa	cAMP↑	NT	Lovenberg et al. (1993a)
m 5-HT_7	COS-7	cAMP↑	NT	Plassat et al. (1993)
guinea pig 5-HT_7	CHO-K1	cAMP↑	NT	Tsou et al. (1994)
Xenopus 5-HT_7	*Xenopus* oocytes	PKA-activated Cl^- current	NT	Nelson et al. (1995)
5-HT_{dro1}	NIH-3T3	cAMP↑	—	Witz et al. (1990)
	Sf9	cAMP↑	NT	Obosi et al. (1996)
Ap5-HT_{B1}	HEK293	IP↑	NT	Li et al. (1995)
Ap5-HT_{B2}	HEK293	IP↑	NT	Li et al. (1995)

h = human, r = rat, m = mouse, ca = dog, Dro = *Drosophila*, Lym = *Lymnaea*, Ap = *Aplysia*, AC = adenylyl cyclase, IP = inositol phosphates, PKA = protein kinase A, PKC = protein kinase C, NT = not tested.
(1) sensitive to botulinum ADP-ribosyltransferase.

nine residues, and exhibits a decreased ability to inhibit cAMP accumulation. Based on experiments with cyclic nucleotide-dependent kinase inhibitors the authors tentatively conclude that phosphorylation and desensitization are mediated through a pathway not involving protein kinase A or -C but rather employing a G-protein-linked receptor kinase.

Abdel-Baset et al. (1992) and Varrault et al. (1992) have shown that the 5-HT_{1A} receptors, when stably transfected into 3T3 fibroblasts exhibit transforming and mitogenic effects upon stimulation with 5-HT. The effects are pertussis toxin-sensitive and seem to be mediated via the capacity of the 5-HT_{1A} receptor to couple to phospholipase C activation, rather than to depend on the ability of the receptor to couple negatively to adenylyl cyclase. These effects correlate well with results of Abdul et al. (1994) who showed that the 5-HT_{1A} receptor antagonist pindobind exerts a marked antiproliferative effect on several human prostatic carcinoma cell lines in vitro. Pindobind also had marked growth-inhibitory effects on the aggressive PC-3 cell line in vivo in athymic nude mice.

2.2. 5-HT_{1B} receptors

5-HT_{1B} receptors are likely to be involved in the contractile response of human arteries to 5-HT (Hamel et al., 1993). Furthermore, these receptors may be involved in anxiety, movement disorders, food intake, sexual activity and aggressive behaviour (Wilkinson and Dourish, 1991). The precise contribution of the 5-HT_{1B} subtype and the related 5-HT_{1D} receptor subtype are, however, still largely unclear. To learn more about the functions of the 5-HT_{1B} receptor Saudou et al. (1994) have generated mutant mice lacking 5-HT_{1B} receptors. These mice develop, breed and feed normally, but are more aggressive than wild-type mice of the same strain in the isolation-induced aggression test, suggesting an involvement of the 5-HT_{1B} receptor in the modulation of aggressive behaviour.

Up to the early 1990s the description of 5-HT_{1B} and 5-HT_{1D} receptor binding sites and the nomenclature of these receptors have been highly confusing. Although both receptor subtypes were pharmacologically distinct they shared a number of common characteristics, most notably a similar distribution in particular brain regions (e.g., substantia nigra and basal ganglia). Surprisingly, the 5-HT_{1B} receptor was only found in rodents, whereas the 5-HT_{1D} receptor was found in human, dog and guinea pig. These observations first led to the believe that 5-HT_{1B} and 5-HT_{1D} receptors were mere species homologues. During the boom of 5-$HT_{1B/D}$ receptor cloning, however, it became apparent that both in rat, as well as in human distinct genes encoding 5-HT_{1B} and 5-HT_{1D} receptors were present.

The nomenclature of the human 5-$HT_{1B/D}$ receptors at first still remained somewhat messy. The same human gene was designated 5-HTS12 by Levy et al. (1992b), 5-$HT_{1D\beta}$ by Demchyshyn et al. (1992) and by Weinshank et al. (1992), just 5-HT_{1D} by Veldman and Bienkowski (1992), but 5-HT_{1B} by Hamblin et al. (1992b) and Jin et al. (1992). These differences in nomenclature were instigated by a different appreciation of (i) the strong amino acid identity (93%) that was noticed between the human 5-$HT_{1B/1D\beta}$ receptor and the cloned rat 5-HT_{1B} receptor (Voigt et al., 1991) and (ii) the rather strong pharmacological differences between the rat 5-HT_{1B} receptor and the human 5-$HT_{1B/1D\beta}$ receptor, as well as the pharmacological resemblance between this 5-$HT_{1B/1D\beta}$ receptor and the cloned 5-$HT_{1D\alpha}$ receptor (Hamblin and Metcalf, 1991; Weinshank et al., 1992). Shortly, it became apparent that the observed pharmacological differences between the human 5-$HT_{1B/D\beta}$ receptor and the rat 5-HT_{1B} receptor could be explained by a single amino acid difference in transmembrane region VII. The human 5-$HT_{1B/D\beta}$ receptor contains a threonine at position 355, whereas the rat 5-HT_{1B} receptor contains an asparagine at the homologous position 351. Mutation of Thr^{355} into an Asn in the human receptor renders the pharmacological profile of the mutated receptor nearly identical to that of the rat receptor (Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993). In view of the above considerations it was recommended by the NC-IUPHAR Subcommittee on 5-Hydroxytryptamine Receptors that the human 5-$HT_{1D\beta}$ receptor be renamed h 5-HT_{1B} receptor (Hartig et al., 1996). In addition to the rat and human cDNAs encoding 5-HT_{1B} receptors, homologues have been cloned from mouse (Maroteaux et al., 1992), from opossum (Cerutis et al., 1994) and from rabbit (Wurch et al., 1996).

No introns have been found in the 5-HT_{1B}-encoding genes (i.e., at least not in the coding regions).

The rat, mouse and opossum 5-HT_{1B} receptors have been expressed in HEK293 (human embryonic kidney), COS-1, COS-7 and CHO cells and were shown to exhibit a 5-HT_{1B} subtype-specific pharmacological profile (cyanopindolol > 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU24969) > 5-CT, 5-HT > (−)-propanolol > methysergide > rauwolscine > 8-OH-DPAT). Ketanserin, mianserin, spiperone and yohimbine have low affinities for these receptors (Voigt et al., 1991; Adham et al., 1992; Hamblin et al., 1992a; Maroteaux et al., 1992; Cerutis et al., 1994). The human 5-HT_{1B} receptor has been expressed in CHO-K1, HeLa, HEK293, Ltk^- and Y1 cells and displays a 5-HT_{1D}-like profile (5CT > methiothepin, 5-HT > metergoline > methysergide > sumatriptan > yohimbine > 8-OH-DPAT > (−)-propanolol) (Demchyshyn et al., 1992; Hamblin et al., 1992b; Jin et al., 1992; Metcalf et al., 1992; Oksenberg et al., 1992; Weinshank et al., 1992). When compared to the 'rodent' 5-HT_{1B} receptors the human receptor has a much lower affinity for β-adrenoceptor antagonists as (−)-propanolol and pindolol, and displays higher affinity to several serotonergic drugs as, e.g., sumatripan and 8-OH-DPAT. The rabbit 5-HT_{1B} receptor expressed in COS-7 cells yields a binding profile

highly similar to the ones found for the human 5-HT_{1B} receptor and unlike the profiles obtained for mouse and rat. This finding may seem somewhat surprising at first glance, but is perfectly explained by the observation that the rabbit receptor has a Thr at position 355 in transmembrane region VII just as the human 5-HT_{1B} receptor (Wurch et al., 1996). Whereas at first it was suggested that the human 5-HT_{1B} and 5-HT_{1D} receptors possess nearly indistinguishable properties based on binding studies with 19 drugs (Hartig et al., 1992), later studies showed that certain drugs (e.g., ritanserin and ketanserin) have a strong preference for the 5-HT_{1D} receptor (Kaumann et al., 1994; Peroutka, 1994; Pauwels and Colpaert, 1995).

Using molecular modeling in combination with receptor mutagenesis, the binding of (−)-propanolol to the 5-HT_{1B} receptor was studied by Glennon et al. (1996). Their results indicate that in the wild-type human 5-HT_{1B} receptor the threonine at position 355 forms a single hydrogen bond to the ether oxygen atom of (−)-propanolol. In a mutant receptor where this Thr is replaced by an Asn, the Asn can form hydrogen bonds with both oxygen atoms of (−)-propanolol resulting in a significantly higher affinity for the mutant receptor ($K_i = 16$ nM in the mutant versus $K_i = 10$ μM in the wild-type receptor).

Like all members of the 5-HT_1 receptor subfamily, activation of the 5-HT_{1B} receptor inhibits adenylyl cyclase. This inhibition can be blocked by antagonists as, for example, methiotepin, and also by pertussis toxin (Maroteaux et al., 1992; Zgombick et al., 1993) indicating a coupling to a G_i/G_o-type of G-protein. There is one report in which, in addition to a negative coupling to adenylyl cyclase, the coupling of the human 5-HT_{1B} receptor to an increase in intracellular Ca^{2+} concentration and an elevation of inositol phosphates has been described in transfected Ltk^- cells (Zgombick et al., 1993). It is interesting to note that the EC_{50} values for the inhibition of cAMP production and stimulation of Ca^{2+} mobilization were similar, suggesting that the receptor couples efficiently to both second messenger systems. Furthermore, it was found that also the coupling to inositol phosphate/Ca^{2+} was sensitive to pertussis toxin. This may indicate a role for $\beta\gamma$ in this coupling.

When the 5-$HT_{1B}/_{1D\beta}$ receptor is permanently expressed in rat C6 glial cells, 5-HT is not only able to inhibit forskolin-stimulated cAMP formation, but it also promotes cell growth (Pauwels et al., 1996b). Dibutyryl-cAMP treatment of cells can inhibit this agonist-promoted cell growth, indicating an inhibitory role for cAMP in the 5-HT_{1B} receptor-mediated cell growth.

It appears that the activity of the 5-HT_{1B} receptor can be regulated in a very peculiar way, until now unprecedented for any other 5-HT receptor or G-protein-coupled receptor. Rousselle et al. (1996) have isolated an endogenous tetrapeptide (Leu–Ser–Ala–Leu) from rat and bovine brain that appears to function as an endogeneous ligand specific for the 5-HT_{1B} receptor. Since interaction of the peptide with the receptor results in a decrease in functional activity of the receptor, possibly via allosteric modulation, the peptide was designated 5-HT-moduline. Later, Massot et al. (1996) could show that 5-HT-moduline is released from a rat brain synaptosomal preparation by a Ca^{2+}-dependent K^+ stimulation, suggesting that the peptide is stored in excitable cells.

2.3. 5-HT_{1D} receptors

As mentioned earlier, the 5-HT_{1D} and 5-HT_{1B} receptors may be involved in a large number of functions (as, e.g., depression, anxiety, migraine and movement disorders), but the precise functions of each receptor subtype remain to be elucidated. At first, it was believed that 5-HT_{1D} binding sites were not present in rodents. The molecular cloning of true 5-HT_{1D} receptor-encoding cDNAs from rat and mouse (see below), however, demonstrated that this assumption was in error.

The first 5-HT_{1D}-encoding gene was isolated from dog as an 'orphan' G-protein-coupled receptor, called RDC4 (Libert et al., 1989). Because of its rather high sequence identity to the 5-HT_{1A} receptor it was suggested to represent a novel member of the 5-HT family. Upon expression in mammalian cells RDC4 exhibited the pharmacological characteristics expected for a 5-HT_{1D} receptor (Maenhaut et al., 1991; Zgombick et al., 1991). Based on the sequence of the canine 5-HT_{1D} receptor species homologues were isolated from human (Hamblin and Metcalf, 1991) and rat (Hamblin et al., 1992b; Bach et al., 1993) as well as a partial cDNA from guinea pig (Weydert et al., 1992).

Just as was established for the 5-HT_{1B} receptor genes, no introns seem to be present in the 5-HT_{1D}-encoding genes.

When expressed in mammalian cells also the human 5-HT_{1D} receptor (Hamblin and Metcalf, 1991) shows affinities for 5-HT drugs closely matching the profile expected for a 5-HT_{1D} receptor (5CT > 5-methoxytryptamine (5-MeOT) = 5-HT = Sumatriptan > RU24969 > Yohimbine > *m*-trifluoromethylphenylpiperazine (TFMPP) > 8-OH-DPAT > Mesulergine > Spiperone). The rat receptor (Bach et al., 1993; Hamblin et al., 1992b) exhibits a very similar profile with the exception that the affinities for dihydroergotamine, methiothepin and spiperone are at least tenfold lower than as measured for the human receptor. The pharmacological profile of the human 5-HT_{1D} receptor was first thought to be indistinguishable from that of the human 5-HT_{1B} receptor (Weinshank et al., 1992). More recent studies, however, show that the 5-HT_{1B} and 5-HT_{1D} subtypes can be differentiated using the 5-HT_2 receptor antagonists ketanserin and ritanserin (Kaumann et al., 1994; Pauwels and Colpaert, 1995; Peroutka, 1994); both drugs show efficient and selective binding to the human 5-HT_{1D} receptor and act as neutral antagonists. Pauwels et al. (1996a) further showed that the human 5-HT_{1D} receptor expressed in rat

C6-glial cells can be selectively activated by 1-naphtylpiperazine, metergoline and the putative 5-HT_{1B} receptor antagonist 2′-methyl-4′-(5-methyl[1,2,4]oxydiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)-2,5-diphenyltetrazolium bromide (GR 127,935).

The 5-HT_{1D} receptor exhibits an interesting coupling to second messenger systems. As expected, the 5-HT_{1D} receptors (when expressed in CHO-K1 and Y1 adrenal cells) couple negatively and in a pertussis toxin-senstitive way to adenylyl cyclase (Hamblin and Metcalf, 1991; Zgombick et al., 1991). In addition, it seems that both the human and the dog receptor can also stimulate adenylyl cyclase via a pertussis toxin-insensitive G-protein (van Sande et al., 1993). Finally, it has been reported that the human receptor is able to evoke dose-dependent elevations in intracellular Ca^{2+} concentrations and inositol phosphates (Zgombick et al., 1993). Both these responses are pertussis toxin-sensitive.

2.4. 5-HT_{1E} receptors

The subfamily of 5-ht_{1E} receptors are recognized by their 5-HT_1-like character, combined with a low affinity for 5-CT (Leonhardt et al., 1989). Little is known about the physiological of this subfamily of receptors. According to the recommendations by the NC-IUPHAR Subcommittee on 5-Hydroxytryptamine Receptors (Vanhoutte et al., 1996) this receptor is referred to by lower case letters.

A human 5-HT_1 receptor gene, first called S31(Levy et al., 1992a), was later identified as the 5-ht_{1E} receptor (Gudermann et al., 1993; McAllister et al., 1992; Zgombick et al., 1992). Until now, the 5-ht_{1E} receptor has not been cloned from any other species. The 5-ht_{1E} receptor has been expressed in several mammalian cells (see Table 1) and displays a pharmacological profile (5-HT > methysergide > ergotamine > 8-OH-DPAT > 5-CT > ketanserin) that resembles quite closely that described by (Leonhardt et al., 1989) for a 5-HT receptor they discovered in human cerebral cortex designated 5-HT_{1E}. Especially the low affinity for 5-CT, TFMPP and 8-OH-DPAT distinguishes the receptor from the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors.

Activation of the 5-ht_{1E} receptor expressed in mammalian cells results in inhibition of forskolin-stimulated adenylyl cyclase (Levy et al., 1992b; McAllister et al., 1992; Zgombick et al., 1992; Gudermann et al., 1993). However, this inhibition is significantly less (30–35%) than that caused by the other 5-HT_1 receptors (50–60%). Adham et al. (1994) found that at higher 5-HT concentrations the 5-ht_{1E} receptor was also able to stimulate adenylyl cyclase. Similarly as was found for the 5-HT_{1D} receptor adenylyl cyclase inhibition is pertussis-toxin-sensitive, whereas adenylyl cyclase stimulation is not. No indications for coupling to phospholipase C or intracellular Ca^{2+} were obtained (Gudermann et al., 1993).

2.5. 5-HT_{1F} receptors

The pharmacology of 5-ht_{1F} receptors is solely based on the isolation and expression of the encoding cDNA. The presence of this receptor was not anticipated on the basis of physiological studies. Therefore, the name of the receptor is written as 5-ht_{1F} rather than 5-HT_{1F} (Vanhoutte et al., 1996). Based on sequence homology, the 5-ht_{1F} receptor is closely related to the 5-ht_{1E} receptor. Therefore, the mouse 5-ht_{1F} receptor has been called 5-$HT_{1E\beta}$ as well (Amlaiky et al., 1992). Homologues from human (Adham et al., 1993b; Lovenberg et al., 1993b) and rat (Lovenberg et al., 1993b) have also been cloned. The pharmacological profiles of the human and the rat receptor are highly similar (5-HT > methysergide > ergotamine > 5-CT), and furthermore resemble the pharmacology of the 5-ht_{1E} receptor, although the latter has a lower affinity for yohimbine and sumatriptan. The pharmacology of the mouse 5-ht_{1F} receptor exhibits some notable differences with its homologues from human and rat, for example the mouse receptor has a much higher affinity for methysergide, ergotamine and sumatriptan.

5-ht_{1F} receptors have been expressed in HeLa (Lovenberg et al., 1993b) and NIH-3T3 cells (Amlaiky et al., 1992; Adham et al., 1993b) in which they inhibit forskolin-stimulated adenylyl cyclase. As with other 5-HT_1 receptors, cell type-dependent promiscuous coupling has been observed: human 5-ht_{1F} receptors expressed in NIH-3T3 cells only inhibit adenylyl cyclase (Adham et al., 1993b), whereas human 5-ht_{1F} receptors expressed in Ltk^- fibroblasts were shown to also mediate phospholipase C activation and Ca^{2+} mobilisation (Adham et al., 1993a).

3. 5-HT_2 receptor family

The 5-HT_2 receptor subclass consists of three subtypes: (i) the 5-HT_{2A} receptor, the ‘classical’ 5-HT_2 receptor, that is still often referred to as 5-HT_2 receptor, (ii) the 5-HT_{2B} receptor, that was formerly called 5-HT_{2F} (F for fundus, because of the high expression level of this particular receptor in the stomach fundus) and (iii) the 5-HT_{2C} receptor; this receptor was originally called 5-HT_{1C} receptor based on its relatively high affinity for 5-HT, but it was later renamed 5-HT_{2C} receptor, based on its pharmacological profile, coupling to second messengers and its amino acid sequence (Humphrey et al., 1993). Although these three receptor subtypes were earlier classified as members of separate 5-HT receptor subfamilies, their similar pharmacological profiles, the fact that they couple to identical signaling pathways and their similarity in (gene) structure clearly indicate that they should be considered as members of a single subfamily, the 5-HT_2 receptor subfamily (Humphrey et al., 1993). The members of this subfamily are recognized by the fact that they do contain introns at

conserved positions within their coding regions and primarily stimulate phospholipase C.

3.1. 5-HT_{2A} receptors

The 5-HT_{2A} receptor has been reported to be involved in many peripheral as well as central, functions (e.g., smooth muscle contraction, platelet aggregation, control of hormone- or transmitter-release, control of sexual activity, regulation of sleep, motor behaviour, and psychiatric disorders like epilepsy, migraine, anxiety, depression, schizophrenia and hallucination).

The first 5-HT_{2A} receptor was cloned from rat brain in 1988 (Pritchett et al., 1988). Two years later the reported rat sequence was corrected by one nucleotide (Julius et al., 1990) leading to a protein of 471 instead of 449 amino acids. Today, 5-HT_{2A} receptors have been cloned from human (Saltzman et al., 1991; Chen et al., 1992; Kao et al., 1992; Stam et al., 1992; Cook et al., 1994), mouse (Foguet et al., 1992b; Yang et al., 1992), hamster (Chambard et al., 1990; Van Obberghen-Schilling et al., 1991), pig (Johnson et al., 1995) and rhesus monkey (Johnson et al., 1995). 5-HT_{2A} receptor-encoding genes contain two introns (Chen et al., 1992; Foguet et al., 1992b; Stam et al., 1992; Yang et al., 1992), the position of which is conserved within all other members of the 5-HT_2 receptor family, including the 5-HT_2 receptors from *Drosophila* (Colas et al., 1995) and *Lymnaea* (Gerhardt et al., 1996) (see below).

The pharmacology of the heterologously expressed 5-HT_{2A} receptors corresponds well to that of the 5-HT_2 binding sites previously reported for, e.g., brain cortex (ketanserin > spiperone > ritanserin > mesulergine > 5-HT). When the pharmacological profile of the human and the rat 5-HT_{2A} receptor are compared, the rat receptor has a much higher affinity for mesulergine than the human receptor ($K_i = \pm 5$ nM in rat versus ± 150 nM in human). Yet, the sequence of the human 5-HT_{2A} receptor is highly homologous to that of the rat. The human and rat sequences differ by only three amino acids in the transmembrane regions and one of these amino acids (Ala^{242} in transmembrane region V) was found to be responsible for the high affinity mesulergine binding in rat (Kao et al., 1992; Johnson et al., 1994). Phe^{340} in transmembrane region VI has also been shown to be involved in the binding of mesulergine (and other ergolines) (Choudary et al., 1993, Choudhary et al., 1995). Additional mutagenesis studies have delineated the role of three aspartate residues (Wang et al., 1993; Sealfon et al., 1995) and Ser^{159} (Almaula et al., 1996) in 5-HT_{2A} receptor activation and a(nta)gonist binding. 5-HT shows a higher affinity for the cloned 5-HT_{2A} receptors than it does for endogeneous receptors, and its affinity depends heavily on the intrinsic activity of the used radioligand. When expressed 5-HT_{2A} receptors are labeled by the (partial) agonists [^{3}H]1-(4-bromo-2,5-di-methoxyphenyl)-2-aminopropane (DOB) or [^{3}H]1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), or by [^{3}H]5-HT (Branchek et al., 1990; Teitler et al., 1990; Sleight et al., 1996), significantly less receptors are labeled than when antagonist radioligands are used, most probably because agonists only bind to the G-protein-precoupled receptors. In contrast, it was found that high affinity agonist binding to the rat 5-HT_{2A} receptor expressed in HEK293 cells was not sensitive to GTP analogues, whereas expression of the same receptor in NIH-3T3 cells was partially sensitive to GTP analogues (Szele and Pritchett, 1993). Apparently, the GTP sensitivity of agonist binding depends on the cell type (i.e., on the G-protein repertoire present within a particular cell type) in which the receptor is expressed, even though stimulation of the 5-HT_{2A} receptor in both cell types strongly stimulated phosphatidyl bisphosphate hydrolysis.

Activation of expressed 5-HT_{2A} receptors in several heterologous cell types (Swiss 3T3, HEK293, NIH-3T3, *Xenopus* oocytes; see Table 1) unequivocally leads to activation of phospholipase C, as measured by phosphatidyl bisphosphate hydrolysis, Ca^{2+}-mobilization, or activation of a Ca^{2+}-induced Cl^- current in *Xenopus* oocytes (Pritchett et al., 1988; Julius et al., 1990; Van Obberghen-Schilling et al., 1991; Apud et al., 1992; Yang et al., 1992). Nevertheless, endogenously expressed 5-HT_{2A} receptors have been reported to couple to additional signaling pathways (e.g., inhibition of adenylyl cyclase (Garnovskaya et al., 1995), activation of adenylyl cyclase (Berg et al., 1994a), or activation of phospholipase A_2 (Felder et al., 1990)). Studies using a chimeric 5-HT_{2A}/5-HT_{1B} receptor have shown that the ability to stimulate the phospholipase C pathway is located in the third cytoplasmic loop of the 5-HT_{2A} receptor (Oksenberg et al., 1995).

Cellular transformation seems to be closely associated with the activation of phospholipase C and protein kinase C. Indeed, the rat 5-HT_{2A} receptor expressed in NIH-3T3 cells (as well as the 5-HT_{2B} and 5-HT_{2C} receptors; see below) has been shown to stimulate cell division (Julius et al., 1990). The growth-potentiating effects of 5-HT_2 receptor stimulation may depend on the cell types studied. As was shown for the hamster 5-HT_{2A} receptors (Van Obberghen-Schilling et al., 1991), 5-HT by itself does not promote cell growth in quiescent cells (although it strongly activates phospholipase C) but rather exerts a synergistic effect on the fibroblast growth factor-induced (or in general, tyrosine kinase-activated pathway induced) DNA synthesis. In addition, both the rat 5-HT_{2A} receptor and the rat 5-HT_{2C} receptor, expressed in NIH-3T3 fibroblasts, have been shown to be linked to $Na^+/K^+/Cl^-$ co-transport (Mayer and Sanders-Bush, 1994). 5-HT_{2A} (and 5-HT_{2C}) receptors are unique among G-protein-coupled receptors in that chronic treatment in vivo with agonists as well as antagonists decreases receptor density. It was found, however, that when rat 5-HT_{2A} receptors were expressed in different heterologous backgrounds (NIH-3T3, MDCK (Madin-Darby canine kidney), or AtT-20 (mouse anterior

pituitary tumor) cells) the effect of treatment with a(nta)gonist on receptor density depends on the used cell type (Grotewiel and Sanders-Bush, 1994). Indeed, when the different studies on desensitization of the 5-HT_{2A} receptor are compared, it is evident that the cellular background can have profound influences.

When hamster 5-HT_{2A} receptors were expressed in PS200 cells (a 5-HT_2 deficient variant of CCL39 cells), it was found that they only show a modest degree of desensitization. This desensitization appears to be independent from receptor phosphorylation, because the receptor is not phosphorylated upon stimulation, and inactivation of protein kinase C or removal of three out of five protein kinase C phosphorylation sites does not influence desensitization. However, even though the receptor is not phosphorylated, it shows agonist-induced internalization. This internalization is not very efficient, it takes 30 min to observe only 50% loss of the receptors (Vouret-Craviari et al., 1995). The process of 5-HT_{2A} receptor internalization was also studied using confocal laser microscopy of immunolabeled 5-HT_{2A} receptors expressed in GF-62 cells (Berry et al., 1996). In contrast to the results described above, it was found that already after five min of agonist incubation, 5-HT_{2A} receptors were internalized via the endosomal pathway. Although 5-HT_{2A} receptors were internalized, no loss of radioligand binding sites or down regulation occurred. Again in contrast to these findings, it has also been reported that the desensitization of the rat 5-HT_{2A} receptor expressed in NIH-3T3 cells is not accompanied by either receptor internalization, receptor down regulation, or G-protein down regulation and that only the intermediate (2–6 h of agonist exposure), but not the long term desensitization (24 h of agonist exposure) is sensitive to down regulation of protein kinase C (Roth et al., 1995). A role of protein kinase C in intermediate term 5-HT_{2A} receptor desensitization has also been suggested by Van Huizen et al. (1993). When the human 5-HT_{2A} receptor was expressed in Swiss 3T3 cells, a time-dependent decrease in [^{3}H]ketanserin binding was observed after pre-incubation of the cells with serotonin or the 5-$HT_{2A/2C}$ receptor agonist DOI. A two h pre-incubation of the cells with 5-HT significantly decreased the 5-HT-mediated phosphatidyl inositol turnover, as well as the potency of 5-HT to stimulate phosphatidyl inositol turnover, processes that were mediated via activation of protein kinase C.

3.2. 5-HT_{2B} receptors

There is not much known about the function of 5-HT_{2B} receptors, mainly due to the lack of specific 5-HT_{2B} receptor ligands. Classically, these receptors are known for their effect on the fundic smooth muscle contraction, therefore they have also been called 5-HT_{2F}. However, after the cDNA of the 5-HT_{2B} receptor became available, 5-HT_{2B} receptor mRNA has been detected in a variety of peripheral tissues and also (although in relatively low amounts) in the brain. The rat brain forms an exception. No transcripts have been found here although several techniques for mRNA detection were used. This indicates that the 5-HT_{2B} receptor in human brain may be involved in higher cognitive brain functions (Kursar et al., 1994). It has also been suggested that the 5-HT_{2B} receptor may play a role in differentiation of serotonergic cells (Loric et al., 1995).

Using a mouse gene fragment that was homologous to the 5-HT_{2A} and 5-HT_{2C} receptors (Foguet et al., 1992b), the rat 5-HT_{2B} receptor was cloned from a rat fundus cDNA library (Foguet et al., 1992a). Around the same time the identical cDNA was reported from rat (Kursar et al., 1992), as well as the mouse homologue (Loric et al., 1992). Two years later the human 5-HT_{2B} receptor was reported (Choi et al., 1994; Kursar et al., 1994; Schmuck et al., 1994). The different 5-HT_{2B} receptors show high amino acid sequence identity ($\pm$80%), however, the mouse 5-HT_{2B} receptor is 25 amino acids longer due to a longer C-terminal part of the mouse receptor. The 5-HT_{2B} genes, like the 5-HT_{2A} genes, contain two introns at conserved positions (Foguet et al., 1992b; Choi et al., 1994; Schmuck et al., 1994).

The pharmacological profile of the 5-HT_{2B} receptor has been extensively studied using displacement of [^{3}H]5-HT (Foguet et al., 1992a; Kursar et al., 1992, 1994; Wainscott et al., 1993, 1996; Bonhaus et al., 1995) or [^{125}I]DOI (Loric et al., 1992, 1995; Choi et al., 1994; Colas et al., in press) binding. It is consistent with the profile described earlier for the rat fundus receptor (ritanserin > 5-HT > yohimbine > ketanserin $\geq$ spiperone) and can be distinguished from that of the 5-HT_{2A} and 5-HT_{2C} receptors by its lower affinity for mianserin and ritanserin and higher affinity for 5-HT and yohimbine. The rat and the human 5-HT_{2B} receptors display a very similar pharmacological profile (Bonhaus et al., 1995; Wainscott et al., 1996), although there are some notable exceptions (e.g., the affinities of the 5-HT_{2A} receptor antagonists ketanserin and spiperone for the human receptor are much higher than for the rat receptor). The mouse 5-HT_{2B} receptor differs from the human and rat receptors by a lower affinity for 5-HT and a higher affinity for spiperone.

When the rat 5-HT_{2B} receptor is expressed in *Xenopus* oocytes, its activation leads to opening of Ca^{2+}-activated chloride channels, due to the activation of phospholipase C (Foguet et al., 1992a). However, this response is less pronounced than that induced by 5-HT_{2A} or 5-HT_{2C} receptors. In several mammalian cells (see Table 1) it was confirmed that 5-HT_{2B} is able to activate phospholipase C. In addition to the activation of phospholipase C, a modest potentiation of cAMP production has been observed when the 5-HT_{2B} receptor was expressed at low density in AV12 cells (Lucaites et al., 1996).

As already mentioned above, 5-HT_2 receptors have been shown to be involved in the growth-stimulatory effects of 5-HT. Recent studies on the mouse 5-HT_{2B} receptor expressed in Ltk$^-$ cells have shown that activa-

tion of this receptor results in activation of $p21^{ras}$ and $p42^{mapk}/p44^{mapk}$ (Launay et al., 1996) and that 5-HT_{2B} receptor-dependent 5-HT-stimulated foci develop in confluent cells which are tumorigenic in nude mice.

3.3. 5-HT_{2C} receptors

5-HT_{2C} receptors are highly expressed in choroid plexus, and function in the control of the exchanges between the central nervous system and the cerebrospinal fluid. $5HT_{2C}$ receptors are also associated with motor behaviour and modulation of appetite. Because of the large overlap in pharmacology between the 5-HT_{2C} and the 5-HT_{2A} receptor, some of the effects attributed to the 5-HT_{2A} receptor (see above) may actually be mediated by 5-HT_{2C} receptors. Clear conclusions about the functions of the 5-HT_{2C} receptor in mice could be drawn from the generation of mutant mice in which the 5-HT_{2C} gene is knocked-out. These mice are overweight as a result of an aberrant feeding behaviour, and exhibit an epileptic phenotype suggesting a role for 5-HT_{2C} receptors in tonic inhibition of neuronal excitability (Tecott et al., 1995).

The 5-HT_{2C} receptor from mouse (Lubbert et al., 1987) and rat (Julius et al., 1988) have been cloned using a system based on injection of choroid plexus-derived mRNA into *Xenopus* oocytes. Subseqently, 5-HT_{2C} receptors were cloned from mouse (Yu et al., 1991) and human (Saltzman et al., 1991; Stam et al., 1994). Interestingly, 5-HT_{2C} receptors contain an additional hydrophobic region at their N-terminus, of which no function has been reported as yet.

In the coding region of the 5-HT_{2C}-receptor gene three introns are present. The second and the third intron are located at the same position as the introns in the 5-HT_{2A} and 5-HT_{2B} receptor genes (Foguet et al., 1992b; Stam et al., 1994). Recently, an alternatively-spliced mRNA encoding a truncated 5-HT_{2C} receptor was identified in rat, mouse and human cDNA (Canton et al., 1996). This splice variant contains a 95 bp deletion in the region encoding the second intracellular loop (IL2) to the fourth transmembrane domain (TM IV), resulting in a shift in the open reading frame. This frameshift generates an alternative open reading frame putatively encoding truncated versions of the rat and human 5-HT_{2C} receptors. After expression in NIH-3T3 cells, the truncated rat receptor does not reveal any serotonergic binding activity or phosphoinositide hydrolysis activity. Clearly, it cannot function as a 5-HT receptor. An interesting suggestion is that the truncated products might form dimers with full-length receptors to regulate localization or function. Whether these alternatively-spliced mRNAs are translated into (functional) proteins in vivo remains to be investigated.

In order to study the glycosylation of 5-HT_{2C} receptors, choroid plexus tissue and 3T3 cells expressing 5-HT_{2C} receptors were treated with tunicamycin (which metabolically inhibits N-linked glycosylation), *N*-glycosidase F (which enzymatically removes N-linked sugars) and neuraminidase (which cleaves sialic acid residues from glycoproteins) (Backstrom et al., 1995). It was concluded that both endogenously and heterologously expressed 5-HT_{2C} receptors contain N-linked sugars, while sialic acid residues only associate with 5-HT_{2C} receptors expressed in the choroid plexus.

The 5-HT_{2C} receptor has a high affinity for 5-HT and has therefore previously been called 5-HT_{1C}. Interestingly, it was found that 5-HT has a high affinity for both the agonist high affinity state (G-protein coupled) and the agonist low affinity (G-protein uncoupled) form of the 5-HT_{2C} receptor (Leonhardt et al., 1992). The pharmacology of the cloned 5-HT_{2C} receptors clearly resembles that of the receptors characterized in the choroid plexus (ritanserin > methysergide > mianserin > 5-HT > ketanserin > *m*-chlorophenylpiperazine (mCPP) > spiperone) and no clear species variations have been reported. Because expression of the rat 5-HT_{2C} receptor in NIH-3T3 cells induces agonist-independent stimulation of phosphatidylinositol bisphosphate hydrolysis, several antagonists could be identified as inverse agonists by their ability to lower the basal levels of inositol phosphates (Barker et al., 1994; Westphal and Sanders-Bush, 1994). In addition, these studies indicated that inverse agonists preferably couple to the G-protein-uncoupled form of the receptor, while agonists prefer the coupled receptors, and neutral antagonists have equal affinities for both receptor forms. Evidently, as was also described above, this has implications for the ligand affinities obtained with either agonist- or antagonist-labeled receptors (Havlik and Peroutka, 1992; Stam et al., 1994).

To compare the atypical activity of a large number of antipsychotics with their affinity for the 5-HT_{2C} receptor, the affinities of a large number of typical and atypical antipsychotics for the rat 5-HT_{2C} receptor (transiently expressed in COS-7 cells) were determined (Roth et al., 1992). It was found that several putative atypical antipsychotic agents have high affinities for the cloned rat 5-HT_{2C} receptor, however, the spectrum of drug binding does not correlate with the atypical nature of these compounds.

Antagonist treatment of 5-HT_{2C} receptors (and 5-HT_{2A} receptors) unexpectedly leads to receptor down regulation (sometimes referred to as atypical down regulation). To study this phenomenon, the rat 5-HT_{2C} receptor was expressed in insect Sf9 cells (Labrecque et al., 1995). Its spontaneous activation of phosphatidylinositol bisphosphate hydrolysis was used to monitor the inverse agonistic properties of several antagonists. Mianserin was found to be the most potent inverse agonist. Interestingly, antagonist treatment results in a decrease in the number of receptors which correlates to their ability to down regulate receptors in vivo. However, although this effect of antagonists was dose-dependent, it did not correlate to their inverse agonistic activity, indicating that the processes underlying both effects (inverse agonism and receptor down regulation) are different. In contrast to the findings of Labrecque et al. (1995), antagonist-mediated down reg-

ulation was not observed when the rat 5-HT_{2C} receptor was expressed in NIH-3T3 cells (Barker et al., 1994), indicating that the process of atypical downregulation may strongly depend on the cellular context.

Xenopus oocytes have often been used to express 5-HT_{2C} receptors and study their functional properties (Lubbert et al., 1987; Julius et al., 1988; Briggs et al., 1991; Panicker et al., 1991; Shichijo et al., 1991; Yu et al., 1991; Chen et al., 1994; Quick et al., 1994). Stimulation of the 5-HT_{2C} receptor leads to activation of phospholipase C and subsequent activation of a Ca^{2+}-induced Cl^- current. The *Xenopus* oocyte system has also been used to determine the specificity of G-protein coupling by the 5-HT_{2C} receptor. Using antisense oligonucleotides against several Gα-subunits and co-expression of mammalian Gα-subunits, it was found that the 5-HT_{2C} receptor couples to G_o (Chen et al., 1994; Quick et al., 1994) and G_{i1} (Chen et al., 1994) and that it activates *Xenopus* phospholipase C-β (Quick et al., 1994). When mammalian Gα-subunits were co-expressed, the 5-HT_{2C} receptor could interact with G_{oa}, G_{ob}, G_q and G_{11} but not with G_s, G_{olf} or G_t (Quick et al., 1994).

A functional coupling to phospholipase C was observed also after expression of the 5-HT_{2C} receptor in NIH-3T3 cells (Julius et al., 1988; Stam et al., 1994), CHO cells (Akiyoshi et al., 1995), COS-7 cells (Tohda et al., 1995), or AV12 cells (Lucaites et al., 1996). The increase in intracellular Ca^{2+} following activation of rat 5-HT_{2C} receptors has been found to cause activation of the α isoform of protein kinase C in COS-7 cells (Lutz et al., 1993) and activation of Ca^{2+}-activated K^+ channels in mouse A9 fibroblasts (Boddeke et al., 1993b). The rat 5-HT_{2C} receptor has also been shown to couple to the activation of Ca^{2+}-independent, inositol phosphate-dependent K^+ channels in *Xenopus* oocytes (Panicker et al., 1991).

Activation of rat 5-HT_{2C} receptors has been shown to induce malignant transformation of transfected NIH-3T3 cells (Julius et al., 1989), and introduction of cells from transformed foci into nude mice results in the generation of tumors. However, the 5-HT_{2C} receptor should not be considered as a real proto-oncogene, since the mitogenic response of the same rat 5-HT_{2C} receptor expressed in hamster CCL39 cells does not induce a transformed phenotype (Kahan et al., 1992). There is, however, an influence of 5-HT on growth, but this response depends on a synergy with tyrosine kinase-activating receptors, and is mediated by endogenous 5-HT_{1B} receptors. The relationship between the activity of 5-HT_{2C} receptors and the capacity to stimulate cell growth was studied measuring [^{3}H]thymidine incorporation, DNA amount, and cell number (Westphal and Sanders-Bush, 1996). It was found that constitutively-active 5-HT_{2C} receptors stimulate cell division in transfected fibroblasts in the absence of agonist. Basal [^{3}H]thymidine incorporation was increased by agonists, decreased by inverse agonists, whereas neutral antagonists were without effect. However, two inverse agonists (mianserin and mesulergine) that eliminate basal phosphatidylinositol bisphosphate hydrolysis, had no effect on [^{3}H]thymidine incorporation, suggesting that the phospholipase C response and the mitogenic response may be responding to different signaling pathways. Moverover, it was found that agonist-mediated, but not constitutive receptor activity is partially sensitive to pertussis toxin. This finding also indicates that agonist-activated receptors couple to different (additional) G-proteins than constitutively active receptors.

When the human 5-HT_{2C} receptor was expressed in CHO cells, it was found that activation of this receptor reduces the inhibition of adenylyl cyclase mediated by activation of endogeneous 5-HT_{1B} receptors that are present on CHO cells (Berg et al., 1994b). This reduction, that was shown to be independent of activation of protein kinase C or increase in intracellular Ca^{2+}, discriminates the signal transduction properties of 5-HT_{2C} and 5-HT_{2A} receptors, since activation of the latter receptor has no effect on the inhibition of adenylyl cyclase. In a follow-up study, it was found that activation of phospholipase A_2, either by 5-HT_{2C} receptor stimulation, purinergic receptor stimulation, or directly with mellitin, mediates the regulation of the CHO/5-HT_{1B} receptor pathway (Berg et al., 1996). However, 5-HT_{2A} receptors also stimulate arachidonic acid release, while they do not influence the 5-HT_{1B}-mediated inhibition of adenylyl cyclase. Interestingly, the simultaneous activation of 5-HT_{2A} receptors and purinergic receptors leads to a block of purinergic receptor-mediated inhibition of adenylyl cyclase. This suggests that the 5-HT_{2A} receptor may couple to a third pathway (in addition to phospholipase C and phospholipase A_2) that can either block the effect, or inhibit the production of arachidonic acid metabolites.

The coupling repertoire of the mouse 5-HT_{2C} receptor expressed in AV12 cells was shown to depend on receptor density (Lucaites et al., 1996). At both high and low density the receptor can activate adenylyl cyclase as well as phospholipase C. The receptor can also inhibit adenylyl cyclase but only at high densities. This must be due to coupling of the receptor to distinct G-proteins because only the adenylyl cyclase inhibition is sensitive to pertussis toxin. The ability to couple to a pertussis toxin-sensitive, G_i/G_o-like G-protein was suggested to be an intrinsic property of the 5-HT_{2C} receptor, since stimulation of the human 5-HT_{2C} receptor, when expressed at high density in AV12 cells, can also inhibit adenylyl cyclase, whereas the rat 5-HT_{2A} or 5-HT_{2B} receptors never showed this response.

The increase in inositol phosphate formation after stimulation of the rat 5-HT_{2C} receptor expressed in COS-7 cells can be inhibited by treatment with botulinum toxin D (Tohda et al., 1995). This indicates that low molecular weight G-proteins (that are specifically ADP-ribosylated by this toxin) may be involved in the signal transduction

pathway leading from the 5-HT_{2C} receptor to the activation of phospholipase C.

In A9 mouse fibroblasts transfected with the rat 5-HT_{2C} receptor Boddeke et al. (1993a) used the Ca^{2+}-induced K^+ current that is activated upon 5-HT-mediated increases in intracellular Ca^{2+} to study receptor desensitization. The rat 5-HT_{2C} receptor appears not to be easily desensitized since a 1 h pretreatment with the 5-HT receptor agonist mCPP did not lead to densitization. Prolonged receptor activation (24 h) did, however, induce desensitization, which appears to be caused by protein kinase C-mediated receptor phosphorylation. Desensitization could be counteracted by dephosphorylation mediated by the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin. Westphal et al. (1995), working with the rat 5-HT_{2C} receptor expressed in NIH-3T3 cells, demonstrated agonist-mediated desensitization of phosphoinositide hydrolysis. These investigators also directly demonstrated that the receptor in its basal state is already pohosphorylated and that phosphorylation is increased by agonist treatment conditions that result in receptor desensitization. The inverse agonist mianserin had no effect on the basal phosphorylation state of the receptor. In contrast to the rat receptor, the human 5-HT_{2C} receptor expressed in CHO cells rapidly desensitizes (measured as a lowering of the 5-HT-induced Ca^{2+} response) (Akiyoshi et al., 1995). The protein kinase C activators phorbol 12-myristate 13-acetate (PMA) and mezerein also inhibit the Ca^{2+} response evoked by 5-HT. However, inhibition of protein kinase C only marginally inhibits desensitization. Rather, indications for a role of calmodulin-dependent protein kinase were obtained.

4. 5-HT_4 receptors

Effects of 5-HT mediated by 5-HT_4 receptors have been described in heart, adrenal, bladder, and alimentary canal (for review, see Bockaert et al. (1992)). Consequently, a role of this receptor subtype in diseases of the gastrointestinal tract, as well as in cardiac, urinary and endocrine functions has been postulated. 5-HT_4 receptors have also been found in the brain, especially in the hippocampus, indicating a role in cognitive enhancement.

Two different cDNAs, probably resulting from alternative splicing of 5-HT_4 receptor pre-mRNA, have been cloned from rat brain by RT-PCR (Gerald et al., 1995). The short and the long isoform (5-$HT_{4(s)}$ and 5-$HT_{4(l)}$) differ in the length and sequence of their C-terminus. Transcripts encoding the 5-$HT_{4(l)}$ receptor are expressed throughout the brain, while transcripts encoding 5-$HT_{4(s)}$ are restricted to the striatum.

Upon transient expression in COS-7 cells, both isoforms of the 5-HT_4 receptor display a high affinity for [^{3}H][1-[2-(methylsulfonylamino)ethyl]-4-piperinidyl]methyl-1-methyl-1*H*-indole-3-carboxylate maleate salt (GR113,808). The displacement profile of both isoforms is similar and corresponds well to that of the native 5-HT_4 receptor: cisapride > 5-HT > renzapride > 5-methoxytryptamine > zacopride > α-methyl-5-HT > 5-CT. Agonist stimulation of the 5-HT_4 receptor expressed in COS-7 leads to an increase in cAMP levels. Interestingly, in contrast to the results from the ligand-binding studies, in functional studies both receptor isoforms behave differentially. In general, the potency of agonists to stimulate cAMP release is greater for the 5-$HT_{4(s)}$ receptor. Despite the fact that the 5-$HT_{4(l)}$ receptor is expressed at a somewhat lower level (1.5-fold), activation of 5-$HT_{4(l)}$ results in a significantly higher adenylyl cyclase stimulation than activation of 5-$HT_{4(s)}$. Some agonists (cisapride, renzapride and zacopride) equipotently activate adenylyl cyclase in cells expressing 5-$HT_{4(l)}$, but have different potencies in cells expressing 5-$HT_{4(s)}$. In addition, GTPγS has a stronger effect on agonist high affinity sites of 5-$HT_{4(l)}$ than of 5-$HT_{4(s)}$. This observation can be explained by the fact that 5-$HT_{4(l)}$ is expressed at lower density, thereby increasing the coupling efficiency with endogenous G-proteins. Because splice variants of G-protein-coupled receptors differing in their C-terminus (e.g., prostaglandin EP3 receptor, pituitary adenylate cyclase activating polypeptide receptors, etc.) have been shown to couple to different G-proteins, it would be interesting to study the possibility that the two isoforms of the 5-HT_4 receptor couple to distinct G-proteins.

5. 5-HT_5 receptors

As yet, no clear function has been associated to the 5-ht_5 receptors. Because of their high affinity for LSD (D-lysergic acid diethylamine) and ergot derivatives, they are postulated to mediate some of the effects of these drugs. Because the chromosomal localization of 5-ht_{5A} receptors (both in mouse and in human), corresponds to regions in which mutations lead to abnormal brain development, it has been suggested that the 5-ht_{5A} receptor is involved in brain development (Matthes et al., 1993). The 5-ht_{5A} and 5-ht_{5B} receptors are differentially expressed in rat brain, with 5-ht_{5A} being expressed throughout the brain and 5-ht_{5B} expression being restricted to the CA1 region of the hippocampus, the medial habenulae and raphe nuclei (Plassat et al., 1992; Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993; Rees et al., 1994).

The 5-ht_{5A} receptor has been cloned from mouse (Plassat et al., 1992), rat (Erlander et al., 1993) and human (Rees et al., 1994) and the 5-ht_{5B} receptor has been cloned from mouse (Matthes et al., 1993) and rat (Erlander et al., 1993; Wisden et al., 1993). Both 5-HT_5 receptor genes show a strong sequence similarity (77%), but similarities to the other 5-HT receptors are low. Both genes contain one intron at a conserved position in the region encoding the third intracellular loop.

The 5-ht_{5A} receptors have been expressed in COS-7, NIH-3T3, COS-M6 and HeLa cells, and the 5-ht_{5B} recep-

tors have been expressed in COS-1, COS-7, COS-M6 and HeLa cells (see Table 1). Both 5-ht$_{5A}$ and 5-ht$_{5B}$ receptors have a very high affinity for LSD, and a similar pharmacological profile: LSD > ergotamine > methiothepin > 5-CT > methysergide > 5-HT. No evident species differences were observed in the pharmacological properties of the 5-ht$_5$ receptors, although the human 5-ht$_{5A}$ receptor has a higher affinity for methiothepin ($pK_i = 8.9$) than the homologous receptors from mouse ($pK_i = 7.0$) and rat ($pK_i = 6.8$).

No effects on adenylyl cyclase or phospholipase C could be detected when the expressed 5-ht$_5$ receptors were activated. However, binding experiments using [^{3}H]5-CT indicate that, both for the 5-ht$_{5A}$ and for the 5-HT$_{5B}$ receptor, high and low affinity binding sites exist, indicating precoupling to G-proteins (Plassat et al., 1992; Wisden et al., 1993). Although the pharmacological profile of the 5-ht$_5$ receptors shows some similarities to that of the 5-HT$_1$ receptors (e.g., a relatively high affinity for 5-CT, methiothepin and several ergolines), they are considered to form a separate subclass because the amino acid sequence similarity between the 5-ht$_5$ and the 5-HT$_1$ receptors is limited, and because both 5-ht$_5$ receptor genes contain an intron whereas 5-HT$_1$ receptor genes do not.

To obtain large amounts of receptor protein for biophysical and structural studies the mouse 5-ht$_{5A}$ receptor has been expressed in the yeasts *Pichia pastoris* (Weiss et al., 1995) and *Saccharomyces cerevisiae* (Bach et al., 1996). In *Pichia* and *Saccharomyces*, receptor concentrations up to 22 pmol/mg protein and 16 pmol/mg protein, respectively, were reached. The pharmacological profiles of the receptors expressed in yeast are comparable to the ones of receptors expressed in mammalian cells. However, the absolute K_i values are approximately one order of magnitude higher. This may be explained by a different lipid composition of the yeast membranes.

6. 5-HT$_6$ receptors

The existence of the 5-ht$_6$ receptor was revealed first by the cloning and characterization of its cDNA. Using this cDNA, the in vivo presence of 5-ht$_6$ receptor-encoding mRNA was confirmed in striatal neurons (Sebben et al., 1994) and in pig caudate putamen membranes (Schoeffter and Waeber, 1995). In addition, the 5-HT receptors that have been characterized in NCB-20 neuroblastoma cells (Conner and Mansour, 1990) and in N18TG2 cells (Unsworth and Molinoff, 1994) might belong to the 5-ht$_6$ receptors. Because 5-ht$_6$ receptors exhibit a high affinity for tricyclic antipsychotic drugs and because 5-ht$_6$ receptor transcripts have been localized to limbic and cortical regions of the brain, it has been suggested that the 5-ht$_6$ receptor may play a role in several neuropsychiatric disorders that involve serotonergic systems (Monsma et al., 1993; Roth et al., 1994). Upon knock-out of the 5-ht$_6$ receptor in rats using antisense oligonucleotides, it was found that such rats reveal a specific behavioral syndrome of yawning, stretching and chewing (Bourson et al., 1995). Because atropine dose-dependently antagonizes this behavioural syndrome, the 5-ht$_6$ receptor may be involved in the control of cholinergic transmission. No influence on locomotion, body weight, food intake, body temperature and nociception was observed.

The 5-ht$_6$ receptor gene has been cloned from rat (Monsma et al., 1993; Ruat et al., 1993a) and human (Kohen et al., 1996). The sequence of the rat 5-ht$_6$ receptor gene, as published by both authors, contained several errors that were caused by the high GC-content of the gene, producing severe sequence compression. The rat sequence was corrected to encode a protein of 438 amino acids (Kohen et al., 1996). The third intracellular loop (IL3) is extremely short (57 residues), while the C-tail is very long (117 residues), features that make it likely that the 5-ht$_6$ receptor couples positively to adenylyl cyclase. The sequence of the 5-ht$_6$ receptor gene is distant from the other 5-HT receptor sequences. Monsma et al. (1993) reported the presence of an intron located between the regions encoding transmembrane domains VI and VII (EL3), while Ruat et al. (1993a) indicated the presence of an intron between the region encoding transmembrane domains V and VI and the intracellular loop 3. Similarly, the human gene contains two introns at the same positions corresponding to intracellular loop 3 and extracellular loop 3. Interestingly, the position of the first intron is conserved in the 5-ht$_{5A}$ receptor, the 5-ht$_{5B}$ receptor and in the dopamine D2 and D3 receptors. The human and rat 5-ht$_6$ receptors show a similar, unique pharmacological profile. They have a high affinity for [^{125}I]LSD and [^{3}H]5-HT, but a low affinity for 5-CT. Various antipsychotics (e.g., loxapine, clozapine) and antidepressants (e.g., amoxapine and clomipramine) show high affinity for the 5-ht$_6$ receptors. Only two of the tested drugs show different affinities for the human and rat receptor: methiothepin (h5-ht$_6$ $pK_i = 9.4$ versus r5-ht$_6$ $pK_i = 8.7$) and metergoline (h5-ht$_6$ $pK_i = 6.4$ versus r5-ht$_6$ $pK_i = 7.5$). When the affinities of 36 typical and atypical antipsychotic agents for the rat 5-ht$_6$ receptor were tested, it was found that atypical antipsychotic activity cannot be predicted on the basis of affinity for the 5-ht$_6$ receptors (Roth et al., 1994).

Although Monsma et al. (1993) found no changes in cAMP levels when the rat 5-ht$_6$ receptor expressed in COS-7 cells was activated, Ruat et al. (1993a) did find a stimulation of adenylyl cyclase in COS-7 cells transfected with the rat 5-ht$_6$ receptor. This positive coupling to adenylyl cyclase was also reported for the rat 5-ht$_6$ receptor upon expression in HEK293 cells (Monsma et al., 1993), and for the human 5-ht$_6$ receptor expressed in HeLa cells (Kohen et al., 1996). Antidepressants and antipsychotics work as antagonists on the increase in cAMP levels.

7. 5-HT$_7$ receptors

The 5-HT$_7$ receptors have been implicated in smooth muscle relaxation in various tissues and have been suggested to play a role in affective behaviour, sensory processes and regulation of circadian phase shifts (for a recent review, see Eglen et al. (1997)). The high affinity of the 5-HT$_7$ receptor for neuroleptic agents suggests that this receptor, just like the 5-ht$_6$ receptor, might play a role in certain neuropsychiatric disorders. The affinities of a large body of typical and atypical antipsychotic drugs for the rat 5-ht$_6$ and 5-HT$_7$ receptors in transfected cells were measured (Roth et al., 1994), but no consistent pattern emerged that enables a discrimination of the role of these two receptors in mediating the effects of these drugs. In the periphery, endogenous 5-HT$_7$ receptors have been identified in human uterine artery smooth muscle cells (Schoeffter et al., 1996).

Genes encoding the 5-HT$_7$ receptor have been cloned from human (Bard et al., 1993), rat (Lovenberg et al., 1993a; Meyerhof et al., 1993; Ruat et al., 1993b; Shen et al., 1993), mouse (Plassat et al., 1993), guinea pig (Tsou et al., 1994), *X. laevis* (Nelson et al., 1995) and *D. melanogaster* (Witz et al., 1990). The published cDNA sequences encoding the rat 5-HT$_7$ receptor are not all identical. The sequences published by Ruat et al. (1993b) matches the one of Meyerhof et al. (1993), but the sequence published by Shen et al. (1993) differs in its amino terminus and predicted start codon, most probably due to sequence errors. The sequence published by Lovenberg et al. (1993a) contains a C-terminus which is 13 amino acids shorter than the C-terminus published by all others. This form results from a 5 bp insertion causing premature termination of the open reading frame. Because Ruat et al. (1993b) found an intron-exon boundary at the exact side of the insertion found by Lovenberg et al. (1993a) this sequence difference is probably due to differential splicing. A similar short splice variant has been found in human cDNA; the corresponding receptor has been called 5-HT$_{7(b)}$ (Jasper et al., 1997). In addition to the intron in the region encoding the C-terminus, the presence of a second intron in the region encoding the second intracellular loop (IL2) was reported (Ruat et al., 1993b; Shen et al., 1993). So, like the genes encoding 5-HT$_5$ and 5-ht$_6$ receptors, the gene encoding the 5-HT$_7$ receptor contains (at least two) introns in its coding region.

When 5-HT$_7$ receptors are expressed in COS-M6 cells (Lovenberg et al., 1993b), COS-7 cells (Bard et al., 1993; Meyerhof et al., 1993; Plassat et al., 1993; Tsou et al., 1994), CHO-K1 cells (Ruat et al., 1993b), or *Xenopus* oocytes (Nelson et al., 1995), they show high affinity binding to [^{3}H]5-HT and [^{125}I]LSD and have a unique pharmacological profile (5-CT > methiothepin > 5-HT > clozapine > 8-OH-DPAT). No extreme differences in pharmacology among the different species exist, although in guinea pig the affinity of 5-HT for the 5-HT$_7$ receptor is 10-fold higher than the affinity of methiotepin. The pharmacological properties of the *Xenopus* receptor were investigated only in a functional assay (see below). The profile is well comparable to those of the mammalian receptors, except for the antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN190) which has a higher affinity for the *Xenopus* receptor (Nelson et al., 1995). Several antipsychotic agents have a high affinity for the 5-HT$_7$ receptor (Roth et al., 1994). However, as was described above for the 5-ht$_6$ receptor, it was not possible to predict the typical or atypical character of antipsychotics on the basis of their affinity for 5-HT$_7$ receptors.

The 5-HT$_7$ receptor unambiguously couples to the activation of adenylyl cyclase, as was demonstrated in a large number of heterologous cell systems (COS-7, HEK-293, CHO, HeLa, NIH-3T3, or insect Sf9 cells; see Table 1). The activity of the *Xenopus* receptor was monitored by co-expressing the receptor with the cystic fibrosis transmembrane conductance regulator (CFTR) in *Xenopus* oocytes. The increased levels of cAMP, caused by activation of the *Xenopus* 5-HT$_7$ receptor, induce CFTR-mediated chloride currents via the activation of protein kinase A (Nelson et al., 1995).

8. Invertebrate 5-HT receptors

8.1. Invertebrate 5-HT$_1$-like receptors

Today, the sequence information for three invertebrate 5-HT$_1$-like receptors is known: two receptors from *Drosophila melanogaster*, (5-HT$_{dro2A}$ and 5-HT$_{dro2B}$; Saudou et al., 1992) and one receptor from *Lymnaea stagnalis* (5-HTLym; Sugamori et al., 1993) (see Fig. 1).

The sequence and signaling properties of the 5-HT$_{dro2A}$ and 5-HT$_{dro2B}$ receptors suggest that they belong to the 5-HT$_1$ receptor family. However, their genes do contain at least four introns in the coding region. A specific function has not yet been ascribed to either of the *Drosophila* receptors, although it was suggested that the 5-HT$_{dro2A}$ receptor might play a role in motor control because it is predominantly expressed in midline motor neurons. 5-HT$_{dro2A}$ and 5-HT$_{dro2B}$ are highly homologous to each other (Saudou et al., 1992). Their genes are located at the same chromosomal position which suggests that they result from a recent duplication event. The 5-HT$_{dro2A}$ receptor contains an additional hydrophobic domain located halfway its first extracellular part. When expressed in NIH-3T3 cells, the 5-HT$_{dro2A}$ and 5-HT$_{dro2B}$ both display a high affinity for [^{125}I]LSD and have a similar pharmacological profile, although 5-HT has a 8-fold higher affinity for 5-HT$_{dro2B}$ than for 5-HT$_{dro2A}$ (2.1 versus 16 μM). Upon activation, both receptors inhibit adenylyl cyclase and moderately activate phospholipase C in a pertussis toxin-sensitive manner. Partial deletion of the large amino termini found in the 5-HT$_{dro2A}$ and 5-HT$_{dro2B}$ receptors (230

and 123 amino acids, respectively) was shown not to influence their pharmacological, nor their signaling properties. However, it did increase their expression levels in NIH-3T3 cells (Saudou et al., 1992).

Sugamori et al. (1993) cloned a 5-HT receptor-encoding gene from the freshwater snail *L. stagnalis*. The receptor specified by this gene, 5-HTLym, shows the highest sequence similarity with the mammalian 5-HT_1 receptors and therefore it is reasonable to classify it as a 5-HT_1-like receptor. Its functional properties are, however, rather unique, probably reflecting the large evolutionary distance between snails and mammals (Sugamori et al., 1993). When expressed in COS-7 cells, 5-HTLym displays a high-affinity binding site for [^{3}H]LSD. The affinity for 5-HT is rather low (1.56 μM) and the pharmacological profile of 5-HTLym does not correspond to any of the other 5-HT receptors (methiothepin > LSD > clozapine > methysergide > 5-CT > 8-OH-DPAT > 5HT > ketanserin). Addition of Gpp(NH)p shifts the receptor into a low affinity state, suggesting that it is able to couple to G-proteins in COS-7 cells. Unfortunately, no experimental data were presented on the coupling of 5-HTLym to second messengers. Because 5-HTLym has the structural characteristics of a receptor that couples negatively to adenylyl cyclase (a long third intracellular loop, and a short C-terminal tail) it is, however, to be expected that it will inhibit adenylyl cyclase.

8.2. Invertebrate 5-HT_2-like receptors

Two 5-HT_2-like receptor cDNAs have been cloned from invertebrates, 5-HT_{2Dro} from *D. melanogaster* (Colas et al., 1995) and 5-HT_{2Lym} from *L. stagnalis* (Gerhardt et al., 1996). In contrast to the other cloned invertebrate receptors, where classification according to the vertebrate classification scheme is not always convincing, the invertebrate 5-HT_2 receptors are closely, both structurally and functionally, related to the mammalian 5-HT_2 receptors. The only evident difference between the vertebrate and invertebrate 5-HT_2 receptors is the size of the amino terminal region, being significantly enlarged in invertebrate receptors. Currently, it is not known if this domain has a special function. Partial deletion of the amino termini of 5-HT_{2Dro} and 5-HT_{2Lym}, as well as that of the 5-HT_{dro2A}, 5-HT_{dro2B} and 5-HT_{dro1} receptors, does not seem to influence their pharmacological or their signaling properties, while it does increase their expression levels in mammalian cell lines (Saudou et al., 1992; Gerhardt et al., 1996; Colas et al., in press).

5-HT_{2Dro} might play an important role in embryogenesis (Colas et al., 1995). In contrast to the 5-HT_{dro2A}, 5-HT_{dro2B} and 5-HT_{dro1} receptors, of which the expression starts in late embryos (Saudou et al., 1992), 5-HT_{2Dro} is expressed in the blastoderm phase of *Drosophila* embryogenesis. Interestingly, this early embyronic expression is organized in a seven-stripe pattern, in phase with the expression of the pair-rule gene *fushi-tarazu*.

The gene encoding 5-HT_{2Dro} contains five introns in the coding regions, of which one, two or three introns are present at conserved positions in the 5-HT_{2Lym}, 5-$HT_{2A/B}$ or 5-HT_{2C} receptors, respectively.

In order to correlate the pharmacological profile of the 5-HT_{2Dro} receptor with that of other mammalian 5-HT_2 receptors, a detailed comparison was made between the profiles of the human and mouse 5-HT_{2B}, the human 5-HT_{2A} and the pig 5-HT_{2C} receptor (Colas et al., in press). All clones were transiently expressed in COS-7 cells, labeled with [^{3}H]DOI and the affinity of 35 compounds was tested. The pharmacology of the 5-HT_{2Dro} receptor was found to correlate with that of the mouse 2B > pig 2C > rat 2A > human 2B receptors and not with any other 5-HT receptors. Furthermore, it was found that deletion of the long N-terminus of 5-HT_{2Dro} only slightly affected the pharmacology. Out of 35 tested drugs, only three showed a significantly increased (5-HT, ketanserin) or decreased (5-CT) affinity for the truncated receptor.

To date, no signaling properties of the 5-HT_{2Dro} receptor have been reported.

The sequence of the 5-HT_2 receptor cloned from *L. stagnalis* clearly suggests that it is a member of the 5-HT_2 receptor subfamily (Gerhardt et al., 1996). The coding region of the 5-HT_{2Lym} gene is interrupted by a single intron, present in the region encoding the first extracellular loop. Interestingly, all members of the 5-HT_2 receptor receptor family contain an intron at this position. When 5-HT_{2Lym} was expressed in HEK293 cells, it was found that stimulation with 5-HT leads to an efficient activation of phospholipase C. However, the levels of receptor expression were too low to be detected using radiolabeled ligands. When the first 220 amino acids were omitted, a high level of receptor expression was obtained, and the pharmacological profile of the receptor could be studied. This profile (ritanserin > methysergide > mianserin > yohimbine > ketanserin > spiperone) indicates that 5-HT_{2Lym} is more closely related to the 5-$HT_{2B/2C}$ than to the 5-HT_{2A} receptors. Mesulergine was found to act as an inverse agonist only when the (truncated) 5-HT_{2Lym} receptor was expressed at high density, at low density it acted as a neutral antagonist.

8.3. Drosophila 5-HT_7-like receptor

When the mammalian 5-HT_7 receptors were cloned, it was found that they shared considerable sequence homology with the 5-HT_{dro1} receptor (Witz et al., 1990). In addition, both the 5-HT_7 receptors and the 5-HT_{dro1} receptor couple positively to adenylyl cyclase, and both have been implicated to be involved in the regulation of circadian rythms. For the *Drosophila* receptor this conclusion was based on the presence of a Gly–Ser repeat in the N-terminal tail of the receptor. Such motifs are considered to function as attachment sites for glycosaminoglycans and are found in biological clock genes like *period* in

Drosophila and *frequency* in *Neurospora*. Because of the structural as well as the functional similarity between the 5-HT_{dro1} receptor and the vertebrate 5-HT_7 receptors the 5-HT_{dro1} receptor can be considered as an invertebrate 5-HT_7-like receptor (Colas et al., 1995).

In contrast to the situation in vertebrates, the gene encoding the 5-HT_{dro1} receptor does not contain any intron in the coding region.

Like many other invertebrate G-protein-coupled receptors, 5-HT_{dro1} contains an extra long amino terminal domain (Witz et al., 1990). Within this domain, an eighth hydrophobic domains can be recognized of which the function is yet unclear.

Upon transient expression of 5-HT_{dro1} in COS-7 cells, displacement of [^{125}I]LSD binding was used to determine the pharmacological properties of 5-HT_{dro1} (dihydroergocryptine > methysergide > 5-HT > prazosin > yohimbine > 8-OH-DPAT) (Saudou et al., 1992). Although this characterization is too limited to determine the pharmacological similarity between the *Drosophila* 5-HT_7-like receptor and the mammalian 5-HT_7 receptors, it is evident that the affinity of 8-OH-DPAT is much lower for the *Drosophila* receptor than for the mammalian receptors. When expressed in mouse NIH-3T3 cells (Witz et al., 1990), or insect Sf9 cells (Obosi et al., 1996), the 5-HT_{dro1} receptor (like the mammalian 5-HT_7 receptor) is positively coupled to adenylyl cyclase.

8.4. 5-HT receptors in Aplysia

Two 5-HT receptors have been cloned from the sea snail *Aplysia californa* that were named Ap5-HT_{B1} and Ap5-HT_{B2} (Li et al., 1995). The sequences of the genes encoding these receptors differ considerably from those of the other 5-HT receptors, but are most similar to the sequences of vertebrate 5-HT_4 receptors (see Fig. 1). It is, however, still unclear whether the *Aplysia* receptors can be classified according to the vertebrate 5-HT receptor subtype classification scheme. According to their sequences they may be considered 5-HT_4-like receptors, their genomic structure and their functional properties are, however, not consistent with such a classification.

The genes encoding the Ap5-HT_{B1} and Ap5-HT_{B2} receptors are intronless and highly homologous to each other. 5-HT is known to exert several functions in *Aplysia* (e.g., control of feeding, locomotion, circadian rhythm, synaptic plasticity) but it is not yet known if the cloned receptors are really involved in any of these functions. Interestingly, Ap5-HT_{B2} is expressed predominantly in the central nervous system, while Ap5-HT_{B1} is expressed predominantly in the reproductive system. No extensive pharmacological characterization was carried out on the *Aplysia* receptors. The EC_{50} values for 5-HT are 1.8 nM (Ap5-HT_{B1}) and 1.5 nM (Ap5-HT_{B2}), respectively. Cyproheptadine (10 μM) is unable to block the inositol phosphate response elicited by 100 nM of 5-HT, while methiothepin and spiperone can do so. When Ap5-HT_{B1} and Ap5-HT_{B2} are expressed in HEK293 cells, stimulation of the receptors with 5-HT leads to a weak stimulation of phospholipase C (1.5-fold over basal levels). No coupling to adenylyl cyclase was observed.

8.5. Nomenclature of invertebrate 5-HT receptors

Unlike the nomenclature of the vertebrate 5-HT receptors, invertebrate 5-HT receptor nomenclature has not (yet) been subject to recommendations from the IUPHAR Serotonin Receptor Nomenclature Committee. As a consequence, invertebrate 5-HT receptor nomenclature is still rather messy. Names of species are either indicated by an abbreviated prefix (e.g., Ap5-HT_{B1}), or as an abbreviated subscript following the agonist abbreviation, 5-HT (e.g., 5-HT_{Dro}). Even more confusing is the fact that numbering of receptor subtypes sometimes is meant to indicate structural and functional homology with an existing vertebrate 5-HT receptor subtype (e.g., 5-HT_{2Dro}), whereas in other cases it merely serves to indicate that two receptors differ from each other (e.g., 5-HT_{dro2A} and 5-HT_{dro2B}). Furthermore, no difference is being made between newly described recombinant receptors and receptors for which a physiological role has been established.

In this review we have, however, refrained from changing the nomenclature for invertebrate 5-HT receptors into one that is more uniform and consistent, because we deem it more appropriate that the responsible committee (i.e., the Serotonin Receptor Nomenclature Committee) first expresses itself with respect to this issue. Therefore, we have rather maintained the conventions and the style of writing that were originally put forward by the authors that first published a particular invertebrate receptor. Recommendations for a uniform nomenclature for invertebrate 5-HT receptors that is fitting as closely as possible with the existing nomenclature for mammalian receptors will be discussed at the next meeting of the Serotonin Receptor Nomenclature Committee.

9. Conclusions

The period that receptors were looked upon solely as ligand binding sites lies far behind us. The isolation of the genes and/or the cDNAs encoding 5-HT receptors has provided us with a complete new set of tools to study receptors. Firstly, it has become clear that classification of a specific receptor subtype on the basis of pharmacological data does not always lead to unambiguous results. Small differences in receptor structure, sometimes as small as a difference in a single amino acid, have occasionally been shown to cause strongly divergent pharmacological profiles. Classifications based on primary receptor sequences, sometimes complemented with data on gene structure and on coupling to particular signal transduction systems, are now generally accepted as the better approach.

The availability of cDNAs encoding a great number of receptor subtypes has allowed a thorough investigation of receptor function and regulation by means of transient or stable expression in heterologous cell systems. Although these studies have greatly increased our knowledge on functional aspects of specific receptor subtypes, it is important to realize that many such aspects depend on the cell type in which the receptor is expressed. Especially the coupling to second messenger pathways can depend on the cell type used and even on the number of receptors expressed per cell. Also other processes as for instance receptor desensitization and receptor down regulation appear to be cell-type specific. These findings indicate that data obtained by the characterization of heterologously expressed receptors must always be viewed with great care and whenever possible should be corroborated by establishing that a given phenomenon also takes place in vivo, or at least in situ.

Since we have access to the genes encoding receptor subtypes, many sophisticated studies can be (and have been) designed. In situ hybridisation studies and Northern blot analysis enable thorough mapping of the cells expressing individual 5-HT receptor subtypes, especially in the brain. Furthermore, mutagenesis and modelling studies provide us with detailed knowledge of structure-function relationships of receptors. Such knowledge may benefit the search for highly subtype-specific drugs that can be used to further elucidate the physiological function(s) of the individual receptor subtypes. Another means to obtain data on the physiological significance of receptor subtypes is to generate genetically modified animals that lack (or overexpress) certain receptor subtypes. The conclusions that could be drawn from the studies of mice in which the 5-HT_{1B} or the 5-HT_{2C} receptor had been knocked out clearly demonstrates the power of this technique. It is to be expected that in the near future data on the effects of knocking out additional 5-HT receptors will become available.

With fifteen 5-HT-receptor subtypes belonging to seven subfamilies this receptor family is the largest neurotransmitter receptor family known. After a real boom in the cloning of novel 5-HT receptor subtypes in the early 1990s the last few years have not resulted in a further enlargement of the family. Nevertheless, one cannot predict with certainty that 'this is all there is'. It may very well be that the forthcoming sequencing of the complete human genome will hold some surprises in store.

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