



Review

A review of central 5-HT receptors and their function

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Abstract

It is now nearly 5 years since the last of the currently recognised 5-HT receptors was identified in terms of its cDNA sequence. Over this period, much effort has been directed towards understanding the function attributable to individual 5-HT receptors in the brain. This has been helped, in part, by the synthesis of a number of compounds that selectively interact with individual 5-HT receptor subtypes—although some 5-HT receptors still lack any selective ligands (e.g. 5-HT_{1E}, 5-HT_{5A} and 5-HT_{5B} receptors). The present review provides background information for each 5-HT receptor subtype and subsequently reviews in more detail the functional responses attributed to each receptor in the brain. Clearly this latter area has moved forward in recent years and this progression is likely to continue given the level of interest associated with the actions of 5-HT. This interest is stimulated by the belief that pharmacological manipulation of the central 5-HT system will have therapeutic potential. In support of which, a number of 5-HT receptor ligands are currently utilised, or are in clinical development, to reduce the symptoms of CNS dysfunction. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

In 1986 the pharmacology of 5-hydroxytryptamine (5-HT; serotonin) was reviewed (Bradley et al.) and the existence of three 5-HT receptor families, 5-HT_{1–3} (comprising five receptors/binding sites in total), was acknowledged although more were suspected. At the time the function of individual 5-HT receptor subtypes in the brain was largely unclear: a 5-HT autoreceptor role was ascribed to a 5-HT₁-like receptor, and it was speculated that the 5-HT₂ receptor mediated a depolarising action on CNS neurones. In the 13 years since this classification, the application of molecular biological techniques has had a major impact on the 5-HT field, allowing the discovery of many additional 5-HT receptors. These are now assigned to one of seven families, 5-HT_{1–7}, comprising a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes (for review see Hoyer et al., 1994; Fig. 1; Table 1).

Intense scrutiny has now revealed much about the functional properties of different 5-HT receptor subtypes. At the molecular level it has been established, largely using recombinant receptor models and hydrophathy profiles, that the 5-HT receptor family are mostly seven putative transmembrane spanning, G-protein coupled metabotropic receptors but one member of the family, the 5-HT₃ receptor, is a ligand-gated ion channel. In the intact brain the function of many 5-HT receptors can now be unequivocally associated with specific physiological responses, ranging from modulation of neuronal activity and transmitter release to behavioural change. The latter work in particular has benefitted considerably from the development of drug tools with 5-HT receptor subtype selectivity, some of which have now progressed to clinical application. Equally important, given that each receptor has a distinct and often limited distribution in the brain, has been the application of experimental approaches to

evaluate receptor expression at the neuroanatomical level.

In this review we outline recent developments in the knowledge of mammalian 5-HT receptor subtypes, specifically their pharmacology, CNS distribution and actions at the molecular level. However, our main focus is the function of these receptors in the brain and, when known, in the *in vivo* situation. We adhere to the current classification and nomenclature of 5-HT receptor subtypes as defined by the serotonin receptor nomenclature sub-committee of IUPHAR. Some of the

most recent changes in 5-HT receptor nomenclature are summarised in Table 2.

2. The 5-HT₁ receptor family

The initial characterisation of the 5-HT₁ receptor came from radioligand binding studies which found high affinity binding sites for [³H]-5HT in rat cortex with low affinity for spiperone (Peroutka and Snyder, 1979). Subsequent studies identified further heterogeneity within the [³H]-5-HT site, which initially accounted for the 5-HT_{1A} and 5-HT_{1B} receptors (Pedigo et al., 1981; Middlemiss and Fozard, 1983), and subsequently the 5-HT_{1C} (now 5-HT_{2C}; Pazos et al., 1984b), 5-HT_{1D} (now recognised as a combination of the species variant of the 5-HT_{1B} receptor and the closely related 5-HT_{1D} receptor; Hoyer et al., 1985a,b; Heuring and Peroutka, 1987), 5-ht_{1E} (Leonhardt et al., 1989) and 5-ht_{1F} (Amalaiky et al., 1992; Adham et al., 1993a,b) receptors. A high affinity binding site for [³H]-5-HT with novel 5-HT_{1-like} pharmacology has recently been detected in mammalian brain (Castro et al., 1997b) but has yet to be sufficiently characterised for inclusion in the 5-HT₁ receptor family. The receptors of the 5-HT₁ family have high amino acid sequence homology (Table 1; Fig. 1) and all couple negatively to adenylate cyclase via G-proteins.

The general pharmacological characteristics of the 5-HT₁ receptors was initially set out in 1986 by Bradley et al. (e.g. effects mimicked by 5-CT, blocked/mimicked by methiothepin/methysergide and not blocked by selective antagonists of the 5-HT₂ and 5-HT₃ receptors). Recently this classification has been revised to take into account several factors, specifically the unusual properties of the 5-ht_{1E} and 5-ht_{1F} receptors (low affinity for 5-CT and methiothepin), the move of the 5-HT_{1C} receptor to the 5-HT₂ receptor family (5-HT_{2C} receptor), and the discovery of additional (5-HT₄₋₇) receptors (Humphrey et al., 1993; Hoyer et al., 1994). The most recent development is a realignment of 5-HT_{1B} and 5-HT_{1D} nomenclature (Hartig et al., 1996; see later).

3. 5-HT_{1A} receptor

Following the identification of the 5-HT_{1A} binding site (Pedigo et al., 1981; Middlemiss and Fozard, 1983) knowledge of the pharmacology and function of the receptor quickly progressed. This was driven by the early identification of a selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (Hjorth et al., 1982; but now known to also agonise 5-HT₇ receptors) and the synthesis of [³H]-8-OH-DPAT and its application to provide the first pharmacological profile of the 5-HT_{1A} binding site (Gozlan et al., 1983). These initial breakthroughs, together with the discovery that buspirone and a series of

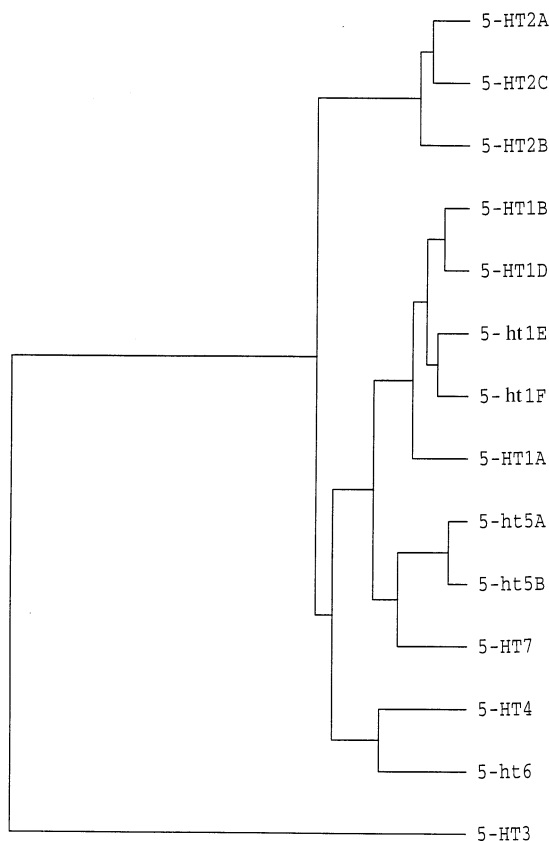


Fig. 1. Dendrogram showing the evolutionary relationship between various human 5-HT receptor protein sequences (except 5-HT_{5A} and 5-HT_{5B} receptors which are murine in origin), created using programs from the GCG package (Genetics Computer Group Inc). Multiple sequence alignments were created using a simplification of the progressive alignment method of Feng and Doolittle (1987). A distance matrix was created from the pairwise evolutionary distances between aligned sequences, expressed as substitutions per 100 amino acids. A phylogenetic tree was constructed from this distance matrix using the unweighted pair group method with arithmetic averages. 5-HT_{1A} receptor (Kobilka et al., 1987), 5-HT_{1B} receptor (Demchyshyn et al., 1992), 5-HT_{1D} receptor (Hamblin and Metcalf, 1991), 5-ht_{1E} (Zgombick et al., 1992), 5-ht_{1F} receptor (Adham et al., 1993a,b), 5-HT_{2A} receptor (Cook et al., 1994), 5-HT_{2B} receptor (Schmuck et al., 1994), 5-HT_{2C} receptor (Xie et al., 1996), 5-HT_{3As} (Hope et al., 1993), 5-HT₄ receptor van den Wyngaert et al., 1997), 5-HT_{5A} receptor (Plassat et al., 1992a,b), 5-ht_{5B} receptor (Matthes et al., 1993), 5-ht₆ receptor (Kohen et al., 1996), 5-HT₇ receptor (Bard et al., 1993).

Table 1
Comparison of the percentage amino acid identity between the different human 5-HT receptor subtypes^a

	1A	1B	1D	1E	1F	2A	2B	2C	3As	4	5A	5B	6	7
1A	100													
1B	43	100												
1D	43	63	100											
1E	40	48	48	100										
1F	42	49	49	57	100									
2A	31	31	29	34	32	100								
2B	35	27	27	30	29	45	100							
2C	32	28	30	32	33	51	42	100						
3As	<<10% ^b	<<10% ^b	<<10% ^b	<<10% ^b	<<10% ^b	<<10% ^b	<<10% ^b	<<10% ^b	100					
4	29	32	31	31	34	28	28	28	<<10% ^b	100				
5A	35	35	36	36	37	25	26	28	<<10% ^b	33	100			
5B	38	34	34	34	35	27	29	29	<<10% ^b	29	69	100		
6	34	31	32	32	32	28	27	27	<<10% ^b	27	30	32	100	
7	38	37	38	39	38	28	28	28	<<10% ^b	32	32	34	33	100

^a The percentages were created using the algorithm of Needleman and Wunsch (1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps (GCG package, Genetics Computer Group, Inc.). Sequences as for Fig. 1.

^b Due to the low percent amino acid identity, the program was unable to align the 5-HT_{3As} receptor with the other 5-HT receptor sequences.

structurally related 5-HT_{1A} ligands were anxiolytic and antidepressant in the clinic (Traber and Glaser, 1987; Robinson et al., 1990), go a long way towards explaining why the 5-HT_{1A} receptor is the best characterised of the 5-HT receptors discovered to date.

3.1. 5-HT_{1A} receptor structure

The 5-HT_{1A} receptor was the first 5-HT receptor to be fully sequenced. Both the human and rat 5-HT_{1A} receptors were identified by screening a genomic library for homologous sequences to the β_2 -adrenoceptor (Kobilka et al., 1987; Fargin et al., 1988; Albert et al., 1990). Experiments on mutated forms of the receptor have established that a single amino acid residue in the 7th transmembrane domain (Asn 385) confers the high affinity of the receptor for certain β -adrenoceptor ligands (Guan et al., 1992).

The rat 5-HT_{1A} receptor (422 amino acids) has 89% homology with the human receptor, and the gene is intronless with a tertiary structure typical of a seven transmembrane spanning protein with sites for glycosylation and phosphorylation. The human receptor is localised on chromosome 5 (5q11.2–q13).

3.2. 5-HT_{1A} receptor distribution

The distribution of the 5-HT_{1A} receptor in brain has been mapped extensively by receptor autoradiography using a range of ligands including [³H]-5-HT, [³H]-8-OH-DPAT, [³H]-ipsapirone, [¹²⁵I]-BH-8-MeO-N-PAT and more recently, [¹²⁵I]-p-MPPI and [³H]-WAY 100 635 (Pazos and Palacios, 1985; Weissmann-Nanopoulos et al., 1985; Hoyer et al., 1986; Verge et al., 1986; Radja et al., 1991; Khawaja, 1995; Kung et

al., 1995). The latter ligand has also been used for the *in vivo* labelling of 5-HT_{1A} receptors in mouse brain (Laporte et al., 1994). Recently, PET studies have used [¹¹C]-WAY100 635 to image 5-HT_{1A} receptors in the living human brain (Pike et al., 1995).

The density of 5-HT_{1A} binding sites is high in limbic brain areas, notably hippocampus, lateral septum, cortical areas (particularly cingulate and entorhinal cortex), and also the mesencephalic raphe nuclei (both dorsal and median raphe nuclei). In contrast, levels of 5-HT_{1A} binding sites in the basal ganglia and cerebellum are barely detectable.

The distribution of mRNA encoding the 5-HT_{1A} receptor is almost identical to that of the 5-HT_{1A} binding site (Chalmers and Watson, 1991; Miquel et al., 1991; Pompeiano et al., 1992; Burnet et al., 1995). The overall pattern of 5-HT_{1A} receptor distribution is simi-

Table 2
Summary of recent important changes in 5-HT receptor nomenclature

Old nomenclature		New nomenclature
Receptor	Species	
5-HT _{1B}	Rat	5-HT _{1B} ^a
5-HT _{1D}	Human, guinea pig	
5-HT _{1Dβ}	All species	
5-HT _{1Dα}	All species	5-HT _{1D}
5-HT ₂	All species	5-HT _{2A}
5-HT _D		
5-HT _{2F}	All species	5-HT _{2B}
5-HT _{1C}	All species	5-HT _{2C}

^a Species equivalent, e.g. r5-HT_{1B} for rodents and h5-HT_{1B} for humans.

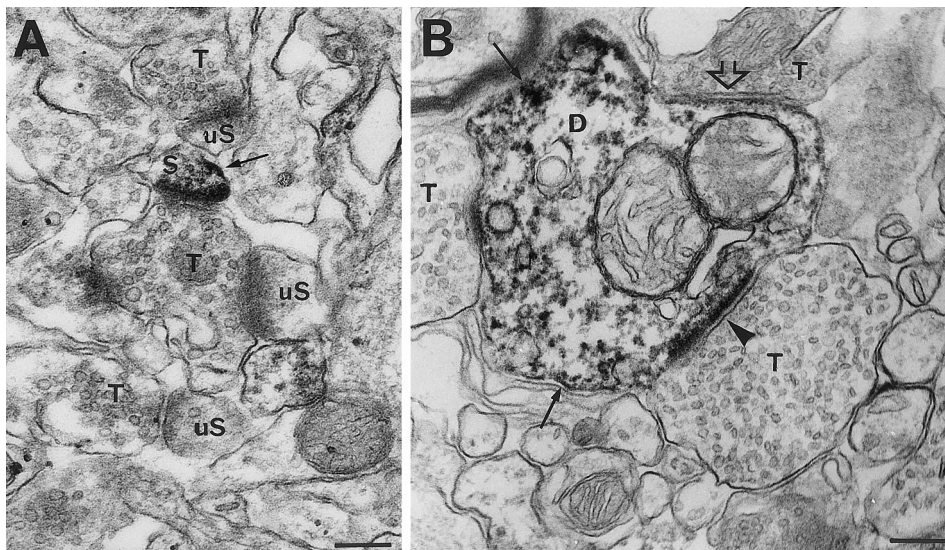


Fig. 2. Electron micrographs showing the dendritic localization of 5-HT_{1A} immunoreactivity in the rat brain. (A) In the hippocampus (dentate gyrus), immunoreactivity is found in the dendritic spines (S). The unlabelled terminal (T) contacting the spine establishes a synapse with another unlabelled spine (uS). Two other immunonegative spines are contacted by two terminals. (B) In the septal complex, immunoreactivity is mainly localised at postsynaptic membrane densities (arrowhead) facing unlabelled terminals filled with pleiomorphic vesicles (T). Another synapse is unlabelled (open arrow). Non-synaptic portions of the plasmalemmal surface also show accumulations of immunoreaction product (small arrows). This figure was kindly supplied by Dr Daniel Vergé, Université Pierre et Marie Curie, Paris.

lar across species although the laminar organisation of the 5-HT_{1A} receptor in cortical and hippocampal areas of humans differs somewhat from that in the rodent (Burnet et al., 1995).

It is clear that 5-HT_{1A} receptors are located both postsynaptic to 5-HT neurones (in forebrain regions), and also on the 5-HT neurones themselves at the level of the soma and dendrites in the mesencephalic and medullary raphe nuclei. This is evident from studies on the effects of neuronal lesions on the abundance of 5-HT_{1A} binding sites and mRNA, and more recently studies of the cellular localisation of the 5-HT_{1A} receptor using immunocytochemistry (see Miquel et al., 1991, 1992; Radja et al., 1991).

At the cellular level, *in situ* hybridisation and immunocytochemical studies demonstrate the presence of 5-HT_{1A} receptors in cortical pyramidal neurones as well as pyramidal and granular neurones of hippocampus (Pompeiano et al., 1992; Burnet et al., 1995; see also Francis et al., 1992). In addition, the 5-HT_{1A} receptor is expressed in 5-HT-containing neurones in the raphe nuclei, cholinergic neurones in the septum and probably glutamatergic (pyramidal) neurones in cortex and hippocampus (Francis et al., 1992; Kia et al., 1996a). A recent detailed study of the ultrastructural location of the 5-HT_{1A} receptor reports evidence that the receptor is present at synaptic membranes, as well as extrasynaptically (Kia et al., 1996b; Fig. 2). Although there are reports of 5-HT_{1A} receptors in brain glial cells (Azmitia et al., 1996) this has not been confirmed in other studies (Burnet et al., 1995; Kia et al., 1996a,b).

3.3. 5-HT_{1A} receptor pharmacology

The pharmacological characteristics of the 5-HT_{1A} receptor clearly set it apart from other members of the 5-HT₁ family and indeed other 5-HT receptors (Hoyer et al., 1994). Selective 5-HT_{1A} receptor agonists include 8-OH-DPAT, dipropyl-5-CT, and gepirone. A number of 5-HT_{1A} ligands, including BMY 7378, NAN-190, MDL 73005 EF, SDZ 216525 were identified as clear antagonists in various models of postsynaptic 5-HT_{1A} receptor function. However, as a group these drugs are not that selective and also demonstrate partial agonist properties (Hjorth and Sharp, 1990; Sharp et al., 1990, 1993; Fletcher et al., 1993a,b; Hoyer and Boddeke, 1993; Schoeffter et al., 1997), which complicates their use as 5-HT_{1A} receptor probes.

After a long search, and during which time various non-selective compounds were the only available antagonist tools (propranolol, spiperone, pindolol), a number of silent 5-HT_{1A} receptor antagonists have now been developed. These include (S)-UH-301, WAY 100 135, WAY 100 635 (Hillver et al., 1990; Björk et al., 1991; Fletcher et al., 1993a,b, 1996) and most recently, NAD-299 (Johansson et al., 1997). WAY 100 635 is the most potent of this group although NAD-299 appears to be somewhat more selective (Fletcher et al., 1996; Johansson et al., 1997). Recent data indicate that WAY 100 135 has partial agonist properties, at least in some models (Davidson et al., 1997; Schoeffter et al., 1997).

Characterisation of putative 5-HT_{1A} receptor-mediated responses has been aided by 5-HT₁ receptor/β-

adrenoceptor antagonists such as pindolol, penbutolol and tertatolol (Tricklebank et al., 1984; Hjorth and Sharp, 1993; Prisco et al., 1993). However, recent data suggest that as a group, these drugs have varying degrees of efficacy at 5-HT_{1A} receptors (pindolol > tertatolol > penbutolol = WAY 100 635; Sanchez et al., 1996; Clifford et al., 1998). The putative partial agonist properties of pindolol at the 5-HT_{1A} receptor may be relevant to the current controversy regarding its use in the adjunctive treatment of depression (see later).

3.4. Functional effects mediated via the 5-HT_{1A} receptor

3.4.1. Second messenger responses

The 5-HT_{1A} receptor couples negatively via G-proteins (α_i) to adenylate cyclase in both rat and guinea pig hippocampal tissue and cell-lines (pituitary GH4C1 cells, COS-7 cells, HeLa cells) stably expressing the cloned 5-HT_{1A} receptor (for reviews see Boess and Martin, 1994; Saudou and Hen, 1994; Albert et al., 1996). In hippocampal tissue (under forskolin- or VIP-stimulated conditions) the rank order of potency of a large number of agonists and antagonists correlates with their affinity for the 5-HT_{1A} binding site (De Vivo and Maayani, 1986; Schoeffter and Hoyer, 1988). Interestingly, despite the high density of 5-HT_{1A} receptors in the dorsal raphe, 5-HT_{1A} receptors do not appear to couple to the inhibition of adenylate cyclase in this region (Clarke et al., 1996).

There are reports of the positive coupling of the 5-HT_{1A} receptor to adenylate cyclase in hippocampal tissue (Shenker et al., 1983; Markstein et al., 1986; Fayolle et al., 1988). However, given similarities between the pharmacology of the 5-HT_{1A} and newer 5-HT receptors (5-HT₇ in particular), it is a possibility that these effects have been inadvertently attributed to the wrong receptor or a combination of receptors.

Other effects of the 5-HT_{1A} receptor in transfected cell-lines include a decrease in intracellular Ca²⁺, activation of phospholipase C and increased intracellular Ca²⁺ (for review see Boess and Martin, 1994; Albert et al., 1996) although as yet there is no firm evidence for such coupling in brain tissue. Experiments utilising antisense constructs targetted against specific G-protein subunits, suggest that the biochemical basis for these diverse effects of the 5-HT_{1A} receptor may reside in both the G-protein complement of the particular cells under investigation but also the particular isoforms of the effector enzymes being expressed (Albert et al., 1996).

The 5-HT_{1A} receptor is reported to induce the secretion of a growth factor (protein S-100) from primary astrocyte cultures (Azmitia et al., 1996), and increase markers of growth in neuronal cultures (Riad et al., 1994). These and other results raise the exciting possi-

bility that the 5-HT_{1A} receptor has a neurotrophic role in the developing brain, and even possibly in the adult (Riad et al., 1994; Azmitia et al., 1996; Yan et al., 1997).

3.4.2. Electrophysiological responses

Electrophysiological experiments have established that 5-HT_{1A} receptor activation causes neuronal hyperpolarisation, an effect mediated through the G-protein-coupled opening of K⁺ channels, and without the involvement of diffusible intracellular messengers such as cAMP (for review see Nicoll et al., 1990; Aghajanian, 1995).

3.4.2.1. Hippocampus and other forebrain regions.

5-HT_{1A} receptor agonists and 5-HT itself inhibit neuronal activity in rat hippocampus, frontal cortex and other brain areas when administered iontophoretically in vivo (e.g. Segal, 1975; De Montigny et al., 1984; Sprouse and Aghajanian, 1988; Ashby et al., 1994a,b). When bath applied to brain slices, 5-HT_{1A} receptor agonists (including 5-HT and 8-OH-DPAT) hyperpolarise neurons in all regions studied so far, including hippocampus, septum and frontal cortex (Andrade and Nicoll, 1987; Araneda and Andrade, 1991; Van den Hooff and Galvan, 1991, 1992; Corradetti et al., 1996). This inhibitory effect is blocked by both non-selective (spiperone and methiothepin) and selective (WAY 100 635) 5-HT_{1A} receptor antagonists.

Compared to 5-HT and 8-OH-DPAT, a number of high affinity 5-HT_{1A} receptor ligands, including MDL 73005 EF, BMY 7378 and buspirone, have low efficacy in specific forebrain regions (Andrade and Nicoll, 1987; Van Den Hooff and Galvan, 1991, 1992) despite behaving as full agonists in the dorsal raphe nucleus (see below). These data are often explained by evidence that these drugs are partial agonists, and that 5-HT_{1A} receptor reserve in the hippocampus and other forebrain regions is low compared to the dorsal raphe nucleus (Meller et al., 1990a,b; Yocca et al., 1992). The pre- and postsynaptic 5-HT_{1A} receptors, however, are different in a number of respects (including regulatory and pharmacological properties as summarised by Clarke et al. (1996)). Although no single difference constitutes convincing evidence for 5-HT_{1A} receptor subtypes, such a discovery would not come as a great surprise. The recent development of a 5-HT_{1A} receptor knockout mouse (Parks et al., 1998) may reveal definitive evidence for the existence of more than one 5-HT_{1A} receptor type.

3.4.2.2. Dorsal raphe nucleus.

Earlier electrophysiological studies demonstrated that systemically and locally applied LSD caused a marked inhibition of 5-HT cell firing in the rat dorsal raphe nucleus (Aghajanian, 1972; Haigler and Aghajanian, 1974). Subsequent studies

found that a wide range of 5-HT_{1A} receptor agonists, including, 8-OH-DPAT and buspirone, have the same

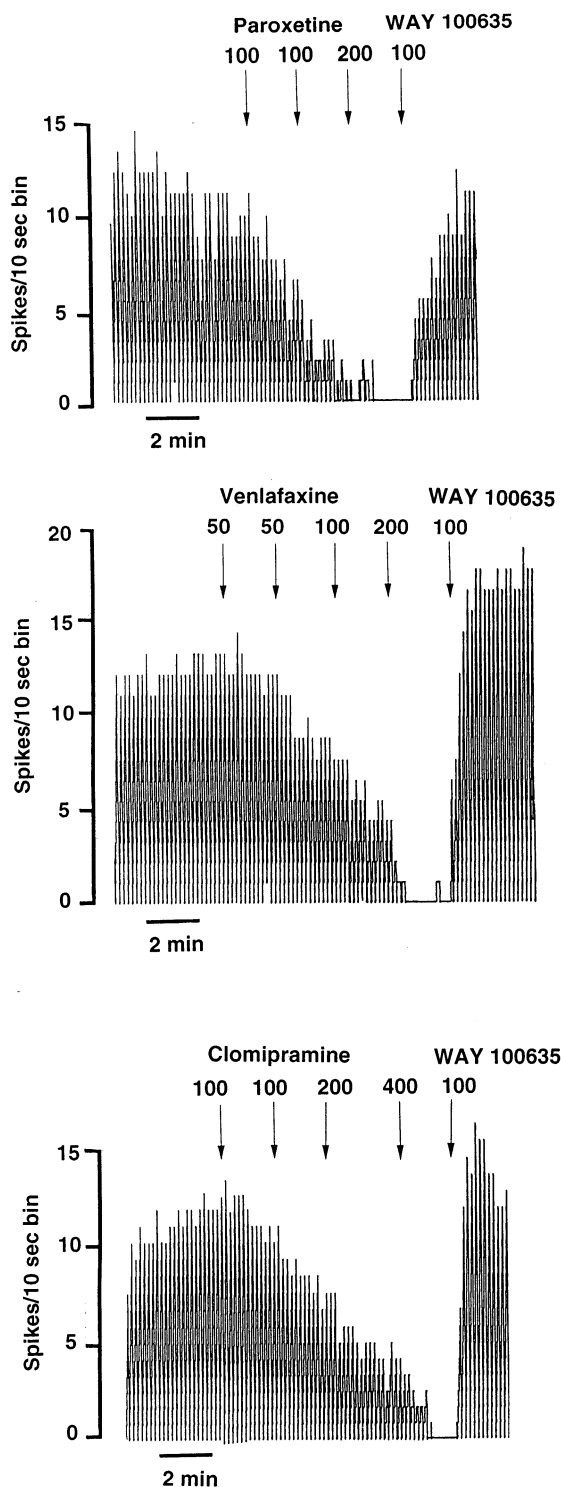


Fig. 3. The inhibition of 5-HT neuronal activity by antidepressant drugs (venlafaxine, clomipramine and paroxetine) and its reversal by a selective 5-HT_{1A} receptor antagonist (WAY 100635). Individual 5-HT neurones were monitored in the dorsal raphe nucleus of the anaesthetised rat using extracellular recording techniques. Drugs were injected at the doses (mg/kg i.v.) and time points indicated. The data are taken from Gartside et al. (1997b).

effect, and that this is blocked by non-selective (spiperone and propranolol) and selective (WAY 100635, WAY 100135 and (S)-UH-301) 5-HT_{1A} receptor antagonists (e.g. Sprouse and Aghajanian, 1988; Arborelius et al., 1994; Craven et al., 1994; Corradetti et al., 1996). Selective 5-HT_{1A} receptor antagonists also reverse the inhibition of 5-HT cell firing induced by both 5-HT itself as well as indirect 5-HT agonists such as 5-HT reuptake inhibitors and 5-HT releasing agents (Craven et al., 1994; Hajós et al., 1995; Gartside et al., 1995, 1997a,b; Fig. 3).

Partial 5-HT_{1A} agonists such as MDL 73005 EF, NAN-190 and SDZ 216525 all inhibit 5-HT cell firing and these effects can be reversed by 5-HT_{1A} receptor antagonists (Sprouse, 1991; Greuel and Glaser, 1992; Lanfumey et al., 1993; Fornal et al., 1994; Munday et al., 1994). Methiothepin, metergoline and methysergide also inhibit 5-HT cell firing (Haigler and Aghajanian, 1974), and this may be due to 5-HT_{1A} receptor activation although α_1 -adrenoceptor blockade may be a contributing factor.

Reports on the effects of WAY 100635 administered alone on 5-HT cell firing are somewhat inconsistent although slight increases have been detected in anaesthetised animals in some studies (Gartside et al., 1995; Munday et al., 1996). However, WAY 100635 seems to have a more clear-cut stimulatory effect on 5-HT cell firing in cats in the active-awake state (Fornal et al., 1996). Therefore, the 5-HT_{1A} autoreceptor appears to be under physiological tone, at least in some conditions.

There is electrophysiological evidence that 5-HT neurones in the dorsal raphe nucleus are more sensitive to 5-HT_{1A} receptor agonists than 5-HT neurones in the median raphe nucleus (Sinton and Fallon, 1988; Blier et al., 1990), although other data do not confirm this (VanderMaelen and Braselton, 1990; Hajós et al., 1995; Gartside et al., 1997a,b). However, recent microdialysis studies report that there are regional differences in the inhibitory effect of 5-HT_{1A} receptor agonists on 5-HT release (Casanovas and Artigas, 1996; McQuade and Sharp, 1996). This suggests that 5-HT_{1A} autoreceptor control may differ between individual 5-HT pathways although the relevance of this to changes in 5-HT cell firing, is not yet clear.

Although the 5-HT_{1A} agonist-induced inhibition of 5-HT cell firing in vivo is generally perceived to reflect a direct action in raphe, recent data raise the possibility of an involvement of postsynaptic 5-HT_{1A} autoreceptors (Ceci et al., 1994; Jolas et al., 1995; Hajós et al., 1999). This issue warrants further investigation since there is potential for the involvement of postsynaptic 5-HT_{1A} autoreceptors, previously attributed to the somatodendritic 5-HT_{1A} autoreceptors.

3.5. 5-HT release

In accordance with the electrophysiological data, microdialysis studies show that 5-HT_{1A} receptor agonists induce a fall in release of 5-HT in the forebrain of the rat *in vivo*, an effect which involves activation of the raphe 5-HT_{1A} autoreceptor (for review see Sharp and Hjorth, 1990). Thus, many 5-HT_{1A} receptor agonists, the low efficacy 5-HT_{1A} receptor ligands (e.g. NAN-190, BMY 7378, SDZ 216525) cause a fall in 5-HT output in the dialysis experiments and these effects are blocked by selective 5-HT_{1A} receptor antagonists (Sharp and Hjorth, 1990; Fletcher et al., 1993a,b; Hjorth et al., 1995; Sharp et al., 1996).

In microdialysis studies, selective 5-HT_{1A} receptor antagonists (specifically WAY 100635, WAY 100135 and (S)-UH-301) do not by themselves consistently increase 5-HT release in either anaesthetised or awake conditions (e.g. Nomikos et al., 1992; Routledge et al., 1993; Sharp et al., 1996). The 5-HT₁ receptor/ β -adrenoceptor antagonists penbutolol and tertatolol, increase 5-HT release in the rat (Hjorth and Sharp, 1993; Assie and Koek, 1996) but a contribution of 5-HT_{1B} receptor blockade to these effects cannot be ruled out.

Recent microdialysis studies have established that 5-HT_{1A} receptor antagonists facilitate the effect of 5-HT reuptake inhibitors, monoamine oxidase inhibitors and certain tricyclic antidepressant drugs on 5-HT release (e.g. Invernizzi et al., 1992; Hjorth, 1993; Gartside et al., 1995; Artigas et al., 1996; Romero et al., 1996; Sharp et al., 1997). This interaction probably relates to the fact that the 5-HT_{1A} receptor antagonists prevent the inhibitory effect of the antidepressants on 5-HT cell firing (Gartside et al., 1995, 1997a,b; Sharp et al., 1997; see Fig. 3). Recent clinical studies have reported evidence that the therapeutic effect of antidepressant drugs can be improved by the adjunctive treatment with pindolol (Artigas et al., 1996) although this has not been confirmed in other studies (Berman et al., 1997; McAskill et al., 1998). Given evidence that pindolol has partial 5-HT_{1A} receptor agonist properties (Sanchez et al., 1996; Clifford et al., 1998), trials with antagonists lacking efficacy may be important.

3.6. Acetylcholine release

8-OH-DPAT increases the release of acetylcholine in the cortex and hippocampus of guinea pigs and rats (Bianchi et al., 1990; Izumi et al., 1994; Wilkinson et al., 1994; Consolo et al., 1996). This effect is blocked by both selective (WAY 100635) and non-selective (methiothepin, propranolol and NAN-190) 5-HT_{1A} receptor antagonists (Bianchi et al., 1990; Wilkinson et al., 1994; Consolo et al., 1996), and appears to involve postsynaptic 5-HT_{1A} receptors (Consolo et al., 1996).

Although the exact location of the postsynaptic 5-HT_{1A} receptors modulating acetylcholine release is not entirely clear, it probably does not involve an action at the cholinergic nerve terminal (Bianchi et al., 1990; Wilkinson et al., 1994; but see Izumi et al., 1994). Recent studies indicate that 5-HT_{1A} receptors are located on cholinergic cell bodies in the septum (Kia et al., 1996a) which probably project to cortical areas, and hippocampus in particular. However, since 5-HT_{1A} receptors are inhibitory in the septum (Van Den Hooff and Galvan, 1992), it is not easy to understand how activation of these receptors could bring about an increase in acetylcholine release from septohippocampal neurones.

3.7. Noradrenaline release

Microdialysis studies in the awake rat demonstrate that 8-OH-DPAT increases the release of noradrenaline in many brain areas including the hypothalamus, hippocampus, frontal cortex and ventral tegmental area (Done and Sharp, 1994; Chen and Reith, 1995; Suzuki et al., 1995). This effect is blocked by WAY 100135 and WAY 100635 (Suzuki et al., 1995; Hajós-Korcsok and Sharp, 1996). Other 5-HT_{1A} ligands also increase noradrenaline, including buspirone, NAN-190 and MDL 73005EF and in each case the effect is probably mediated by 5-HT_{1A} receptor activation (Done and Sharp, 1994; Hajós-Korcsok et al., 1999).

The noradrenaline response to administration of 5-HT_{1A} receptor agonists is still present in rats pretreated with either a 5-HT neurotoxin (Suzuki et al., 1995) or a 5-HT synthesis inhibitor (Chen and Reith, 1995; Hajós-Korcsok et al., 1999), indicating the involvement of 5-HT_{1A} receptors located postsynaptically. Interestingly, 5-HT_{1A} receptor agonists induce a striking expression of the intermediate-early gene, *c-fos*, in the locus coeruleus which is the main source of the ascending noradrenergic projections (Hajós-Korcsok and Sharp, 1999). Since 5-HT_{1A} receptor levels in the locus coeruleus are low, the 5-HT_{1A} receptor agonists may stimulate noradrenergic activity via an action on locus coeruleus afferents.

There are interesting parallels between the effect of 8-OH-DPAT on noradrenaline and its effect on acetylcholine (see above). One can speculate that the 5-HT_{1A} receptor has an important role in mediating the influence of 5-HT on both noradrenergic and cholinergic pathways. This would provide a route for the modulation by 5-HT of brain functions in which both noradrenaline and acetylcholine have a recognised role (e.g. attention, mood and cognition).

3.7.1. Behavioural and other physiological responses

In the rat, administration of 8-OH-DPAT and other 5-HT_{1A} receptor agonists causes a wide range of be-

Table 3
Summary of the functional responses associated with activation of the brain 5-HT_{1A} receptor

Level	Response	Mechanism
Cellular	Adenylate cyclase (–)	Post
Electrophysiological	Hyperpolarisation	Post
Behavioural	5-HT syndrome	Post
	Hypothermia	Pre/post
	Hyperphagia	Pre
	Anxiolysis	Pre/post
	Sexual behaviour (+)	Pre/post
Neurochemical	Discriminative stimulus	Pre/post
	5-HT release (–)	Pre
	Noradrenaline release (+)	Post
	Acetylcholine release (+)	Post
Neuroendocrine	Glutamate release (–)	?
	ACTH (+)	Post
	Prolactin (+)	Post

havioural and physiological effects including the induction of the 5-HT behavioural, hyperphagia, hypothermia, altered sexual behaviour and a tail flick response (for review see Green and Grahame-Smith, 1976; Green and Heal, 1985; Glennon and Lucki, 1988; Millan et al., 1991; Lucki, 1992). 5-HT_{1A} receptor agonists also induce a strong discriminative stimulus (Glennon and Lucki, 1988). In addition, there is a large literature of basic and clinical data attesting to the anxiolytic and antidepressant activity of 5-HT_{1A} receptor agonists (Traber and Glaser, 1987; Charney et al., 1990; Handley, 1995).

Whilst the involvement of the 5-HT_{1A} receptors in many of these responses is clear, particularly on the basis of more recent studies with selective 5-HT_{1A} receptor antagonists (e.g. Fletcher et al., 1993a,b, 1996), in some cases controversy exists regarding the involvement of pre- (5-HT_{1A} autoreceptors) or postsynaptic mechanisms. The 5-HT behavioural syndrome is undoubtedly mediated via activation of postsynaptic 5-HT_{1A} receptors (Tricklebank et al., 1984; Lucki, 1992) and this also seems to be the case for the tail flick response which is mediated spinally (Bervoets et al., 1993). A role for the presynaptic 5-HT_{1A} receptor (autoreceptor) in the hyperphagia response seems likely on the basis of several lines of evidence but particularly experiments demonstrating that intra-raphe injection of 5-HT_{1A} receptor agonists induces this effect (see Simansky, 1996). Studies of the mechanisms underlying the anxiolytic properties of 5-HT_{1A} receptor agonists tend to favour a presynaptic action, at least in some models, although an involvement of postsynaptic mechanisms cannot be ruled out (for review see Handley, 1995; De Vry, 1995; Jolas et al., 1995).

In rats, intra-raphe injection of 5-HT_{1A} receptor agonists also evokes hypothermia (Higgins et al., 1988; Hillegaart, 1991), however inhibition of 5-HT synthesis

and 5-HT lesions do not prevent hypothermia when the agonists are injected systemically (Bill et al., 1991; O'Connell et al., 1992; Millan et al., 1993). In comparison, in the mouse, 5-HT lesions abolish the hypothermic response to 5-HT_{1A} receptor agonists (Goodwin et al., 1985; Bill et al., 1991). Therefore, there appears to be a species difference in the mechanism underlying the hypothermic effect of 5-HT_{1A} receptor agonists; in the mouse it appears presynaptic, whereas in the rat it can be mediated via both pre- and postsynaptic mechanisms (presumably involving different neural circuits). Both pre- and postsynaptic mechanisms also seem to be able to mediate the discriminative stimulus effects of 5-HT_{1A} receptor agonists (Schreiber and De Vry, 1993).

Recently, there has been an interest in the cognition-enhancing properties of 5-HT_{1A} antagonists on the basis of evidence that learning impairments induced in rats and primates by cholinergic antagonists or lesions can be reversed by WAY 100135 and WAY 100635 (Carli et al., 1995; Harder et al., 1996; Carli et al., 1997). This action of the antagonists is currently interpreted as being mediated through blocking a 5-HT_{1A}-mediated inhibitory input to cortical pyramidal neurones which compensates for the loss of an excitatory cholinergic input. Findings from microdialysis studies that application of WAY 100135 to the cortex increases glutamate release in the striatum (Dijk et al., 1995) is taken as evidence that blockade of 5-HT_{1A} receptors activates cortical pyramidal neurones (in this case, specifically corticostriatal neurones). However, recent electrophysiological recordings of cortical neurones in awake rats have failed to confirm this idea (Hajós et al., 1998).

Neuroendocrine studies in rats have found that 5-HT_{1A} receptor agonists cause an elevation of plasma ACTH, corticosteroids and prolactin (e.g. Gilbert et al., 1988; Gartside et al., 1990), and in man there is also increased secretion of growth hormone (Cowen et al., 1990). Both the animal and human work shows that these neuroendocrine responses are blocked by 5-HT_{1A} receptor antagonists (Gilbert et al., 1988; Cowen et al., 1990; Gartside et al., 1990; Critchley et al., 1994). Data showing that the ACTH response is intact in rats with 5-HT lesions suggests that it is mediated by postsynaptic 5-HT_{1A} receptors (Fuller, 1996).

The functional effects associated with activation of central 5-HT_{1A} receptors are summarised in Table 3.

4. 5-HT_{1B} receptor

The 5-HT_{1B} receptor was initially characterised as a [³H]-5HT binding site with low affinity for spiperone in rodent brain tissue (Pedigo et al., 1981). The finding that this site had low affinity for 8-OH-DPAT established that this receptor had pharmacological properties

different from the 5-HT_{1A} (and 5-HT₂) sites (Middlemiss and Fozard, 1983). Another binding site for [³H]-5HT was detected in bovine brain, and was originally classified as a 5-HT_{1D} site on the basis of it being pharmacology distinguishable from the rodent 5-HT_{1B} site (Heuring and Peroutka, 1987). It is now generally accepted that the originally defined 5-HT_{1D} site is in fact a species variant of the 5-HT_{1B} receptor (Hartig et al., 1996; Table 2).

4.1. 5-HT_{1B} receptor structure

The 5-HT_{1B} binding site was found in high levels in rodents (rat, mouse, hamster) while the 5-HT_{1D} site was high in other species (calf, guinea pig, dog, human). The fact that the CNS distributions of the originally defined 5-HT_{1B} and 5-HT_{1D} sites were similar led some workers to speculate at an early stage (i.e. prior to cloning data) that the two sites were species equivalents which displayed different pharmacology (Hoyer and Middlemiss, 1989).

This initial idea (which turned out to be correct) became complicated by the discovery of two related human receptor genes which were isolated on the basis of their sequence homology with an orphan receptor (dog RDC4) with 5-HT₁ receptor-characteristics. When expressed these two genes demonstrated the pharmacology of the originally described 5-HT_{1D} site, and not the rodent 5-HT_{1B} site, and they were designated 5-HT_{1D α} and 5-HT_{1D β} (77% sequence homology in the transmembrane domain) (see Hartig et al., 1992 for review). However, the rodent 5-HT_{1B} receptor was eventually cloned (Voigt et al., 1991; Adham et al., 1992; Maroteaux et al., 1992) and found to have high sequence homology (96% homology in the transmembrane domains) with the human 5-HT_{1D β} receptor (Jin et al., 1992).

These findings, together with the discovery of a rat gene homologous to the human 5-HT_{1D α} receptor and which encoded a receptor with a 5-HT_{1D} binding site profile (Hamblin et al., 1992a,b), has led to a recent reassessment of the nomenclature for the 5-HT_{1B/D} receptors (Hartig et al., 1996; Table 2). This nomenclature change recognised that despite differing pharmacology, the human 5-HT_{1D β} receptor is a species equivalent of the rodent 5-HT_{1B} receptor. Therefore, the 5-HT_{1D β} receptor was realigned to the 5-HT_{1B} classification (Table 2). To take account of the fact that the pharmacology of the 5-HT_{1B} receptor shows significant differences across species, prefixes are used to denote species specific 5-HT_{1B} receptors: the rat becomes r5-HT_{1B} and the human becomes h5-HT_{1B}. Advice on prefix nomenclature for other species is given by Vanhoutte et al. (1996). The genes encoding the mouse and human 5-HT_{1B} receptors are located on chromosome 9 (position 9E) and 6 (6q13), respectively (see Saudou and Hen, 1994).

With the recent realignment, the 5-HT_{1D α} receptor expressed in the rat and human, and other species, became the 5-HT_{1D} receptor. It is important to note that the latter receptor is expressed in very low amounts in the brain (see Section 5). Moreover, the vast majority of functional responses to attributed to the 5-HT_{1D} receptor prior to the nomenclature realignment, now needs reappraisal. It seems likely that most, if not all, of these responses were mediated by the 5-HT_{1B} receptor.

4.2. 5-HT_{1B} receptor distribution

Autoradiographic studies using [³H]-5-HT (in the presence of 8-OH-DPAT), [¹²⁵I]-cyanopindolol (in the presence of isoprenaline) or [¹²⁵I]-GTI (serotonin-5-*O*-carboxymethyl-glycyl-[¹²⁵I]tyrosinamide) demonstrate a high density of 5-HT_{1B} sites in the rat basal ganglia, (particularly the substantia nigra, globus pallidus, ventral pallidum and entopeduncular nucleus), but also many other regions (Pazos et al., 1985; Verge et al., 1986; Bruinvels et al., 1993). With appropriate displacing agents, both [¹²⁵I]-cyanopindolol and [¹²⁵I]-GTI allow discrimination of 5-HT_{1B} binding sites from 5-HT_{1D} binding sites in rodents but currently there are no selective radioligands that allow this in non-rodent species. The discrimination of 5-HT_{1B} and 5-HT_{1D} receptors in both rodent and non-rodent species looks likely to become much more straight forward with the availability of a new 5-HT_{1B/D} radioligand, [³H]-GR-125 743 (Doménech et al., 1997) as well as cold ligands which discriminate 5-HT_{1B} and 5-HT_{1D} receptors (see below).

Evidence from radioligand binding experiments using 5-HT neuronal lesions is equivocal regarding the synaptic location of the rat 5-HT_{1B} receptor, with some studies finding that the lesion causes an upregulation of 5-HT_{1B} binding sites and others finding a downregulation in the same areas (see Middlemiss and Hutson, 1990; Bruinvels et al., 1994a,b for review). However, in situ hybridisation studies (Voigt et al., 1991; Boschert et al., 1994; Bruinvels et al., 1994a,b; Doucet et al., 1995) have located mRNA encoding the 5-HT_{1B} receptor in the dorsal and median raphe nuclei. Furthermore, 5-HT_{1B} receptor mRNA in the raphe nuclei is markedly reduced by a 5-HT neuronal lesion (Doucet et al., 1995).

Some forebrain areas with high levels of 5-HT_{1B} binding sites (e.g. striatum) also express 5-HT_{1B} receptor mRNA. However, other areas with high levels of 5-HT_{1B} binding sites have little detectable mRNA (e.g. substantia nigra, globus pallidus and entopeduncular nucleus). Similar mismatches between brain distribution of 5-HT_{1B} receptor mRNA and binding sites have been found in the primate and human brain (Jin et al., 1992).

Together, these data suggest that 5-HT_{1B} receptors are located with presynaptically and postsynaptically

relative to the 5-HT neurones. It is speculated that in some brain areas (including substantia nigra and globus pallidus), 5-HT_{1B} binding sites may be located on non-5-HT nerve terminals, having been synthesised and then transported from cell bodies in other regions (see Boschert et al., 1994; Bruinvels et al., 1994a,b). Overall, the anatomical location of the 5-HT_{1B} receptor provides strong evidence to support the idea that the 5-HT_{1B} receptor has a role as both a 5-HT autoreceptor and 5-HT heteroreceptor, i.e. controlling transmitter release (see below).

At the cellular level, *in situ* hybridisation studies have localised 5-HT_{1B} receptor mRNA to granule and pyramidal cells within hippocampus (Doucet et al., 1995), and medium spiny neurones of the caudate putamen which are probably GABAergic (Boschert et al., 1994). Immunocytochemical studies are now necessary to reveal the synaptic location of the receptors.

4.3. 5-HT_{1B} receptor pharmacology

There are a large number of available ligands with high affinity for the 5-HT_{1B} receptor but most are not selective (see Hoyer et al., 1994 for review). The most potent agonists include L-694247, RU 24969, 5-CT and CP 93129; methiothepin is a potent antagonist. Although as a group these compounds have affinity for other 5-HT receptor subtypes (particularly 5-HT_{1A}), the low affinity for 5-HT_{1B} sites of drugs such as 8-OH-DPAT, WAY 100635, ritanserin and tropisetron, aids the discrimination of the 5-HT_{1B} receptor. However, the compound GR 127935 has high selectivity for 5-HT_{1B/1D} versus other 5-HT receptors and is a potent antagonist in functional models (Skingle et al., 1995). Recently, the first antagonists, SB-224289 and SB-216641, with high affinity and selectivity for the 5-HT_{1B} over the 5-HT_{1D} receptor were reported (Price et al., 1997; Roberts et al., 1997a). These drug tools are going to be essential for future studies aiming to characterise the function of 5-HT_{1B} or 5-HT_{1D} receptors.

Despite their high sequence homology and similar brain distribution, the rat and mouse 5-HT_{1B} receptors are pharmacologically distinct from the human (Hamblin et al., 1992a,b). The most striking difference is that certain β -adrenoreceptor antagonists including cyanopindolol, SDZ 21009, isamoltane, pindolol and propranolol have higher affinity for the 5-HT_{1B} receptor in the rodent than human (see Boess and Martin, 1994). This difference can be accounted for by a single amino acid difference in the putative 7th transmembrane region at position 355 where it is asparagine for the rat and threonine for the human (Metcalf et al., 1992; Oksenberg et al., 1992; Parker et al., 1993).

The most difficult problem at present is discriminating between human 5-HT_{1B} and 5-HT_{1D} receptors. Despite early evidence to the contrary (Weinshank et al.,

1992), a number of studies now indicate that there are clear differences in the pharmacology of these receptors. In particular the 5-HT₂ receptor antagonists, ketanserin and ritanserin, show selectivity (15–30-fold) for the human 5-HT_{1D} versus 5-HT_{1B} receptor (Kauermann et al., 1994; Pauwels et al., 1996). More recently, the first antagonists with selectivity (at least 25-fold) for the human 5-HT_{1B} (SB-216641, SB-224289) and human 5-HT_{1D} (BRL-15572) receptors have been developed (Price et al., 1997; Roberts et al., 1997a).

4.4. Functional effects mediated via the 5-HT_{1B} receptor

4.4.1. Second messenger responses

Studies on cells transfected with either the rat or human 5-HT_{1B} receptor have established that the receptors couple negatively to adenylate cyclase under forskolin-stimulated conditions (Adham et al., 1992; Levy et al., 1992a; Weinshank et al., 1992). A receptor with 5-HT_{1B} receptor pharmacology and negatively coupled to adenylate cyclase, has also been detected in the rat and calf substantia nigra (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989). Recent studies (Pauwels et al., 1997; Roberts et al., 1997a) using increased binding of [³⁵S]-GTP γ S as a measure of ligand efficacy at recombinant h5-HT_{1B} and h5-HT_{1D} receptors, have revealed that a number of compounds (methiothepin, ketanserin, SB-224289) demonstrate inverse agonist properties in this model. Moreover, GR 127935 is a potent but partial 5-HT_{1B} receptor agonist in some functional tests ([³⁵S]-GTP γ S and cAMP accumulation) using recombinant receptors (Pauwels et al., 1996; Price et al., 1997) although little evidence for this has yet come out in models based on native receptors.

4.4.2. 5-HT_{1B} autoreceptors

There is now convincing evidence that the 5-HT_{1B} receptor functions as a 5-HT autoreceptor at the 5-HT nerve terminal (for review see Middlemiss and Hutson, 1990; Bühlen et al., 1996). *In vitro* 5-HT release studies using rat brain tissue demonstrate that there is a strong correlation between the potency with which 5-HT receptor agonists inhibit 5-HT release, and their affinity for the rat 5-HT_{1B} binding site. A similar correlation holds for the potency with which antagonists block the receptor (Middlemiss, 1984; Engel et al., 1986; Limberger et al., 1991). It is also clear that the pharmacology of the nerve terminal 5-HT autoreceptor in the human fits that of a 5-HT_{1B} receptor (5-HT_{1D β} in old nomenclature) (Galzin et al., 1992; Maura et al., 1993; Fink et al., 1995). In addition, evidence that the pharmacology of the 5-HT autoreceptor in the guinea pig matches that of a 5-HT_{1B} receptor has recently been reported (Bühlen et al., 1996). Recent experiments demonstrate that 5-HT agonist-induced inhibition of

5-HT release in both the guinea pig and human cortex *in vitro*, is reversed by the 5-HT_{1B} selective antagonist, SB-216641 but not the 5-HT_{1D} selective antagonist, BRL-15572 (Schlicker et al., 1997; Fig. 4).

Microdialysis studies show that drugs with 5-HT_{1B} receptor agonist properties such as 5-CT, RU 24969, and CP 93129, which inhibit 5-HT release *in vitro*, all cause a fall in 5-HT release in the rat and guinea pig brain *in vivo* (Sharp et al., 1989; Hjorth and Tao, 1991; Lawrence and Marsden, 1992; Martin et al., 1992). Although the pharmacology underlying these effects is complicated by the fact that the drugs are not selective, the involvement of the terminal 5-HT_{1B} autoreceptor seems likely given the fact that the effects occur follow-

ing local perfusion in the terminal regions and can be antagonised by methiothepin.

Recent data suggest that 5-HT_{1B} receptor antagonists by themselves increase 5-HT release *in vivo* although the region of study may be important. A number of microdialysis studies have found that GR 127935 does not increase 5-HT in frontal cortex (Hutson et al., 1995; Skingle et al., 1995; Sharp et al., 1997), but this drug was found to increase 5-HT in hypothalamus (Rollema et al., 1996). GR 127935 did not enhance the effect on 5-HT release of a selective 5-HT reuptake inhibitor (systemically administered) in frontal cortex (Sharp et al., 1997) although an enhancement was detected in hypothalamus (Rollema et al., 1996). Finally, there is preliminary microdialysis evidence that the selective 5-HT_{1B} receptor antagonist, SB-224289, increases 5-HT release in dorsal hippocampus but not frontal cortex or striatum of the guinea pig (Roberts et al., 1997b). Therefore, there may be regional differences in the effect of 5-HT_{1B} receptor antagonists on 5-HT release which could reflect regional differences in 5-HT autoreceptor tone.

Data from recent *in vitro* voltammetric studies indicate the presence of 5-HT_{1B} autoreceptors in the region of the 5-HT cell bodies in the DRN (Starkey and Skingle, 1994; Davidson and Stamford, 1995). Specifically, electrically-evoked release of 5-HT in this region is enhanced by GR 127935 and inhibited by 5-HT_{1B} receptor agonists, including CP 9129 which is 5-HT_{1B} receptor selective in the rat. Although the precise cellular location of these receptors is unclear, the occurrence of 5-HT_{1B} receptor mRNA in the DRN (see above) suggests that some of the 5-HT_{1B} receptors may be located on the 5-HT soma and dendrites in the DRN. Alternatively, and perhaps more likely, these receptors are located on the terminals of 5-HT afferents to the DRN.

Previous electrophysiological studies have concluded that 5-HT_{1B} receptors do not play a role in the regulation of 5-HT cell firing in the rat DRN (Sprouse and Aghajanian, 1988). Although this work utilised what are now recognised as non-selective 5-HT_{1/2} receptor agonists (TFMPP, mCPP), recent experiments find that the inhibitory effect of exogenous 5-HT on 5-HT cell firing in the raphe slice preparation is not blocked by 5-HT_{1B/1D} receptor antagonist GR 127935 whereas the response is abolished by WAY 100635 (Craven et al., 1994). Interestingly, Craven et al. (1997) have recently reported that in the presence of WAY 100635, 5-HT has an excitatory effect on 5-HT cell firing. Although the pharmacology of this response is not yet certain, it appears to be of the 5-HT₂ family.

4.4.3. 5-HT_{1B} heteroreceptors

A mismatch in the distribution of 5-HT_{1B} binding sites and 5-HT_{1B} receptor mRNA has led to specula-

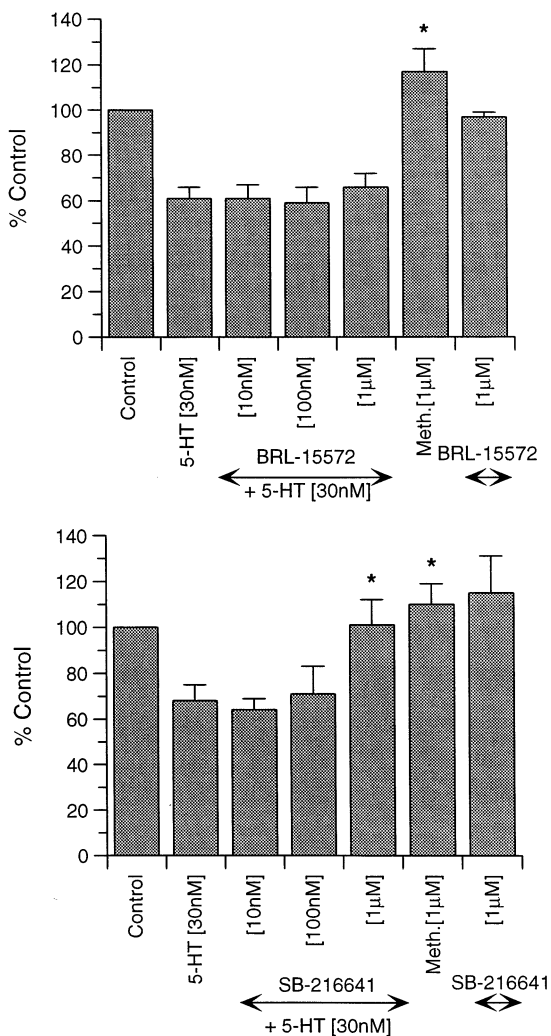


Fig. 4. Effect of 5-HT_{1B} and 5-HT_{1D} selective antagonists, SB-216641 and BRL-15572 respectively, on the electrically evoked release of [³H]-5-HT from superfused slices of the guinea pig cortex. Electrical stimuli (120 pulses at 1 Hz) were applied twice (S1 and S2) and data are expressed as a percentage of the control S2/S1 ratio. 5-HT was applied 12 min, and the antagonists 32 min prior to S2. Note the reversal of the inhibitory effect of 5-HT by SB-216641 and the lack of effect of BRL-15572. The data were kindly supplied by Dr Gary Price, SmithKline Beecham, Harlow.

tion (e.g. Bruinvels et al., 1994a,b) that in some brain regions, the 5-HT_{1B} receptor is transported to the nerve terminals and functions as a 5-HT heteroreceptor (i.e. a modulatory receptor located on non-5HT terminals).

Functional evidence to support a heteroreceptor role for the 5-HT_{1B} receptor comes from *in vitro* studies on rat hippocampus which demonstrate an inhibitory influence on acetylcholine release of a 5-HT receptor with 5-HT_{1B}-like pharmacology (Maura and Raiteri, 1986; Cassel et al., 1995). In comparison, microdialysis studies have detected a facilitatory effect of 5-HT_{1B} receptor agonists on release of acetylcholine in rat frontal cortex (Consolo et al., 1996). A facilitatory effect of 5-HT and 5-HT_{1B} receptor agonists on release of dopamine in rat frontal cortex has also been reported in a recent microdialysis study (Lyer and Bradberry, 1996). Given the inhibitory nature of the 5-HT_{1B} receptor, it seems likely that the latter effects are mediated indirectly.

Clear functional evidence of a heteroreceptor role for the 5-HT_{1B} receptor comes from electrophysiological studies. Earlier work by Bobker and Williams (1989) found evidence that various non-selective 5-HT₁ receptor agonists inhibited depolarizing synaptic potentials in neurones of the rat locus coeruleus *in vitro*. It was concluded that the effects were mediated via a 5-HT_{1B} receptor-induced inhibition of glutamate release. Activation of presynaptic 5-HT_{1B} receptors and inhibition of glutamate release, is also believed to underlie the 5-HT-induced inhibition of synaptic potentials evoked in neurones of the rat subiculum (Boeijinga and Boddeke, 1993, 1996) and cingulate cortex (Tanaka and North, 1993). The location of presynaptic 5-HT_{1B} receptors in subiculum is consistent with the presence of 5-HT_{1B} receptor mRNA in hippocampal CA1 neurones which are a source of glutamatergic projections to the subiculum (Bruinvels et al., 1994a,b).

It is thought that 5-HT_{1B} heteroreceptors underlie the 5-HT-induced suppression of GABA_B receptor-mediated IPSPs in rat midbrain dopamine neurones *in vitro* (Johnson et al., 1992). This idea is consistent with the high levels of 5-HT_{1B} binding sites in the substantia nigra which lesion experiments indicate are located on striatonigral GABAergic afferents rather than the dopamine cells themselves (Waeber et al., 1990a,b).

4.4.4. Behavioural and other physiological responses

Studies on the *in vivo* effects of 5-HT_{1B} receptor activation have been hampered by the lack of drug tools with sufficient selectivity or brain penetration. Some of the agonists and antagonists that have been used to study the *in vivo* neuropharmacology of the 5-HT_{1B} receptor have been reviewed (Lucki, 1992; Middlemiss and Tricklebank, 1992).

Early studies used the strong locomotor response of rats and mice to RU 24969 as a model of postsynaptic 5-HT_{1B} receptor function (Green and Heal, 1985), al-

though studies by Tricklebank et al. (1986) implicated an involvement of 5-HT_{1A} receptors. More recent work using selective receptor antagonists (WAY 100635, GR 127935) confirm that, at least in the rat, the response is likely to be mediated by the 5-HT_{1A} receptor (Kalkman, 1995). However, in the mouse, the evidence for an involvement of the 5-HT_{1B} receptor in the locomotor stimulant effects of RU 24969 is more certain. This is based not only on antagonist pharmacology (Cheetham and Heal, 1993) but also the fact that the response is abolished in 5-HT_{1B} knock-out mice (Saudou et al., 1994; see also below). The locomotor activating effects of 5-HT releasing agents, including MDMA, may also be mediated via activation of the postsynaptic 5-HT_{1B} receptor (Geyer, 1996).

Other behavioural and physiological effects of RU 24969 and non-selective 5-HT_{1B} receptor agonists in the rat that have been attributed provisionally to activation of central 5-HT_{1B} receptors, include increased corticosterone and prolactin secretion, hypophagia, hypothermia, penile erection and a stimulus cue in drug discrimination tests (reviewed by Glennon and Lucki, 1988; Middlemiss and Hutson, 1990). The precise role of the 5-HT_{1B} receptor these effects will become clearer once more widely selective agonists and antagonists become more widely available.

Given the lack of suitable drug tools, an important development is the production of 5-HT_{1B} knock-out mice (Saudou et al., 1994). That these mice lack 5-HT_{1B} receptors was confirmed by receptor autoradiography and *in vitro* studies of 5-HT_{1B} autoreceptor function (Saudou et al., 1994; Piñeyro et al., 1995a). As noted above, RU 24969 does not evoke locomotor activation in these mice. One other prominent behavioural change noted in these animals is that compared to wild-type mice, the mutants are more aggressive towards intruder mice. This finding fits in with findings that certain 5-HT_{1B} receptor agonists (serenics) have antiaggressive properties (Olivier et al., 1995). Although there is a consistent line of evidence associating reduced brain 5-HT transmission with high levels of impulsivity and aggression, drugs with 5-HT_{1B} receptor antagonist properties (e.g. pindolol, cyanopindolol) are not widely seen as aggression-enhancing compounds.

In the guinea pig intranigral injection of 5-HT_{1B/1D} receptor agonists induces contralateral rotation (Higgins et al., 1991; Skingle et al., 1996). Thus, 5-CT, sumatriptan, RU 24969 and GR 56764 (but not 8-OH-DPAT, DOI or 2-methyl-5-HT) all increased rotational behaviour and the effects were antagonised by GR 127935, methiothepin and metergoline (but not ritanserin or ondansetron). This work followed on from earlier experiments by Oberlander et al. (1981), injecting RU 24969 into the substantia nigra of the rat. Because of the system of nomenclature prevailing at the time, these responses were originally attributed to the

Table 4
Summary of the functional responses associated with activation of the brain 5-HT_{1B} receptor

Level	Response	Mechanism
Cellular	Adenylate cyclase (–)	Post
Electrophysiological	Inhibition of evoked synaptic potentials	Pre (heteroreceptor)
Behavioural	Locomotion/rotation (+)	Post
	Hypophagia	Pre
	Hypothermia (g. pig)	?
Neurochemical	Myoclonic jerks (g. pig)	?
	5-HT release (–)	Pre (autoreceptor)
	Acetylcholine release (–)	Pre (heteroreceptor)

5-HT_{1D} receptor. However, given the recent receptor realignment, it is probable that the rotational response is mediated by the 5-HT_{1B} receptor since levels of this receptor are very high in the substantia nigra of both the rat and guinea pig. The 5-HT_{1B} receptor located on the terminals of striatonigral GABA pathway (see above) is a clear candidate substrate for the behavioural response and this may also involve indirect activation of the nigrostriatal dopamine pathway (Oberlander, 1983; Higgins et al., 1991; see also Johnson et al., 1992).

Interestingly, in the guinea pig the 5-HT₁ receptor agonists, 5-CT and GR 46611 evoke hypothermia (Skingle et al., 1996). Although these agonists are not selective for the various 5-HT₁ receptor subtypes, the effect appears to be mediated by 5-HT_{1B/D} receptors in that it is abolished by GR 127935 and metergoline but not other 5-HT receptor antagonists, including WAY 100635 (Skingle et al., 1996). Furthermore, in the guinea pig (unlike the rat) 5-HT_{1A} receptor agonists like 8-OH-DPAT do not evoke hypothermia. This functional response was originally classified as mediated by the 5-HT_{1D} receptor, with the recent nomenclature realignment, the 5-HT_{1B} receptor seems a more likely candidate although this now needs confirming with the 5-HT_{1B}-selective agents coming available. Another *in vivo* functional response in the guinea pig that is probably mediated by the 5-HT_{1B} receptor (despite the original classification as a 5-HT_{1D} receptor response) is the potentiation of 5-HTP-induced myoclonic jerks (Hagan et al., 1995).

The functional effects associated with activation of central 5-HT_{1B} receptors are summarised in Table 4.

5. 5-HT_{1D} receptor

Using membranes prepared from bovine brain, Heuring and Peroutka (1987) detected a high affinity binding site for [³H]-5HT in the presence of ligands blocking the 5-HT_{1A} and 5-HT_{1C} (now 5-HT_{2C}) binding sites, which had a pharmacology distinct from that of the 5-HT_{1B}

site detected in rodent brain. Similar sites were detected in other species including humans (Waeber et al., 1988). These findings were taken as evidence of a new 5-HT₁ receptor and the binding site was given the 5-HT_{1D} classification. It is now generally recognised that this binding site was in fact the species equivalent of the rat 5-HT_{1B} receptor (see Section 4). However, during the search for gene sequences with 5-HT₁ homology, a novel 5-HT₁ receptor was uncovered (5-HT_{1D α}) and today is classified as the 5-HT_{1D} receptor.

5.1. 5-HT_{1D} receptor structure

At the beginning of the 1990's, two homologous human 5-HT₁ receptor clones were isolated on the basis of their sequence homology with the orphan receptor (canine RDC4 gene) which was suspected to be a 5-HT receptor. When expressed both clones demonstrated the pharmacology of the originally defined 5-HT_{1D} site, and were termed 5-HT_{1D α} and 5-HT_{1D β} (Hamblin and Metcalf, 1991; Levy et al., 1992a,b; Weinschank et al., 1992). However, on the basis of its similar distribution and gene sequence to the rodent 5-HT_{1B} receptor, the human 5-HT_{1D β} receptor was redefined as a species homologue of the 5-HT_{1B} receptor (see above; Table 2). With the subsequent discovery of a rat gene which was homologous to the human 5-HT_{1D α} receptor and encodes a receptor with a 5-HT_{1D} binding site profile (Hamblin et al., 1992b), the 5-HT_{1D α} receptor was renamed 5-HT_{1D} (Hartig et al., 1996; Table 2). In the human, the genes encoding the 5-HT_{1D} and 5-HT_{1B} receptors are on different chromosomes (1p34.3–36.3 and 6q13, respectively).

5.2. 5-HT_{1D} receptor distribution

It has been difficult to determine the distribution of the 5-HT_{1D} receptor because levels appear to be low and there is a lack of radioligand that is able to discriminate this receptor from the 5-HT_{1B} receptor. Receptor autoradiographic studies in rat utilising [¹²⁵I]-GTI (in the presence of CP 93129 to mask the rat 5-HT_{1B} binding site) suggest that in rat the 5-HT_{1D} site is present in various regions but especially the basal ganglia (particularly the globus pallidus, substantia nigra and caudate putamen) and also the hippocampus and cortex (Bruinvels et al., 1993). A recent study of the distribution of 5-HT_{1D} receptors in human brain, as defined by the ketanserin-sensitive component of [³H]-sumatriptan binding site, detected their presence in the basal ganglia (globus pallidus and substantia nigra) as well as specific regions of the midbrain (periaqueductal grey) and spinal cord (Castro et al., 1997a).

In situ hybridisation experiments have detected 5-HT_{1D} mRNA (5-HT_{1D α}) in various rat brain regions including the caudate putamen, nucleus accumbens,

olfactory cortex, dorsal raphe nucleus and locus coeruleus (Hamblin et al., 1992a,b; Bruinvels et al., 1994a,b). The mRNA had low abundance in all regions but interestingly was undetectable in certain regions, including the globus pallidus, ventral pallidum and substantia nigra where 5-HT_{1D} binding sites appear to be present. Together, these data are reminiscent of the findings with the 5-HT_{1B} receptor, and indicative of the 5-HT_{1D} receptor being located predominantly on axon terminals of both 5-HT and non-5-HT neurones.

5.3. 5-HT_{1D} receptor pharmacology

The human 5-HT_{1D} and 5-HT_{1B} receptors are homologous in their amino acid sequence (77% within the transmembrane regions; Table 1; Fig. 1) and have drug binding profiles that are almost indistinguishable (Weinshank et al., 1992; see Boess and Martin, 1994). In addition, the pharmacological characteristics of the rat and human 5-HT_{1D} receptors are very close (Hamblin et al., 1992a,b; Boess and Martin, 1994). Overall, comparing the pharmacology of 5-HT_{1D} and 5-HT_{1B} receptors across various species, it is only the rodent 5-HT_{1B} receptor that stands out, specifically in terms of its higher affinity for certain β -adrenergic ligands and the compound CP 93 129, and marginally lower affinity for sumatriptan.

Thus, many of the ligands with high affinity for 5-HT_{1B} binding site listed above (see section on 5-HT_{1B} receptor pharmacology) also have high affinity for the 5-HT_{1D} binding site (e.g. Pauwels et al., 1996). Some of these compounds (e.g. GR 127 935, GR 125 743) are, however, selective for the 5-HT_{1B/1D} binding sites versus other sites, which makes them extremely useful tools. There are, nevertheless, detectable differences in the pharmacology of the human 5-HT_{1D} and 5-HT_{1B} receptors, ketanserin and ritanserin having some selectivity (15–30-fold) for the 5-HT_{1D} receptor (Kaumann et al., 1994; Pauwels et al., 1996). Moreover, the novel compound BRL-15572 is reported to have 60-fold higher affinity for the 5-HT_{1D} than 5-HT_{1B} receptor (Price et al., 1997).

5.4. Functional effects mediated via the 5-HT_{1D} receptor

5.4.1. Second messenger responses

In transfected cells, the cloned 5-HT_{1D} receptor couples negatively to adenylate cyclase (Hamblin and Metcalf, 1991; Weinshank et al., 1992). The pharmacology of the 5-HT_{1D} receptor determined in these functional assays agrees with that determined in the radioligand binding studies. For example, ketanserin and ritanserin show antagonist properties with selectivity for the cloned human 5-HT_{1D} versus 5-HT_{1B} receptor (Pauwels and Colpaert, 1996; Pauwels et al., 1996). The moderate

affinity of 8-OH-DPAT for the 5-HT_{1D} receptor displayed in the radioligand binding studies, shows up as agonist properties in these functional tests. GR 127 935 behaves as a partial 5-HT_{1D} receptor agonist in some tests using cloned receptors (Pauwels et al., 1996; Price et al., 1997).

Although the originally defined 5-HT_{1D} receptor was reported to couple negatively to adenylate cyclase in the substantia nigra of the calf and guinea pig (Schoeffter and Hoyer, 1989; Waeber et al., 1990a), it now seems clear that the receptor being detected in these earlier studies was in fact the species equivalent of the 5-HT_{1B} receptor. Therefore, as yet no second messenger response can be safely attributed to the 5-HT_{1D} receptor expressed in native tissue.

5.4.2. 5-HT_{1D} autoreceptors

There is clear evidence from receptor autoradiography and in situ hybridisation studies that the 5-HT_{1D} receptor, like the 5-HT_{1B} receptor, is located presynaptically on both 5-HT and non-5-HT neurones (see above). There are several reports suggesting that the 5-HT_{1D} receptor may have a 5-HT autoreceptor role in both the raphe nuclei and 5-HT nerve terminal regions.

Using voltammetric measurements of 5-HT efflux from brain slices, Starkey and Skingle (1994) reported the presence of a 5-HT_{1B/1D} autoreceptor in the guinea pig DRN but were unable to discriminate between the two subtypes. A subsequent in vitro voltammetric study on the rat DRN concluded that both 5-HT_{1B} and 5-HT_{1D} autoreceptors were present in this region (Davidson and Stamford, 1995). Moreover, Piñeyro et al. (1995b) also concluded that the pharmacology of the 5-HT agonist-induced inhibition of [³H]-5HT from slices of the rat mesencephalon indicated the presence of a 5-HT_{1D} (but not 5-HT_{1B}) autoreceptor in this preparation. Recently, the Blier laboratory claimed evidence of a 5-HT_{1D} receptor in the rat DRN based on in vivo electrophysiological observations (Piñeyro et al., 1996). As with most work in this area, the conclusion of the latter study relies on interpreting the actions of non-selective drugs. This is made all the more difficult in in vivo studies.

The presence of a 5-HT_{1D} autoreceptor on 5-HT nerve terminals has been proposed on the basis of in vitro evidence of the persistence of 5-HT agonist-induced inhibition of 5-HT release in the cortex, hippocampus and DRN of 5-HT_{1B} knock-out mice (Piñeyro et al., 1995a). However, a recent in vivo microdialysis study of 5-HT_{1B} knock-out mice found that the inhibitory effect of 5-HT_{1B/1D} receptor agonists on 5-HT release in cortex and hippocampus was absent in the mutants (Trillat et al., 1997).

In contrast to the above, studies using classical in vitro models are largely unequivocal regarding the identity of the terminal 5-HT autoreceptor. Thus, utilising a

model of the 5-HT autoreceptor in the guinea pig cortex, Göthert and colleagues (Bühlen et al., 1996) concluded on the basis of correlations between agonist/antagonist potencies and binding affinities, that the 5-HT autoreceptor belongs to the 5-HT_{1B} rather than 5-HT_{1D} subtype. Similar conclusions were arrived at regarding the pharmacology of the 5-HT autoreceptor in the human cortex (Fink et al., 1995). These results are corroborated by recent *in vitro* studies showing that the 5-HT autoreceptor in guinea pig and human cortex is blocked by the 5-HT_{1B}-selective antagonist, SB-216641, but not the 5-HT_{1D}-selective antagonist, BRL-15572 (Schlicker et al., 1997; Fig. 4).

In conclusion, on the basis of available data, the presence of a 5-HT_{1D} autoreceptor in brain remains a possibility although such a receptor may be restricted to some brain regions and/or be present amongst higher levels of 5-HT_{1B} autoreceptors. This issue can now be pursued further with experiments using drugs which can discriminate between the 5-HT_{1D} and 5-HT_{1B} receptor.

5.4.3. 5-HT_{1D} heteroreceptors

There is limited functional data regarding a possible heteroreceptor role for the 5-HT_{1D} receptor that certain anatomical data suggest. There is, however evidence supportive of this idea. Thus, Raiteri and colleagues (Maura and Raiteri, 1996) described evidence for a 5-HT_{1D}-like receptor mediating an inhibition of glutamate release from rat cerebellar synaptosomes, and the same group have recently reported a similar findings using tissue from human cerebral cortex (Maura et al., 1998). Furthermore, Feuerstein et al. (1996) reported that [³H]-GABA released from human (but not rabbit) cortex *in vitro* may be modulated by an inhibitory 5-HT_{1D}-like receptor. An earlier study found *in vitro* evidence for a 5-HT_{1D}-like receptor with an inhibitory influence on acetylcholine release in guinea pig hippocampus (Harel-Dupas et al., 1991). As with the issue of the pharmacology of the 5-HT_{1D} autoreceptor, it will be very important that selective 5-HT_{1D} and 5-HT_{1B} receptor ligands are tested in these models before a heteroreceptor function can be attributed unequivocally to the 5-HT_{1D} receptor.

5.4.4. Behavioural and other physiological responses

As yet no *in vivo* functional response can be safely ascribed to activation of the CNS 5-HT_{1D} receptor. The lack of drug tools which are brain penetrating and can discriminate between the 5-HT_{1D} and 5-HT_{1B} receptors is a particular problem. In addition, studies in all species are faced by the low levels of the 5-HT_{1D} versus 5-HT_{1B} receptor in brain. Although a number of behavioural responses in the guinea pig have been attributed to the 5-HT_{1D} receptor, this was only relevant to the old nomenclature which did not recognise that

the presence of species homologues of the 5-HT_{1B} receptor (see Section 4).

6. 5-ht_{1E} receptor

The 5-ht_{1E} receptor was first detected in radioligand binding studies which found that [³H]-5-HT, in the presence of blocking agents for other 5-HT₁ subtypes that were known at that time (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}), demonstrated a biphasic displacement curve to 5-CT (Waeber et al., 1988; Leonhardt et al., 1989). The site with high affinity for 5-CT was thought to represent the 5-HT_{1D} receptor. The low affinity site had a novel pharmacology and was seen as a novel 5-HT receptor (5-HT_{1E}; Leonhardt et al., 1989). A 5-CT-insensitive [³H]-5-HT binding site was found in cortex and caudate membranes of human as well as other species, e.g. guinea pig, rabbit, dog (Waeber et al., 1988; Leonhardt et al., 1989; Beer et al., 1992). Although we now know that other 5-HT receptor subtypes also have high affinity for [³H]-5-HT but are 5-CT insensitive (5-ht_{1F}, 5-ht₆), and could therefore have contributed to the initially described 5-ht_{1E} binding site, a human gene encoding for a receptor with 5-ht_{1E} pharmacology (and structural features typical of a 5-ht₁ receptor) was subsequently isolated (McAllister et al., 1992; Zgombick et al., 1992).

6.1. 5-ht_{1E} receptor structure

The human 5-ht_{1E} receptor gene is intronless, encodes a protein of 365 amino acids (McAllister et al., 1992; Zgombick et al., 1992; Gudermann et al., 1993) and locates to human chromosome 6q14–q15 (Levy et al., 1992b). The 5-ht_{1E} receptor has highest homology with the 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1E} receptors (Table 2).

6.2. 5-ht_{1E} receptor distribution

Although currently there are no available selective radioligands for the 5-ht_{1E} receptor, autoradiographic studies have provided a picture of the distribution of non-5-HT_{1A/1B/1D/2C} [³H]-5-HT binding sites in human, rat, mouse and guinea pig brain (Miller and Teitler, 1992; Barone et al., 1993; Bruinvels et al., 1994c). These studies indicate that in all species, higher levels of these binding sites were present in the cortex (particularly entorhinal cortex), caudate putamen and claustrum but detectable levels were found in other areas, including hippocampus (subiculum) and amygdala.

Although the above receptor autoradiography studies may be detecting a combination of 5-ht_{1E} and 5-ht_{1F} binding sites, in the human and monkey brain 5-ht_{1E} mRNA is present in cortical areas (including entorhinal cortex) and the caudate and putamen, with lower but

detectable levels in amygdala and hypothalamic regions (Bruinvels et al., 1994a,b). It has been pointed out that this pattern to some extent follows that of the 5-ht_{1B} and 5-HT_{1D} receptor mRNA (Bruinvels et al., 1994a,b) although there is as yet no firm evidence for the existence of 5-ht_{1E} mRNA in the raphe nuclei. Thus, the 5-ht_{1E} mRNA would appear to have a postsynaptic location which is consistent with receptor autoradiography studies finding no change in levels of the 5-ht_{1E} binding site in rat forebrain following 5-HT neuronal lesions (Barone et al., 1993).

6.3. 5-ht_{1E} receptor pharmacology

Currently, there are no 5-ht_{1E} receptor selective ligands available. The 5-ht_{1E} receptor (like the 5-ht_{1F} receptor) is characterised by its high affinity for 5-HT and lower affinity for 5-CT. A relatively low affinity for sumatriptan sets it apart from the 5-ht_{1F} binding site. As with the 5-HT_{1D}, human 5-HT_{1B} and 5-ht_{1F} receptors, the 5-ht_{1E} receptor has little affinity for beta-adrenergic ligands due to a single amino acid residue (threonine) in the 7th transmembrane domain (Adham et al., 1994a).

6.4. Functional effects mediated via the 5-ht_{1E} receptor

Little is known about the physiological role of the 5-ht_{1E} receptor and its effects on neurones although in expression systems the receptor (human) has been shown to mediate a modest inhibition of forskolin-stimulated adenylate cyclase (Levy et al., 1992a; McAllister et al., 1992; Zgombick et al., 1992; Adham et al., 1994b). The rank order of potency of agonists for the inhibition of cyclase is compatible with the pharmacological profile of the 5-ht_{1E} receptor determined in radioligand binding studies in both human brain tissue (Leonhardt et al., 1989) and heterologous expression systems (Levy et al., 1992a; McAllister et al., 1992; Zgombick et al., 1992). In these studies methiothepin appears to behave as an antagonist, albeit a weak one, and 5-CT has very low potency as an agonist (Adham et al., 1994a).

7. 5-ht_{1F} receptor

This 5-ht_{1F} receptor gene was originally detected in the mouse on the basis of its sequence homology with the 5-HT_{1B/1D} receptor subtypes (Amlaiky et al., 1992); the human gene followed shortly afterwards (Adham et al., 1993b). Initially, the receptor was designated 5-ht_{1EB} (Amlaiky et al., 1992; Table 2). This was based on findings that the cloned 5-ht_{1F} receptor had a pharmacological profile close to that of the

5-ht_{1E} receptor (including low affinity for 5-CT) but that 5-ht_{1F} receptor mRNA showed quite a different distribution in the brain compared to 5-ht_{1E} receptor mRNA.

7.1. 5-ht_{1F} receptor structure

The structural characteristics of the 5-ht_{1F} receptor are similar to those of other members of the 5-HT₁ receptor family (e.g. intronless, seven transmembrane spanning regions), and it has a high degree of homology with the 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1E} receptors (Amlaiky et al., 1992; Adham et al., 1993b; Table 1; Fig. 1). In the human the 5-ht_{1F} receptor gene is located on chromosome 3q11 (Saudou and Hen, 1994).

7.2. 5-ht_{1F} receptor distribution

Initial studies located 5-ht_{1F} mRNA in the mouse and guinea pig brain using *in situ* hybridisation (Amlaiky et al., 1992; Adham et al., 1993b). 5-HT_{1F} mRNA abundance was found in hippocampus (CA1–CA3 cell layers), cortex (particularly cingulate and entorhinal cortices) and dorsal raphe nucleus. These results were confirmed in a subsequent more detailed mapping in the guinea pig brain (Bruinvels et al., 1994a,b), although levels of 5-ht_{1F} mRNA in the raphe nuclei appeared to be in a much lower level of abundance than in the initial report.

Brain regions containing 5-ht_{1F} mRNA also display 5-CT-insensitive 5-HT₁ but non-5-HT_{1A/1B/1C/1D} sites as detected in autoradiography studies (Bruinvels et al., 1994a,b). In a more recent autoradiography study, Waeber and Moskowitz (1995a,b) utilised [³H]-sumatriptan in the presence of 5-CT in an attempt to label 5-ht_{1F} binding sites in the guinea pig and rat brain. Pazos and colleagues (Pascual et al., 1996; Castro et al., 1997a) have also used this method to study 5-ht_{1F} binding sites in human forebrain and brain stem. The distribution of 5-CT-insensitive [³H]-sumatriptan binding sites demonstrates a very good correlation with the distribution of 5-ht_{1F} mRNA in the guinea pig (Bruinvels et al., 1994a,b) with the highest levels of binding in cortical and hippocampal areas, claustrum and the caudate nucleus. Although the receptor is located in parts of the basal ganglia, in contrast to 5-HT_{1B} and 5-ht_{1D} binding sites, 5-ht_{1F} binding sites appear to be barely detectable in the substantia nigra (Waeber and Moskowitz, 1995a,b). The brain distribution of 5-ht_{1F} binding sites labelled by a novel, selective 5-ht_{1F} radioligand, [³H]LY334370, was recently reported (Lucaites et al., 1996). The preliminary results of the latter study fit with there being a low abundance of 5-ht_{1F} receptors with a restricted distribution.

7.3. 5-HT_{1F} receptor pharmacology

The pharmacology of the human receptor in transfected cells has a close similarity to that of the 5-HT_{1E} receptor with its characteristic high affinity for 5-HT but low affinity for 5-CT (Amlaiky et al., 1992; Adham et al., 1993a,b; Lovenberg et al., 1993a,b). However, its high affinity for sumatriptan discriminates the 5-HT_{1F} receptor from the 5-HT_{1E} receptor. Until recently, there were no selective ligands for the 5-HT_{1F} receptor. However, data on two novel and selective 5-HT_{1F} receptor agonists, LY344864 and LY334370 have recently appeared (Overshiner et al., 1996; Johnson et al., 1997; Phebus et al., 1997; Table 5).

7.4. Functional effects mediated via the 5-HT_{1F} receptor

When expressed in cultured cells, the cloned human and mouse 5-HT_{1F} receptors couple to the inhibition of forskolin-stimulated adenylate cyclase (Amlaiky et al., 1992; Adham et al., 1993a; Lovenberg et al., 1993a,b). In these conditions 5-HT acts as a potent agonist (EC₅₀ value 7–8 nM) and methiothepin acts as a silent but weak (pK_B 6.3) receptor antagonist. The recently reported selective 5-HT_{1F} receptor agonists, LY334370 and LY344864 are full and potent agonists in cells expressing the 5-HT_{1F} receptor with EC₅₀ values of 1.5 and 3 nM, respectively (Johnson et al., 1997; Phebus et al., 1997; Table 1). The effects of activation of the native 5-HT_{1F} receptor is currently unknown although on the basis of its anatomical location, it is speculated that this receptor may play roles in visual and cognitive function and as a 5-HT autoreceptor (Waeber and Moskowitz, 1995a,b).

Initial reports on the novel 5-HT_{1F} receptor agonist, LY334370, suggest that it does not evoke overt behavioural effects (5-HT behavioural syndrome, locomotion, changes in body temperature) when administered

to rats (Overshiner et al., 1996). Furthermore, the compound did not decrease brain levels of the 5-HT metabolite, 5-HIAA. However, both this drug and its partner, LY344864, are active in the rat dural extravasation model at very low doses, indicating a possible use in the treatment of migraine (Johnson et al., 1997; Phebus et al., 1997).

8. The 5-HT₂ receptor family

The 5-HT₂ receptor family currently accommodates three receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which are similar in terms of their molecular structure, pharmacology and signal transduction pathways. In recent nomenclature updates (Humphrey et al., 1993; Hoyer et al., 1994; Table 2), the 5-HT_{2A} receptor was aligned with the 5-HT D receptor (also called 5-HT₂) originally defined by Gaddum and Picarelli (1957) as mediating contractions in the guinea pig ileum. In addition, the 5-HT_{2C} appellation replaced 5-HT_{1C} to carry the latter receptor from the 5-HT₁ to the 5-HT₂ receptor family, also the 5-HT_{2B} receptor classification took on the properties of what was previously classified as the 5-HT₂-like receptor in the stomach fundus (also called 5-HT_{2F} and SRL receptor).

The amino acid sequences of the 5-HT₂ receptor family have a high degree of homology within the seven transmembrane domains but they are structurally distinct from other 5-HT receptors (see Baxter et al., 1995). A characteristic of all genes in the 5-HT₂ receptor family is that they have either two introns (in the case of both the 5-HT_{2A} and 5-HT_{2B} receptors) or three introns (5-HT_{2C} receptors) in the coding sequence (Yu et al., 1991; Chen et al., 1992; Stam et al., 1992), and all are coupled positively to phospholipase C and mobilise intracellular calcium.

Table 5
Receptor binding and potency data for 5-HT_{1F} agonists^a

Compound	5-HT _{1B}	5-HT _{1D}	5-HT _{1F}	Extravasation potency (–log ID ₅₀)	
	Binding (pK _i)	Binding (pK _i)	Binding (pK _i)	Function (pEC ₅₀)	
LY334370	6.9	6.9	8.8	8.8	13.2
LY302148	7.3	7.7	8.6	8.6	12.1
Naratriptan	8.5	8.6	8.4	8.7	12.4
LY334864	6.3	6.2	8.2	8.5	11.7
LY306258	5.8	6.1	8.0	8.0	11.1
Zolmitriptan	8.3	9.0	7.6	7.5	11.1
Sumatriptan	8.0	8.3	7.6	7.5	10.4
DHE	9.2	9.4	6.6	6.9	10.6
Rizatriptan	8.0	8.4	6.6	7.6	9.7

^a The pK_i and pEC₅₀ (to inhibit the forskolin-stimulated adenylate cyclase) values are for the human 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors. The ID₅₀ values (expressed as the –log M/kg) were determined in the guinea pig neurogenic dural extravasation model of migraine. These data are taken from Johnson et al. (1997) and Phebus et al. (1997), and kindly provided by Dr Lee Phebus, Eli Lilly and Co., IN.

The receptors are well characterised at the molecular level and their distribution in the brain is established (although levels of the 5-HT_{2B} receptor seem low). The development of selective receptor antagonists is at an advanced stage but there is a need for selective agonists. Some 5-HT₂ receptor antagonists are currently undergoing clinical assessment as potential treatments for a range of CNS disorders including schizophrenia, anxiety, sleep and feeding disorders, and migraine (Baxter et al., 1995).

9. 5-HT_{2A} receptor

The brain 5-HT_{2A} receptor was initially detected in rat cortical membranes as a binding site with high affinity for [³H]-spiperone, a relatively low (micromolar) affinity for 5-HT, but with a pharmacological profile of a 5-HT receptor (Leysen et al., 1978; Peroutka and Snyder, 1979). Although this receptor was originally termed the 5-HT₂ receptor (Peroutka and Snyder, 1979), it has now been attributed to the 5-HT_{2A} receptor classification.

9.1. 5-HT_{2A} receptor structure

By the mid-1980's it had already been recognised that the 5-HT₂ and 5-HT_{1C} receptors (old nomenclature) had similar pharmacological properties and second messenger systems, and that the receptors were probably structurally related. Both the rat and human 5-HT_{2A} receptor genes were isolated by homologous screening very shortly following the first reports of the 5-HT_{2C} (now 5-HT_{2C} receptor sequence (Pritchett et al., 1988a,b; Julius et al., 1990). The human 5-HT_{2A} receptor is located on chromosome 13q14–q21 and has a relatively high amino acid sequence identity with the human 5-HT_{2C} receptor, although this is lower when compared with the human 5-HT_{2B} receptor (Table 1; Fig. 1). The human 5-HT_{2A} receptor is 87% homologous with its rat counterpart.

The amino acid sequence of the 5-HT_{2A} receptor has potential sites for glycosylation (5), phosphorylation (~11) and palmitoylation (1) (Saltzman et al., 1991). Experiments involving site-directed mutagenesis have identified individual amino acid residues which have major effects on the ligand binding and effector coupling properties of the 5-HT_{2A} receptor (for review see Boess and Martin, 1994; Saudou and Hen, 1994; Baxter et al., 1995).

9.2. 5-HT_{2A} receptor distribution

The CNS distribution of 5-HT_{2A} receptor has been mapped extensively by receptor autoradiography, in situ hybridisation and, more recently, immunocyto-

chemistry. Receptor autoradiography studies using [³H]-spiperone, [³H]-ketanserin, [¹²⁵I]-DOI and more recently [³H]-MDL 100907 as radioligands, find high levels of 5-HT_{2A} binding sites in many forebrain regions, but particularly cortical areas (neocortex, entorhinal and pyriform cortex, claustrum), caudate nucleus, nucleus accumbens, olfactory tubercle and hippocampus, of all species studied (Pazos et al., 1985, 1987; López-Giménez et al., 1997). There is a generally close concordance between the distribution of 5-HT_{2A} binding sites, 5-HT_{2A} mRNA and 5-HT_{2A} receptor-like immunoreactivity (Mengod et al., 1990a; Morilak et al., 1993, 1994; Pompeiano et al., 1994; Burnet et al., 1995), suggesting that the cells expressing 5-HT_{2A} receptors are located in the region where the receptors are present (and postsynaptic to the 5-HT neurone). A number of 5-HT_{2A}-selective radioligands are currently under development for imaging 5-HT_{2A} receptors in humans, one of the most promising being the PET ligand [¹¹C]-MDL 100907 (Lundkvist et al., 1996; Ito et al., 1998 Fig. 5).

Various studies have investigated the cellular location of the 5-HT_{2A} receptor in the brain. So far 5-HT_{2A} receptor-like immunoreactivity or 5-HT_{2A} mRNA have been found in neurones (Morilak et al., 1993, 1994; Pompeiano et al., 1994; Burnet et al., 1995), although the receptor is expressed in cultured astrocytes and glioma cells (e.g. Deecher et al., 1993; Meller et al., 1997). Converging evidence from immunocytochemical, in situ hybridisation and receptor autoradiography studies suggests that in various brain areas including cortex, the 5-HT_{2A} receptor is located on local (GABAergic) interneurons (Francis et al., 1992; Morilak et al., 1993, 1994; Burnet et al., 1995; see also Sheldon and Aghajanian, 1991). Recent data from in situ hybridisation studies also indicate the presence of 5-HT_{2A} receptor in cortical pyramidal (projection) neurones (Burnet et al., 1995; Wright et al., 1995), which are known to be glutamatergic. It is reported that 5-HT_{2A} receptor-like immunoreactivity may be located in cholinergic neurones in the basal forebrain and specific nuclei in the brain stem (Morilak et al., 1993).

It has been noted that the distribution of 5-HT_{2A} binding sites appears to map onto the distribution of 5-HT axons arriving from the DRN (Blue et al., 1988). For example in the rat, the DRN 5-HT innervation of the frontal cortex seems to follow the laminar distribution of 5-HT_{2A} binding sites in this region. That 5-HT_{2A} receptors receive a selective innervation from DRN, however, may not generalise to other regions. Thus, it has been found in electrophysiological experiments that 5-HT_{2A} receptor-mediated responses in the prefrontal cortex can be evoked by stimulation of the MRN (Godbout et al., 1991).

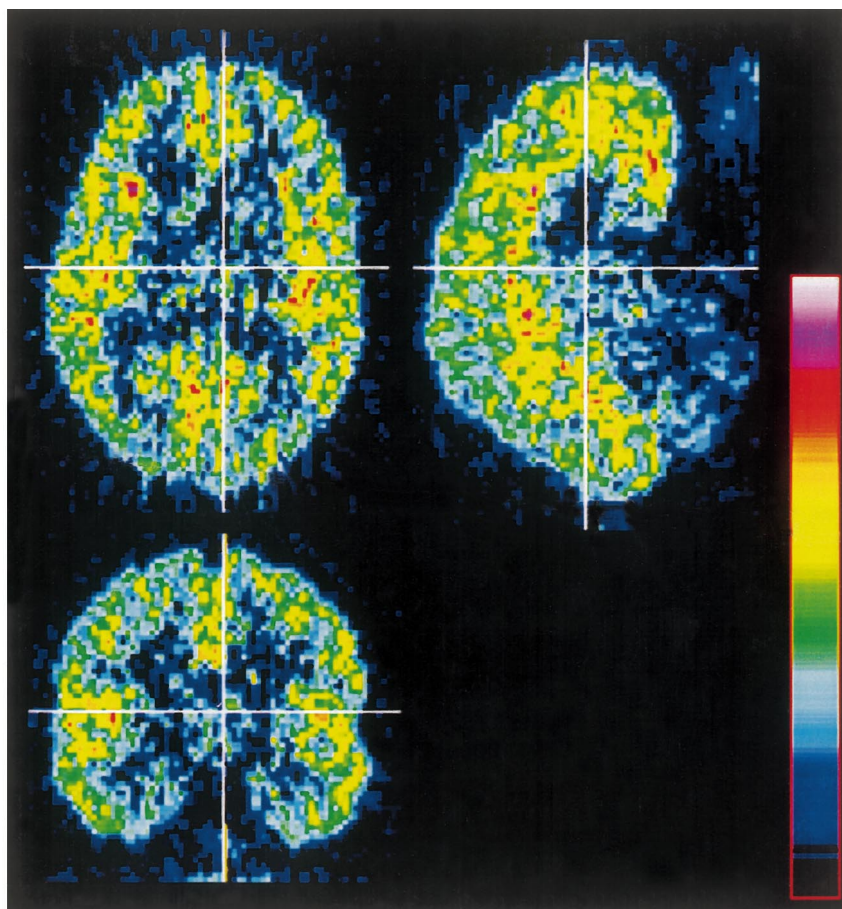
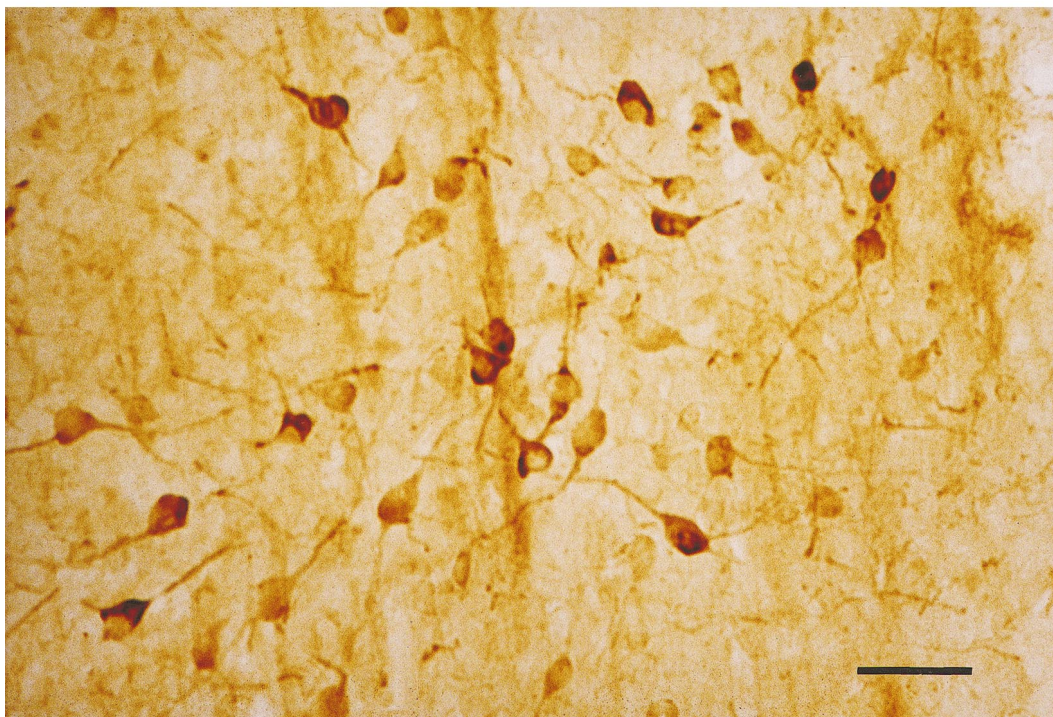
**Fig. 5****Fig. 6**

Fig. 5. Horizontal (upper) and vertical (lower) PET sections showing the distribution of radioactivity in the brain of a human volunteer following intravenous injection of a tracer dose of the 5-HT_{2A} receptor ligand [¹¹C]MDL 100907. The data were kindly supplied by Dr Svante Nyberg, Karolinska Institute, Stockholm, and are part of a study reported by Ito et al. (1998).

Fig. 6. Localisation of 5-HT_{2B} receptor-like immunoreactivity in multipolar and unipolar neurones of the rat medial amygdala. Scale bar, 40 μ m. The data were taken from Duxon et al. (1997a,b) and kindly provided by Dr Kevin Fone, University of Nottingham.

Table 6
Affinity (pK_i) of various ligands for 5-HT₂ receptors

	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}
<i>5-HT_{2A} receptor</i>			
Spiperone	8.8	5.5	5.9
MDL 100 907	9.4	n.d.	6.9
Ketanserin	8.9	5.4	7.0
<i>5-HT_{2B} receptor</i>			
5-MeOT	7.4 ^a	8.8 ^a	6.2 ^a
a-Methyl-5-HT	6.1 ^a	8.4 ^a	7.3 ^a
SB 2044741	<5.3	7.8	<6.0
BW 723C86	<5.4 ^a	7.9 ^a	<6.9
<i>5-HT_{2C} receptor</i>			
SB 242084	6.8	7.0	9.0
RS-102221	6.0	6.1	8.4
RO 60-0175	6.0	5.8	8.8
<i>5-HT_{2B/2C} receptors</i>			
SB 200646A	5.2	7.5	6.9
mCPP	6.7	7.4 ^a	7.8
SB 206553	5.8	8.9	7.9
<i>Non-selective</i>			
LY 53857	7.3	8.2	8.1
ICI 170809	9.1	n.d.	8.3
Ritanserin	8.8	8.3	8.9
Mianserin	8.1	7.3	8.0
DOI	7.3 ^a	7.4 ^a	7.8 ^a

^a pEC₅₀ value for agonist. 5-MeOT, 5-methoxytryptamine; n.d., not determined. Data were taken from Baxter et al. (1995) with additions from Bonhaus et al. (1997), Millan et al. (1997) and Kennett et al. (1996a,b, 1997a,b).

9.3. 5-HT_{2A} receptor pharmacology

The 5-HT₂ family of receptors is characterised by a relatively low affinity for 5-HT, a high affinity for the 5-HT₂ receptor agonist, DOI, and its structural analogues (DOB, DOM), and a high affinity for various 5-HT₂ receptor antagonists, including ritanserin and ICI 170809. Until recently, it has been difficult to discriminate between the 5-HT₂ family members; although ketanserin and spiperone are about two orders of magnitude more selective for the 5-HT_{2A} versus 5-HT_{2B/2C} receptors, these drugs have affinity for other monoamine receptors. However, a number of selective antagonists are now available which greatly aid the delineation of the 5-HT₂ receptors in both in vitro and in vivo models (Table 6; see Baxter et al., 1995 for review). MDL 100907 is a newly developed, potent and selective antagonist of the 5-HT_{2A} receptor which has lower affinity for the 5-HT_{2C} receptor or other receptors (Sorensen et al., 1993; Kehne et al., 1996).

Discrimination of the 5-HT_{2A} receptor from other members of the 5-HT₂ receptor family has also become considerably more straightforward by the development of antagonists which distinguish between 5-HT_{2A} and 5-HT_{2C/2B} receptors (SB 200 646A and SB 206 553) and

more recently by the arrival of potent antagonists with selectivity for both 5-HT_{2B} (SB 204 741) and 5-HT_{2C} receptors (SB 242 084 and RS-102 221) (Baxter et al., 1995; Baxter, 1996; Kennett et al., 1996a,b, 1997a,b; Bonhaus et al., 1997; Table 6).

At present, there is no suitably selective agonist for the 5-HT₂ receptor subtypes although certain tryptamine analogues (in particular BW 723C86 and 5-methoxytryptamine) have some selectivity for the 5-HT_{2B} receptor in in vitro preparations (Baxter et al., 1995; Baxter, 1996). An agonist, RO 60-0175, with selectivity for the 5-HT_{2C} receptor was recently reported (Millan et al., 1997).

9.4. Functional effects mediated via the 5-HT_{2A} receptor

9.4.1. Second messenger responses

All three 5-HT₂ receptor subtypes couple positively to phospholipase C and lead to increased accumulation of inositol phosphates and intracellular Ca²⁺ (for review see Boess and Martin, 1994; Sanders-Bush and Canton, 1995). Stimulation of the 5-HT_{2A} receptor has been demonstrated to activate phospholipase C in both heterologous expression systems (Pritchett et al., 1988a,b; Julius et al., 1990; Stam et al., 1992) and brain tissue (Conn and Sanders-Bush, 1984; Godfrey et al., 1988), via G-protein coupling (Sanders-Bush and Canton, 1995).

In these second messenger studies, DOI, DOB and DOM (and LSD) have partial agonist properties (Sanders-Bush et al., 1988). The non-selective 5-HT₂ receptor agonists, mCPP and TFMPP, have even lower efficacy and usually display only 5-HT_{2A} receptor antagonist activity in functional models (Conn and Sanders-Bush, 1986; Grotewiel et al., 1994). All 5-HT₂ receptors desensitise following prolonged exposure to 5-HT and other agonists (Sanders-Bush, 1990), although the sensitivity to agonists and mechanisms underlying desensitisation of each subtype (particularly 5-HT_{2A} versus 5-HT_{2C}) may be different (Bridson et al., 1995). A curious property of 5-HT_{2A} receptors is that in some in vitro and in vivo models they downregulate in the face of constant exposure to certain antagonists (mianserin, spiperone and mesulergine) (e.g. Sanders-Bush, 1990; Roth and Ciaranello, 1991; Grotewiel and Sanders-Bush, 1994). One of several explanations put forward to account for this phenomenon is that under certain conditions, 5-HT_{2A} receptors are constitutively active, and that some of the ligands act as inverse agonists.

Of current interest is evidence that stimulation of the 5-HT_{2A} receptor causes activation of a biochemical cascade leading to altered expression of a number of genes including that of brain-derived neurotrophic factor (BDNF) (Vaidya et al., 1997). These changes may

be linked at least in part to the increase in expression of BDNF seen following repeated treatment with antidepressants (Duman et al., 1997). There is the exciting but as yet unproven possibility that the latter changes lead to altered synaptic connectivity in the brain, and that this may even contribute to the therapeutic effect of antidepressants.

9.4.2. Electrophysiological responses

5-HT₂ receptor activation results in neuronal excitation in a variety of brain regions. Although few of these responses have been analysed using newly available 5-HT₂ receptor subtype-selective agents, evidence suggests that in some of these cases the responses are mediated by the 5-HT_{2A} receptor while others involve the 5-HT_{2C} receptor (Aghajanian, 1995).

Clear evidence for a 5-HT_{2A} receptor-mediated excitation in the cortex comes from intracellular recordings of interneurons in slices of rat pyriform cortex. Thus, the 5-HT-induced activation of these cells is blocked by both selective (MDL 100 907) and non-selective 5-HT_{2A} receptor antagonists (Sheldon and Aghajanian, 1991; Marek and Aghajanian, 1994). Furthermore, LSD and DOI are potent but partial agonists in this preparation (Marek and Aghajanian, 1996). 5-HT-induced neuronal depolarisations have also been detected in slice preparations of the nucleus accumbens (North and Uchimura, 1989), neocortex (Araneda and Andrade, 1991; Aghajanian and Marek, 1997), dentate gyrus of the hippocampus (Piguet and Galvan, 1994), and all have the pharmacological characteristics which bear the hallmark of the 5-HT_{2A} receptor. The excitatory responses to 5-HT_{2A} receptor activation are associated with a reduction of potassium conductances (Aghajanian, 1995), although whether the phosphoinositide signalling pathway has a role in this effect is not certain.

Electrophysiological studies also implicate the 5-HT₂ receptor in the regulation of noradrenergic neurones in the locus coeruleus (LC). Evidence from recordings in anaesthetised rats suggests that 5-HT₂ receptor activation results in both the facilitation of sensory-evoked activation of noradrenergic neurones, and inhibition of their spontaneous activity (Aghajanian, 1995). The inhibitory effect of 5-HT₂ receptor activation on noradrenergic transmission has also been detected in microdialysis studies which demonstrate a decrease in noradrenaline release in rat hippocampus following administration of DOI and DOB, and the reversal of this effect by ritanserin and spiperone (Done and Sharp, 1992). There is evidence from microdialysis studies in the awake rat that 5-HT₂ receptor antagonists increase noradrenaline release (Done and Sharp, 1994). Although earlier data indicates that the pharmacology of the 5-HT₂ receptor modulating noradrenaline is of 5-HT_{2A} subtype, this idea needs reappraisal in view of new findings that 5HT_{2C} antagonists increase nona-

drenaline in microdialysis experiments (Millan et al., 1998).

The effects of 5-HT₂ receptor activation on noradrenergic neurones are likely to be indirect, possibly involving afferents to the LC from the brain stem (Gorea et al., 1991; Aghajanian, 1995). Interestingly, there is evidence for a 5-HT₂ receptor-mediated excitation of neurones in the nucleus prepositus hypoglossi which is a major source of inhibitory input to the LC (Bobker, 1994).

9.4.3. Behavioural and other physiological responses

The behavioural effects of 5-HT₂ receptor agonists in rodents are many, ranging from changes in both unconditioned (e.g. increased motor activity and hyperthermia) and conditioned responses (e.g. punished responding, drug discrimination) (for review see Glennon and Lucki, 1988; Koek et al., 1992). The delineation of the involvement of specific 5-HT₂ receptor subtypes in these behaviours has not been straightforward due to the fact that most 5-HT₂ receptor agonists studied so far, are not selective. Nevertheless certain behaviours can be attributed, with some degree of confidence, to activation of either 5-HT_{2A} or 5-HT_{2C} receptors (see below).

Head twitches (mice) and wet dog shakes (rats) induced by drugs such as DOI and its structural analogues, as well as 5-HT releasing agents and precursors like 5-HTP, have long been thought to be mediated via a receptor of the 5-HT₂ type (for review see Green and Heal, 1985). It now seems clear that this response is 5-HT_{2A} receptor-mediated. Thus, the potency with which 5-HT₂ antagonists inhibit agonist-induced head shakes closely correlates with their affinity for the 5-HT_{2A} binding site but not other binding sites, including the 5-HT_{2C} binding site (Arnt et al., 1984; Schreiber et al., 1995). Furthermore, 5-HT_{2A} receptor selective antagonists such as MDL 100907 inhibit the head shake response while 5-HT_{2B/2C} receptor selective antagonists (SB 200 646A) do not (Kennett et al., 1994; Schreiber et al., 1995). It should be pointed out, however, that the use of this model as an *in vivo* test of 5-HT_{2A} receptor pharmacology is complicated by the fact it is sensitive to drugs active on other transmitter receptors (5-HT₁ and catecholamine receptors, in particular) which presumably interact indirectly with the neural pathways expressing the headshake/twitch response (Koek et al., 1992).

Activation of the 5-HT₂ receptor leads to a discriminative stimulus in rats. For example, animals trained to discriminate 5-HT₂ receptor agonists such as DOM, recognise its structural derivatives (DOI, DOB) but not 5-HT₁ receptor agonists (Glennon and Lucki, 1988). The DOM stimulus is blocked by 5-HT₂ receptor antagonists such as ketanserin and LY 53857, suggesting that the discriminative cue is 5-HT_{2A} receptor-medi-

ated. Recent data show that the potency of 5-HT₂ receptor antagonists to block the DOM cue correlates strongly with their affinity for the 5-HT_{2A} but not 5-HT_{2C} binding site (Fiorella et al., 1995). In addition, there is a significant correlation between the potency of a wide range of 5-HT₂ receptor agonists in the DOM-discrimination model and their affinity for the 5-HT_{2A} binding site (Glennon, 1990). The non-selective 5-HT₂ receptor agonist, mCPP, also evokes a discriminative stimulus in rats, but this appears to involve a dopaminergic mechanism and not 5-HT₂ or other 5-HT receptors (Bourson et al., 1996).

An agonist action at 5-HT₂ receptors is likely to be involved in hallucinogenic mechanisms since there is a close correlation between the human hallucinogenic potency of 5-HT₂ receptor agonists and their affinity for the 5-HT₂ binding sites (Glennon, 1990). Although this correlation fitted best for the 5-HT_{2A} binding site, 5-HT_{2C} sites were also strongly correlated. Despite the latter correlation, it has been argued that the 5-HT_{2C} receptor may not be important as mCPP, which acts as a 5-HT_{2C} agonist in many models (see below) is not an hallucinogen in humans. However, this argument is complicated by the fact that mCPP has 5-HT_{2A} receptor antagonist properties in some models.

Currently there is considerable interest in the role of the 5-HT_{2A} receptor in antipsychotic drug action. This interest is based on many findings including the relationship between 5-HT_{2A} receptor and hallucinogens discussed above; also clozapine, olanzepine and other atypical antipsychotic drugs have high affinity for the 5-HT_{2A} binding site e.g. (Leysen et al., 1993), and the evidence of an association between schizophrenia and treatment outcome and certain polymorphic variants of the 5-HT_{2A} receptor (Busatto and Kerwin, 1997). Moreover, selective 5-HT_{2A} receptor antagonists (especially MDL 100907) appear to be active in animal models predictive of atypical antipsychotic action (Kehne et al., 1996). Whether selective 5-HT_{2A} receptor antagonists are as clinically effective as antipsychotics

compared to the available mixed 5-HT₂/dopamine receptor antagonists (e.g. sertindole, risperidone) is clearly a critical question.

Finally, other responses to 5-HT₂ receptor agonists that may be mediated by the 5-HT_{2A} receptor include hyperthermia (Gudelsky et al., 1986), and neuroendocrine responses such as increased secretion of cortisol, ACTH, renin and prolactin (e.g. Fuller, 1996; Van de Kar et al., 1996). The functional effects associated with activation of central 5-HT_{2A} receptors are summarised in Table 7.

10. 5-HT_{2B} receptor

The receptor mediating the 5-HT-induced contraction of the rat stomach fundus (Vane, 1959) was originally classified as a 5-HT₁-like receptor (Bradley et al., 1986). Although the receptor had pharmacological profile reminiscent of the 5-HT_{1C} (now 5-HT_{2C}) receptor (Buchheit et al., 1986), the rat fundus did not contain detectable amounts of 5-HT_{2C} receptor mRNA (Baez et al., 1990). The situation was resolved by the isolation of the mouse and rat fundus receptor gene by low stringency screening for sequences homologous to the 5-HT_{2C} receptor (Foguet et al., 1992a,b; Kursar et al., 1992). The human equivalent to the rat fundus 5-HT receptor followed not long after (Schmuck et al., 1994). Although originally termed the 5-HT_{2F} receptor (Kursar et al., 1992), it was reclassified as 5-HT_{2B} to become the third member of the 5-HT₂ receptor family (Humphrey et al., 1993; Table 2).

10.1. 5-HT_{2B} receptor structure

The human 5-HT_{2B} receptor (481 amino acids) is relatively homologous with the human 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Table 1; Fig. 1). The 5-HT_{2B} receptor gene has two introns which are present at positions corresponding to those of the 5-HT_{2A} and 5-HT_{2C} receptor genes (Foguet et al., 1992b). The human 5-HT_{2B} receptor gene is located at chromosomal position 2q36.3–2q37.1.

10.2. 5-HT_{2B} receptor distribution

The presence of the 5-HT_{2B} receptor in the brain (especially that of the rat) has been controversial but the picture emerging is that of a distribution of 5-HT_{2B} mRNA and its protein product which is very limited (relative to 5-HT_{2A} and 5-HT_{2C} for example) but potentially of functional importance. Thus, 5-HT_{2B} mRNA transcripts have been detected (albeit in low levels) in brain tissue of both the mouse and human (Loric et al., 1992; Kursar et al., 1994; Bonhaus et al., 1995) although several groups have failed confirm this for the

Table 7
Summary of the functional responses associated with activation of the brain 5-HT_{2A} receptor

Level	Response	Mechanism
Cellular	Phosphatidyl inositide turnover (+)	Post
Electrophysiological	Neuronal depolarisation	Post
Behavioural	Head twitch (mouse)	Post
	Wet dog shake (rat)	Post
	Hyperthermia	Post
	Discriminative stimulus	Post
Neurochemical	Noradrenaline release (–)	Post
Neuroendocrine	Cortisol	Post
	ACTH	Post

rat (Foguet et al., 1992a,b; Kursar et al., 1992; Pompeiano et al., 1994).

However, the presence of 5-HT_{2B} receptor-like immunoreactivity has recently been reported in rat brain (Duxon et al., 1997a; Fig. 6). What is striking about the distribution of the reported immunostaining is that it is restricted to a few brain regions, and particularly cerebellum, lateral septum, dorsal hypothalamus and medial amygdala. In this study the cells expressing 5-HT_{2B} receptor-like immunoreactivity have a neuronal and not astrocytic morphology. These findings will provide an important guide for receptor autoradiographic studies once a suitable 5-HT_{2B} radioligand becomes available.

10.3. 5-HT_{2B} receptor pharmacology

There is a close pharmacological identity between the cloned 5-HT_{2B} receptor and that of the 5-HT receptor in the rat stomach fundus (Wainscott et al., 1993; Baxter et al., 1994). As expected from the homologous sequences of the 5-HT₂ receptor family, the receptor binding properties of the human 5-HT_{2B} receptor compare well with those of the 5-HT_{2A} and 5-HT_{2C} receptors, although the 5-HT_{2B} receptor is clearly distinct (Bonhaus et al., 1995; Table 5). For instance, the 5-HT_{2B} receptor has a low affinity for ritanserin but higher affinity for yohimbine than either the 5-HT_{2A} or 5-HT_{2C} receptor. SB 200 646 and SB 206 553 have high affinity for the 5-HT_{2C/2B} receptors and low affinity for the 5-HT_{2A} receptor, while spiperone shows the reverse. Most importantly, the novel antagonist, SB 204 741 is more than 20–60-fold more selective for the 5-HT_{2B} receptor versus the 5-HT_{2A}, 5-HT_{2C} and other receptors at which it has been tested (Baxter et al., 1995; Bonhaus et al., 1995; Baxter, 1996). In addition, the agonist BW 723C86 has about 10-fold selectivity for the 5-HT_{2B} receptor versus the 5-HT_{2A/2C} and other sites (Baxter et al., 1995; Baxter, 1996). The affinity of a variety of 5-HT₂ ligands for the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} sites are shown in Table 4.

Several studies have highlighted the possibility of species differences in the pharmacology of the 5-HT₂ receptors, particularly between the rat and human (see Hoyer et al., 1994). In the case of the 5-HT_{2B} receptor, rat/human differences appear to be minimal (Bonhaus et al., 1995).

10.4. Functional effects mediated via the 5-HT_{2B} receptor

10.4.1. Signal transduction

In heterologous expression systems the cloned rat and human 5-HT_{2B} receptors stimulate phosphatidylinositol hydrolysis (Wainscott et al., 1993; Kursar et al., 1994; Schmuck et al., 1994), in common with the other two members of the 5-HT₂ receptor family. In these

functional models the potency of a range of agonists at the transfected 5-HT_{2B} receptor correlated with that predicted by radioligand binding studies (Wainscott et al., 1993). 5-HT itself and various tryptamine analogues (including 5-methoxytryptamine) behaved as full agonists while TFMPP and quipazine were partial agonists. mCPP is a weak partial agonist in the rat fundus preparation (Baxter et al., 1995).

One interesting putative function of the 5-HT_{2B} receptor is to mediate the mitogenic effects of 5-HT during neural development. This idea comes from new studies demonstrating the presence of 5-HT_{2B} receptor expression in the neural crest of the mouse embryo and a report of severe neural abnormalities in 5-HT_{2B} knock-outs (Choi et al., 1997; Nebigil et al., 1998).

10.4.2. Behavioural responses

There are little available data on the functional effects of activation of the central 5-HT_{2B} receptor. Indeed, it has not yet been proven that the native 5-HT_{2B} receptor in brain couples to phosphatidylinositol hydrolysis. However, recent experiments investigating the actions of the 5-HT₂ receptor agonist BW 723C86 are suggestive of a role for the 5-HT_{2B} receptor in anxiety. Thus, BW 723C86 is reported to have anxiolytic properties in the rat social interaction test (an effect reversed by SB 200 646A), although it was weakly active in conflict and x-maze tests (Kennett et al., 1996a,b). Interestingly, BW 723C86 has an anxiolytic effect when injected directly into the medial amygdala (Duxon et al., 1997b) which contains detectable amounts of 5-HT_{2B} receptor-like immunoreactivity (Duxon et al., 1997a).

11. 5-HT_{2C} receptor

The 5-HT_{2C} receptor was identified as a [³H]-5-HT binding site in choroid plexus of various species that could also be labelled by [³H]-mesulergine and [³H]-LSD but not by [³H]ketanserin (Pazos et al., 1984b). Originally this site was seen as a new member of the 5-HT₁ receptor family, and termed 5-HT_{1C}, because of its high affinity for [³H]-5-HT (Pazos et al., 1984b). However, once the receptor was cloned and more information about its characteristics became available, a shift to the 5-HT₂ receptor family and reclassification as 5-HT_{2C} receptor became unavoidable (Humphrey et al., 1993; Table 2).

11.1. 5-HT_{2C} receptor structure

The partial cloning of the mouse 5-HT_{2C} receptor by Lubbert et al. (1987) was shortly followed by the sequencing of the full length clone in, initially the rat (Julius et al., 1988), and then the mouse (Yu et al.,

1991) and human (Saltzman et al., 1991). A splice variant of the 5-HT_{2C} receptor has been isolated and found to be present in brain tissue of the rat, mouse and human (Canton et al., 1996). The functional significance of this variant is however, unclear since the protein product is a truncated 5-HT_{2C} receptor without a 5-HT binding site. More recently, it has been reported that 5-HT_{2C} mRNA undergoes post-transcriptional editing to yield multiple 5-HT_{2C} receptor isoforms with different distributions in brain (Burns et al., 1997). In functional terms, this is potentially of great significance as the amino acid sequences predicted from the mRNA transcripts indicates that the isoforms (if expressed endogenously in significant amounts in brain tissue) may have different regulatory and pharmacological properties.

The 5-HT_{2C} receptor is X-linked (human chromosome Xq24, mouse chromosome X D-X F4). The 5-HT_{2C} receptor gene has three introns (rather than two in the case of the 5-HT_{2A} and 5-HT_{2B}) and may produce a protein product with eight rather than seven transmembrane regions which, if proven, would be unusual for a G-protein coupled receptor (Yu et al., 1991). There is high sequence homology (> 80% in the transmembrane regions) between the mouse, rat and human 5-HT_{2C} receptors. The mouse and rat 5-HT_{2C} receptors possess six potential *N*-glycosylation sites (Yu et al., 1991), four of which are conserved in the human sequence (Saltzman et al., 1991). The rat 5-HT_{2C} receptor has eight serine/threonine residues representing possible phosphorylation sites, all of which are conserved in the human sequence.

11.2. 5-HT_{2C} receptor distribution

Unlike the 5-HT_{2A} and 5-HT_{2B} receptors, there is little evidence for expression of 5-HT_{2C} receptors outside the CNS. Autoradiographic studies, using a variety of ligands including [³H]-5-HT, [³H]mesulergine and [³H]-LSD, have provided a detailed map of the distribution of 5-HT_{2C} binding sites in rat and many other species (for review see Palacios et al., 1991; Radja et al., 1991). In addition to the very high levels detected in the choroid plexus, 5-HT_{2C} binding sites are widely distributed and present in areas of cortex (olfactory nucleus, pyriform, cingulate and retrosplenial), limbic system (nucleus accumbens, hippocampus, amygdala) and the basal ganglia (caudate nucleus, substantia nigra). The presence of 5-HT_{2C} binding sites in the pyriform cortex and substantia nigra is relevant to findings of 5-HT_{2C} receptor-mediated electrophysiological response in these regions (Sheldon and Aghajanian, 1991; Rick et al., 1995; see below).

By and large there is a good concordance between the distribution of 5-HT_{2C} receptor mRNA and 5-HT_{2C} binding sites (Mengod et al., 1990b). One notable ex-

ception is that there is a high level of 5-HT_{2C} receptor mRNA in the lateral habenular nucleus whereas levels of 5-HT_{2C} binding sites are very low. The 5-HT_{2C} receptor may therefore be located presynaptically, at least in the case of projections from the habenula. It was recently reported that the distribution of 5-HT_{2C} receptor-like immunoreactivity also follows the binding data (Abramowski et al., 1995).

Although two studies have reported 5-HT_{2C} receptor mRNA in the midbrain raphe nuclei (Hoffman and Mezey, 1989; Molineaux et al., 1989), this was not confirmed in another study (Mengod et al., 1990b). However, both 5-HT_{2C} receptor mRNA and immunoreactivity have been found in the central grey which is adjacent to the DRN (Mengod et al., 1990b; Abramowski et al., 1995). Thus, whilst the 5-HT_{2C} receptor is clearly located postsynaptically, the possibility of a presynaptic location needs further study.

11.3. 5-HT_{2C} receptor pharmacology

The pharmacological profile of the 5-HT_{2C} receptor is close to but distinguishable from other members of the 5-HT₂ receptor family (Baxter et al., 1995). Most 5-HT₂ ligands (e.g. antagonists—ritanserin, LY 53857, mesulergine, mianserin; agonists—DOI, mCPP) do not discriminate sufficiently between the receptors (Hoyer et al., 1994). However, the 5-HT_{2B/2C} receptors can be distinguished from the 5-HT_{2A} receptor by their high affinity for SB 200646A and SB 206553, and their lower affinity for the antagonists MDL 100907, ketanserin and spiperone (Table 2). In addition, the novel compound SB 204741 is about 20–60-fold more selective for 5-HT_{2B} over the 5-HT_{2C} receptor. The most important recent event in the pharmacology of the 5-HT_{2C} receptor is the development of the selective antagonists SB 242084 and RS-102221 which are at least 2 orders of magnitude more selective for 5-HT_{2C} versus 5-HT_{2B}, 5-HT_{2A} and other binding sites (Bonhaus et al., 1997; Kennett et al., 1997a,b).

A number of atypical and typical antipsychotic agents (including clozapine, loxapine, and chlorpromazine, have a relatively high affinity for 5-HT_{2C} binding sites (5-HT_{2A} also) as do some conventional and atypical antidepressants (e.g. tricyclics, doxepin, mianserin and trazadone) (Canton et al., 1990; Roth et al., 1992; Jenck et al., 1993).

11.4. Functional effects mediated via the 5-HT_{2C} receptor

11.4.1. Signal transduction

Activation of the 5-HT_{2C} receptor increases phospholipase C activity in choroid plexus of various species (Sanders-Bush et al., 1988) and stably transfected cells (Julius et al., 1988) via a G-protein coupled mechanism

(for review see Boess and Martin, 1994). In the choroid plexus preparation, the non-selective 5-HT₂ receptor agonists, TFMPP, quipazine, DOM, mCPP, and MK 212 behave as agonists but only the latter compound had an efficacy equal to 5-HT (Conn and Sanders-Bush, 1987; Sanders-Bush et al., 1988). It has been suggested that 5-HT_{2C} receptors in choroid plexus may regulate CSF formation as a result of their ability mediate cGMP formation (Kaufman et al., 1995).

5-HT_{2C} receptors, in common with 5-HT_{2A} receptors, also down-regulate in response to chronic exposure to both agonists and antagonists, which could in part relate to apparent inverse agonist properties (Barker et al., 1994; Labrecque et al., 1995).

11.4.2. Electrophysiological responses

There is evidence for the 5-HT_{2C} receptor-mediated excitation of neurones in several brain regions. In particular, neurones of the rat substantia nigra reticulata *in vitro* are excited by 5-HT and this effect is blocked by ketanserin and methysergide but not spiperone or selective antagonists of 5-HT₁, 5-HT₃ or 5-HT₄ receptors (Rick et al., 1995). Activation of 5-HT_{2C} receptors also appears to depolarise pyramidal neurones in rat pyriform cortex (Sheldon and Aghajanian, 1991). Thus, the response of these neurones to 5-HT was blocked by spiperone, ritanserin and LY 53857 but at concentrations that appeared to be somewhat higher than those needed to block 5-HT_{2A} receptor-mediated responses (activation of interneurons) in the same preparation. It should be noted that the 5-HT-induced depolarisation of pyramidal neurones in rat neocortex is mediated via the 5-HT_{2A} receptor (Araneda and Andrade, 1991; Aghajanian and Marek, 1997).

Motoneurons of the facial nucleus *in vitro* and *in vivo* are activated by the local application of 5-HT and 5-HT₂ receptor agonists and this effect may be mediated via the 5-HT_{2C} receptor (for review see Aghajanian, 1995; Larkman and Kelly, 1991). Thus, in earlier *in vitro* studies the response of these neurones to 5-HT was blocked by methysergide (albeit at high concentrations) and not ketanserin or spiperone (Larkman and Kelly, 1991). In other studies, however, the excitation of facial motoneurons by 5-HT and other 5-HT agonists was blocked by ritanserin and spiperone (Aghajanian, 1995). However, the absence of 5-HT_{2A} receptor-like immunoreactivity in the rat facial nucleus (Morilak et al., 1993) argues against the involvement of 5-HT_{2A} receptor. Clearly the application of the newly developed ligands specific for the 5-HT₂ receptor subtypes should help resolve the issue.

11.4.3. Behavioural and other physiological responses

There are several behavioural responses that have been associated with activation of central 5-HT_{2C} receptors. These include hypolocomotion, hypophagia, anxi-

ety, penile erections and hyperthermia (see Koek et al., 1992 for review). To a large extent these associations are based on the behavioural effects in rats of non-selective 5-HT₂ receptor agonists such as mCPP, TFMPP and MK 212, and their antagonism by non-selective 5-HT₂ receptor antagonists, such as ritanserin and mianserin. Nevertheless, evidence for the involvement of the 5-HT_{2C} receptor in many of these *in vivo* responses is now compelling.

Thus, whilst the most commonly used agonists mCPP and TFMPP are partial agonists at the 5-HT_{2C} receptor, these drugs usually display antagonist properties at the 5-HT_{2A} receptor (Conn and Sanders-Bush, 1987; see Baxter et al., 1995). Also, those 5-HT_{2A} receptor antagonists tested to date (e.g. ketanserin) are generally inactive against mCPP-induced responses (see Koek et al., 1992), and at least in the case of mCPP-induced hypophagia and penile erection, there is a good correlation between the potency of antagonists to block these effects and their affinity for the 5-HT_{2C} and not the 5-HT_{2A} or other binding site (Berendsen et al., 1990; Kennett and Curzon, 1991). A role for the 5-HT_{2C} receptor in mCPP-induced hypophagia is further supported by evidence that 5-HT_{2C} knock out mice are overweight (Tecott et al., 1995). Importantly, mCPP-induced hypophagia, hypolocomotion and anxiety are antagonised by the 5-HT_{2C/2B} receptor antagonists, SB 200646A or SB 206553 (Kennett et al., 1994, 1996a,b). The selective 5-HT_{2C} receptor antagonist, SB 242084 also potently antagonises mCPP-induced hypolocomotion and hypophagia (Kennett et al., 1997a,b; Fig. 7). Somewhat surprisingly, RS-102221 does not antagonise the hypolocomotor response, possibly due to a restricted brain penetration (Bonhaus et al., 1997).

When administered alone, 5-HT_{2C} receptor antagonists are anxiolytic in various animal models (Kennett et al., 1996a,b, 1997a,b). Available evidence suggests that animals treated with these drugs do not over-eat or have a propensity for epileptic convulsions, even though both of these features are characteristic of 5-HT_{2C} knock out mice (Tecott et al., 1995). Thus, the abnormalities in the knock-out mice may be developmental in nature and not due to the loss in the adult of 5-HT_{2C} receptor function *per se* (Kennett et al., 1997a,b but see Bonhaus et al., 1997).

There are recent reports that 5-HT_{2C} antagonists increase the release of noradrenaline and dopamine in microdialysis experiments (Millan et al., 1998; Di Matteo et al., 1998). These data suggest that 5-HT_{2C} receptors exert a tonic inhibitory influence on mesocortical/mesolimbic dopaminergic and noradrenergic projections. In rats corticosterone and ACTH responses to mCPP and similar agonists may be mediated via the 5-HT_{2C} receptor (see Fuller, 1996). There is evidence that in humans, mCPP-induced prolactin secretion also involves 5-HT_{2C} receptor activation (see Cowen et al.,

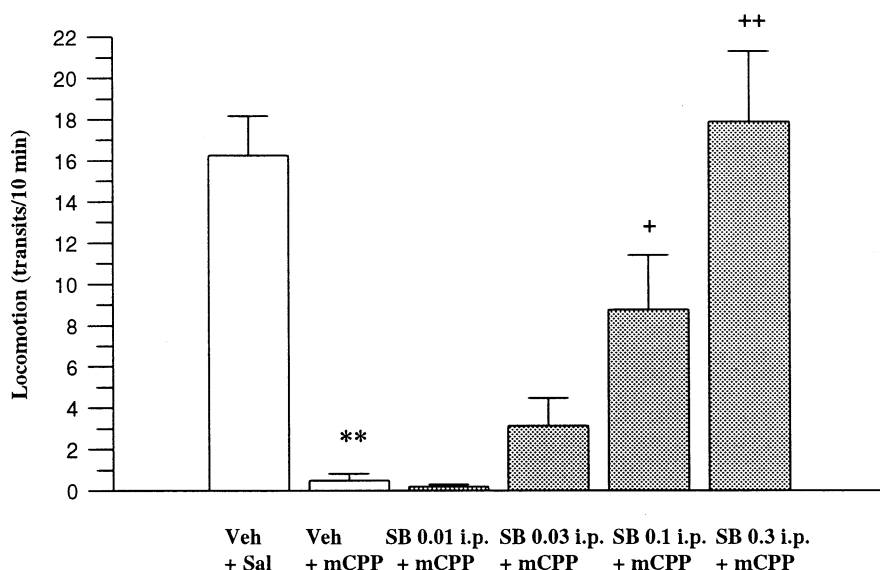


Fig. 7. Effect of the selective 5-HT_{2C} receptor antagonist, SB 242084, on mCPP-induced hypolocomotion in the rat. Drugs were injected 20 min (lower) or 30 min (upper) pre-test. mCPP was injected at 7 mg/kg i.p. and SB 242084 was injected at the doses (mg/kg i.p.) shown. ** $P < 0.01$, versus vehicle treatment. + $P < 0.05$ and ++ $P < 0.01$, versus mCPP alone. Data were taken from Kennett et al. (1997a,b) and kindly provided by Dr Guy Kennett, SmithKline Beecham, Harlow.

1996). Finally, in humans blockade of 5-HT_{2C} receptors is thought to increase slow wave sleep (Sharpley et al., 1994). The functional effects unequivocally associated with activation of central 5-HT_{2C} receptors are summarised in Table 8.

12. 5-HT₃ receptor

Responses mediated via the 5-HT₃ receptor have been documented for over half a century, although the nomenclature has been subject to modification. For instance, it is now appreciated that Gaddum's M receptor, responsible for indirect contraction of the guinea pig ileum, equates to the currently recognised 5-HT₃ receptor.

12.1. 5-HT₃ receptor structure

At the molecular level, the 5-HT₃ receptor is a ligand-gated ion channel (e.g. Derkach et al., 1989; Maricq et al., 1991) which is likely to be comprised of multiple subunits in common with other members of this superfamily (e.g. Cockcroft et al., 1990; Burt and Kanatchi, 1991; Heinemann et al., 1991; Barnes and Henley, 1992). However, to date, only one gene has been recognised which encodes a 5-HT₃ receptor subunit (5-HT_{3A} receptor subunit; Maricq et al., 1991) comprised of 487 amino acids which display highest levels of identity with other members of the Cys–Cys loop ligand-gated ion channel superfamily (e.g. nicotinic, GABA_A and glycine receptors).

The initial identification of the 5-HT_{3A} receptor subunit cDNA resulted from screening an expression library, derived from murine NCB-20 cells, for 5-HT-induced ion currents in *Xenopus* oocytes (Maricq et al., 1991). Subsequently, an alternatively spliced variant (5-HT_{3As}; the principle difference with respect to the long form is a deletion of six amino acids from the putative large intracellular loop; Hope et al., 1993) and species homologues have been reported (rat, guinea pig and human; Johnson and Heinemann, 1992; Isenberg et al., 1993; Belelli et al., 1995; Miyake et al., 1995; Lankiewicz et al., 1998). Interestingly, mRNA for the short form of the 5-HT_{3A} subunit (5-HT_{3As}) predominates (4–6-fold) in both mouse neuronal tissue and murine derived cell lines (Werner et al., 1994). Furthermore the splice acceptor site that results in the expression of the long form of the 5-HT_{3A} receptor subunit is

Table 8

Summary of the functional responses associated with activation of the brain 5-HT_{2C} receptor

Level	Response	Mechanism
Cellular	Phosphatidyl inositide turnover (+)	Post
Electrophysiological	Neuronal depolarisation	Post
Behavioural	Hypolocomotion	Post
	Hypophagia	Post
	Anxiogenesis	Post
	Penile erection	Post
Neurochemical	Noradrenaline/Dopamine release (-)	Post

absent in the human gene, indicating that this form is not expressed by humans (Werner et al., 1994).

The 5-HT binding site is associated with the extracellular N-terminal of the 5-HT_{3A} subunit (Eiselé et al., 1993) which would appear to be glycosylated. The receptor can be phosphorylated at a number of putative intracellular sites (Maricq et al., 1991; Hope et al., 1993; Belelli et al., 1995; Miyake et al., 1995; Lankiewicz et al., 1998), one of which is lost in the deletion associated with the short variant of the A subunit (e.g. Hope et al., 1993).

Analysis of the murine 5-HT₃ receptor gene demonstrates the presence of nine exons spanning approximately 12 Kbp of DNA, which range in size from 45 to 829 bp (Uetz et al., 1994). The expressed spliced variants of the 5-HT_{3A} receptor subunit derive from the variable use of two splice acceptor sites in exon 9, resulting in the deletion of the 18 nucleotides from the short form (Uetz et al., 1994). Detailed chromosomal mapping of the 5-HT_{3A} receptor gene has yet to be reported although the human gene maps to chromosome 11 (Uetz et al., 1994; Miyake et al., 1995).

Hydrophobicity analysis of the predicted amino acid sequence of both spliced variants of the 5-HT_{3A} receptor subunit indicate that it possesses four hydrophobic domains which may form α -helical membrane spanning regions (Hovius et al., 1998) analogous to the historically recognised secondary structure of proteins within this family (e.g. Ortells and Lunt, 1995). More recent studies with the nicotinic receptor, the closest family member to the 5-HT₃ receptor (for review see Ortells and Lunt, 1995), have questioned this secondary structure (Unwin, 1993). Thus, only the putative M2 membrane spanning region of the nicotinic subunit appears to form an α -helix (Unwin, 1993); this part of the subunit forms the lining of the ion channel which is gated by an inward kink at the hydrophobic leucine residue (Leu²⁵¹) which is well conserved by members of this receptor family, including the 5-HT_{3A} receptor subunit (Unwin, 1993, 1995). Despite the similarities in the primary structure of the 5-HT₃ receptor and the nicotinic receptor, however, recent evidence indicates the secondary structure of the murine homomeric 5-HT_{3A} receptor displays relatively more α -helical content, and less β -strand, compared to the nicotinic receptor from *Torpedo* (Hovius et al., 1998). Both functional and immunological investigations indicate that the N- and C-termini of the 5-HT_{3A} receptor subunit is extracellular (e.g. Eiselé et al., 1993; Mukerji et al., 1996).

Ultrastructural studies with the purified receptor from NG108-15 cells indicate that the quaternary structure of the 5-HT₃ receptor complex can be modelled as a cylinder with a gated central cavity, 3 nm in diameter, which results from the symmetrical assembly of five subunits (Boess et al., 1995).

The electrophysiological properties of the ion channel integral with the 5-HT₃ receptor complex have been reviewed extensively (e.g. Peters et al., 1992; Jackson and Yakel, 1995). The ion channel is cation selective (with near equal permeability to both Na⁺ and K⁺) and is prone to rapid desensitisation.

12.2. 5-HT₃ receptor distribution

A number of studies using various radioligands have mapped the distribution of 5-HT₃ receptors in the CNS. Highest levels of 5-HT₃ receptor binding sites are within the dorsal vagal complex in the brainstem (for review see Pratt et al., 1990). This region comprises the nucleus tractus solitarius, area postrema and dorsal motor nucleus of the vagus nerve which are intimately involved in the initiation and coordination of the vomiting reflex; antagonism of the 5-HT₃ receptors in these nuclei is therefore likely to contribute to the antiemetic action of 5-HT₃ receptor antagonists.

Relative to the dorsal vagal complex, 5-HT₃ receptor expression in the forebrain is low. Highest levels are expressed in regions such as the hippocampus, amygdala and superficial layers of the cerebral cortex. In addition to pharmacological differences, the available evidence indicates that the relative distribution of 5-HT₃ receptor recognition sites within the forebrain displays some species variation. For instance, within the human forebrain, relatively high levels of 5-HT₃ receptor recognition sites have been located within the caudate nucleus and putamen (Abi-Dargham et al., 1993; Bufton et al., 1993; Parker et al., 1996a) whereas relatively low levels are detected within cortical regions (Barnes et al., 1989a,b; Waeber et al., 1989; Abi-Dargham et al., 1993; Bufton et al., 1993; Parker et al., 1996a). This pattern of expression is reversed in rodents. The majority of species investigated so far, however, express high levels of 5-HT₃ receptors within the hippocampus relative to other forebrain regions (e.g. mouse, rat, man; Parker et al., 1996a).

In situ hybridisation studies indicate that 5-HT_{3A} receptor transcripts are similarly distributed in the rodent brain to radiolabelled 5-HT₃ receptor binding sites (e.g. piriform cortex, entorhinal cortex, hippocampus; Tecott et al., 1993). Within the hippocampal formation, mRNA is detected primarily within interneurons; this distribution indicating that the 5-HT₃ receptor may mediate the indirect inhibition of excitatory pyramidal neurons via activation of GABAergic interneurons (Tecott et al., 1993). This hypothesis was subsequently supported by reports of elegant studies from Bloom's laboratory. This group have developed a polyclonal antibody recognising the 5-HT₃ receptor (Morales et al., 1996b) and demonstrated, using double labelling, that 5-HT₃ receptor-like immunoreactivity is primarily associated with GABA containing neurons in the cere-

bral cortex and hippocampus (Morales et al., 1996a; Morales and Bloom, 1997) that often co-localise with CCK (but not somatostatin; Morales and Bloom, 1997). Further study indicated that the 5-HT₃ receptor-like immunoreactivity was located within a subpopulation of GABAergic interneurons that contain the Ca²⁺-binding protein calbindin (but not parvalbumin) and these are preferentially present in the CA1–CA3 fields of the hippocampus (Morales and Bloom, 1997).

Attempts to define the cellular location of the 5-HT₃ receptor expressed in the human basal ganglia indicate that they are not principally located on dopaminergic neurones since their density is not influenced by the neurodegeneration within this region associated with Parkinson's disease (Steward et al., 1993a). However, at least a significant population of the 5-HT₃ receptors in this region are associated with neurones that degenerate in Huntington's disease (Steward et al., 1993a). This disease is neuropathologically characterised by the degeneration of neurones that have their cell bodies within the caudate-putamen which include the GABAergic projection neurones (Bird, 1990).

12.3. 5-HT₃ receptor pharmacology

Pharmacological definition of responses mediated via the 5-HT₃ receptor has been facilitated by a large number of ligands that interact selectively with the receptor (e.g. the antagonists granisetron, ondansetron and tropisetron). It is well established, however, that the 5-HT₃ receptor displays some marked inter-species pharmacological differences. For instance, it has long been recognised that the selective 5-HT₃ receptor antagonist MDL 72222 and the agonist PBG display considerably lower affinity for the guinea pig variant of the 5-HT₃ receptor (e.g. Kilpatrick and Tyers, 1992; Lankiewicz et al., 1998). In addition, the affinity with which D-tubocurarine interacts with the mouse, rat, guinea pig and human 5-HT₃ receptor differs by over three orders of magnitude (e.g. Bufton et al., 1993), and the affinity of the partial agonist *m*-CPBG differs approximately 300-fold between rat and rabbit native 5-HT₃ receptors (Kilpatrick et al., 1991).

In addition to the recognition site for 5-HT, the 5-HT₃ receptor possesses additional, pharmacologically distinct, sites which mediate allosteric modulation of the receptor complex. For instance, electrophysiological data demonstrate that both ethanol and the active metabolite of chloral hydrate, trichloroethanol, increase the potency with which agonists activate the 5-HT₃ receptor complex (Lovinger and Zhou, 1993; Downie et al., 1995; for review see Parker et al., 1996b). Furthermore, some anaesthetic agents (in addition to trichloroethanol) also modify the function of the 5-HT₃ receptor (for review see Parker et al., 1996b). Indeed, it may be pertinent to note that many of the compounds

which appear to allosterically interact with the 5-HT₃ receptor also modulate the function of other members of the ligand-gated ion channel receptor superfamily (e.g. alcohols, anaesthetic barbiturates and steroids; e.g. Olsen, 1982; Miller et al., 1996; Lovinger et al., 1989; Lambert et al., 1990; Sieghart, 1992) which further emphasises the common ancestry of these receptors.

12.4. Are there additional 5-HT₃ receptor subunits? (See note added in proof)

Given the relatively long period of time which has elapsed since the disclosure of the cDNA for the 5-HT_{3A} receptor subunit, it would appear significant that only an alternatively spliced variant and species homologues of the 5-HT_{3A} receptor subunit have been reported (Maricq et al., 1991; Johnson and Heinemann, 1992; Isenberg et al., 1993; Belelli et al., 1995; Miyake et al., 1995; Lankiewicz et al., 1998). However, the minimal pharmacological and functional differences that have been reported when comparing the alternatively spliced variants of the 5-HT₃ receptor suggests their differences do not sufficiently account for the diversity of the properties of native 5-HT₃ receptors (e.g. Hope et al., 1993; Downie et al., 1995; Werner et al., 1994; Niemeyer and Lummis, 1996; see below). Whilst the failure to identify additional 5-HT₃ receptor subunits may indicate that they do not exist, other reasons may explain the apparent lack of success. For instance, additional 5-HT₃ receptor subunits may not form functional homomeric receptors, and/or they may display relatively low levels of sequence homology with the 5-HT_{3A} receptor subunit and are therefore unlikely to be detected by either expression cloning or sequence homology screening, respectively. Given the precedence of other ligand-gated ion channels, the presence of other 5-HT₃ receptor subunits remains attractive.

More important than mere precedence within this receptor family, however, are investigations providing direct evidence that certain populations of native 5-HT₃ receptor are unlikely to be simply homomeric 5-HT_{3A} receptor complexes. For instance, whilst it had been appreciated for some time that the major differences in conductances which were apparent with 5-HT₃ receptors from different sources (recombinant and native 5-HT₃ receptors from various species) may point to the presence of multiple distinct 5-HT₃ receptor subunits, it remained a possibility that this merely reflected inter-species differences analogous to the inter-species pharmacological differences (see above).

In an attempt to directly investigate this prospect, Hussy and colleagues (1994) performed a comparative study of the whole-cell and single-channel properties of 5-HT₃ receptors in three preparations; (i) heterologously expressed murine 5-HT_{3As} receptors (designated 5-HT_{3A} by the authors but actually representing the short variant of the 5-HT_{3A} receptor subunit; Hussy

et al., 1994); (ii) the murine neuroblastoma cell line N1E-115; and (iii) murine superior cervical ganglion neurones. Whilst no pharmacological differences could be detected between the different preparations, current–voltage relationships of whole-cell currents displayed inward rectification in all three preparations but the rectification was stronger in the N1E-115 cells and the cells expressing the recombinant 5-HT_{3As} receptor subunit (Hussy et al., 1994). Whilst the different membrane environments could account for these differences, stronger evidence towards a structural difference between the receptor complexes was forwarded when comparisons were made between the 5-HT₃ receptor mediated conductances. Thus, the conductances mediated by either the heterologously expressed 5-HT_{3As} receptor or the 5-HT₃ receptor expressed by N1E-115 cells could not be individually resolved; necessitating current fluctuation analysis to estimate the conductance (0.4–0.6 pS for both preparations). This led the authors to make the logical assumption that the 5-HT₃ receptors expressed in each of these two preparations are structurally similar, i.e. the 5-HT₃ receptor expressed by N1E-115 cells is a homomeric receptor (Hussy et al., 1994). In contrast, activation of 5-HT₃ receptors from mouse superior cervical ganglion resulted in resolvable individual channels of about 10 pS (Hussy et al., 1994). Interestingly, however, whole-cell noise analysis in this latter preparation resulted in a lower unitary conductance (3.4 pS) suggesting that these cells may express additionally 5-HT₃ receptors of lower conductance (e.g. homomeric 5-HT_{3A} or _{As} receptors; Hussy et al., 1994). A similar phenomenon was reported by Hille's group when investigating 5-HT₃ receptor channels expressed by rat superior cervical ganglion neurones (Yang et al., 1992). These latter authors also indicated that the most plausible explanation for their results was the presence of a low conductance 5-HT₃ receptor, comparable to that expressed in N1E-115 cells, in addition to the 5-HT₃ receptor mediating the relatively high conductance (Yang et al., 1992). Furthermore, hippocampal neurones from both mice and rats express 5-HT₃ receptors with high conductances (approximately 10 pS; Jones and Surprenant, 1994). The corollary of these studies is that not all native 5-HT₃ receptors are structurally similar to the low conductance recombinant 5-HT_{3A} receptor nor the 5-HT₃ receptor expressed by neuroblastoma cell lines (e.g. N1E-115, N18 and NCB-20 cells; for review see Peters et al., 1992); although the conductance of 5-HT₃ receptors expressed by NG108-15 cells, which, similar to NCB-20 cells, are derived from N18 cells [see Kelly et al., 1990], would appear to be intermediate [approximately 4 pS; for review see Peters et al., 1992]. Whilst the conductance of the 5-HT₃ receptor has been shown to be influenced by post-translational modifications such as phosphorylation (Van Hooft and Vijverberg, 1995) and site-directed

mutagenesis studies indicate that minor structural changes may have a major functional effect (single amino acid substitutions; e.g. Yakel et al., 1993), efforts continue in the search for an additional 5-HT₃ receptor subunit(s), or the presence of a modulatory protein associated with the 5-HT₃ receptor (analogous to proteins associated with other members of the ligand gated ion channel family; e.g. Yu et al., 1997), which may contribute to the functional diversity of the 5-HT₃ receptor.

Of direct relevance to the existence of multiple 5-HT₃ receptor subunits (or an associated modulatory protein since these may be co-purified with the receptor), SDS-PAGE analysis of the 5-HT₃ receptor protein purified from pig cerebral cortex has revealed multiple protein bands with differing molecular weights; not all of these are recognised by a polyclonal antibody raised against a 128 amino acid polypeptide which represents the putative long intracellular loop of the 5-HT_{3A} receptor subunit (Fletcher and Barnes, 1997). It is unlikely that these proteins represent cleaved 5-HT_{3A} or _{As} receptor subunits which have lost their putative large intracellular loop since these potential fragments would be considerably smaller (≤ 35 kDa) than the detected non-5-HT_{3A} proteins (Fletcher and Barnes, 1997; for review see Fletcher and Barnes, 1998).

These non-5-HT_{3A} like protein bands might represent an additional subunit(s) of the 5-HT₃ receptor which, although not influencing the pharmacology of the ligand gated ion channel, may affect its conductance, and hence represents a structural subunit for which there are numerous precedents within the ligand-gated ion channel superfamily (e.g. Ortells and Lunt, 1995). Alternatively, the non-5-HT_{3A}-like protein may be an accessory protein which co-purifies with the 5-HT₃ receptor. Again a number of precedents are available such as the 43 kDa protein of the nAChR which is involved in receptor clustering (e.g. Froehner et al. 1990) or the 93 kDa protein gephyrin, which is likely to anchor the glycine receptor to microtubules in the postsynaptic membrane (e.g. Kirsch et al. 1991), or an endogenous tyrosine kinase such as that which co-purifies with, and modulates the function of, the NMDA receptor (Yu et al. 1997).

A further potential explanation is that the non-5-HT_{3A}-like proteins may be a subunit(s) from another ligand gated ion channel which displays sufficient promiscuity to assemble with the 5-HT_{3A} receptor subunit, or indeed, may have been wrongly assigned to a different neurotransmitter receptor family. It is therefore of interest that a recent report demonstrates that in a heterologous expression system, the 5-HT_{3A} receptor is able to co-assemble with the α_4 subunit of the nicotinic acetylcholine receptor to confer Ca²⁺ permeability on the channel which displays 5-HT₃ receptor pharmacology (Van Hooft et al., 1998). Indeed, Ca²⁺ perme-

ability has also been shown to distinguish native central 5-HT₃ receptors from those expressed by NG108-15 cells (Rondé and Nichols, 1998; Fig. 9). However, at least in pig brain, native 5-HT₃ receptors do not appear to contain the α_4 nicotinic receptor subunit (nor the α_1 , α_3 , α_5 , α_7 or β_2 nicotinic receptor subunits; Fletcher et al., 1998) but the presence of another structural component within (or associated with) the 5-HT₃ receptor may confer this property of central 5-HT₃ receptors.

12.5. Functional effects mediated via the 5-HT₃ receptor

Most of the initial evidence indicating the presence of functional 5-HT₃ receptors in the brain were reports of the behavioural effects of 5-HT₃ receptor ligands (mostly antagonists) although it is now appreciated that this receptor is the only monoamine receptor to be associated with fast synaptic transmission in the brain (Sugita et al., 1992). The behavioural pharmacology of the 5-HT₃ receptor antagonists initially generated much optimism in the search for novel psychotropic agents. Thus, 5-HT₃ receptor antagonists were forwarded as potential therapeutic agents for a number of CNS disorders including anxiety, cognitive dysfunction and psychosis (for review see Bentley and Barnes, 1995). However, most of the clinical reports do not substantiate the predicted efficacy from the preclinical investigations (for review see Bentley and Barnes, 1995). Furthermore, a number of the preclinical behavioural actions of 5-HT₃ receptor antagonists remain controversial (see Greenshaw, 1993; Bentley and Barnes, 1995). Subsequent to the initial behavioural reports, a number of neurochemical and electrophysiological responses mediated via central 5-HT₃ receptors were documented; some of these responses were proposed as mechanisms underlying the behavioural actions of the 5-HT₃ receptors ligands. Since the behavioural pharmacology of 5-HT₃ receptor ligands has been reviewed extensively (e.g. Costall and Naylor, 1992; Greenshaw, 1993; Bentley and Barnes, 1995) this will not be described in detail here.

The generation and use of 5-HT_{3A} receptor knock-out mice has, so far, not contributed much information concerning the function of 5-HT₃ receptors. However, a preliminary report indicates that the behavioural response to certain forms of pain is reduced in these animals (Guy et al., 1997).

12.6. Functional actions of the 5-HT₃ receptor relevant to anxiety

In a variety of animal models, a range of structurally unrelated 5-HT₃ receptor antagonists display the potential to reduce levels of anxiety, although they do not generally induce a benzodiazepine receptor agonist-like

response in conflict models (for review see Bentley and Barnes, 1995). One of the initial compounds to be systematically investigated was ondansetron (Jones et al., 1988). This compound displayed activity in models utilising the mouse (light–dark test), rat (social interaction, elevated plus maze) and marmoset (human threat test).

On the basis of results from central injections of 5-HT₃ receptor ligands, the amygdala has been forwarded as a site of action. This brain region expresses relatively high levels of the 5-HT₃ receptor (e.g. Steward et al., 1993) and direct intra-amygdaloid injection of 5-HT₃ receptor agonists and antagonists increase and decrease, respectively, aversive-like behaviour in animals (Costall et al., 1989; Higgins et al., 1991a).

The search for potential neurochemical mechanisms underlying the ability of the 5-HT₃ receptor to modulate anxiety-like behaviour in animals have forwarded a number of candidate neurotransmitters.

It has long been recognised that 5-HT function in the brain is associated closely with animal behaviours indicative of anxiety (e.g. Andrews and File, 1993; Cadogan et al., 1994; Andrews et al., 1997; for reviews see Iversen, 1984; Handley and McBlane, 1993), and the 5-HT₃ receptor modulates the release of 5-HT in relevant regions of the brain. Thus, using the *in vivo* microdialysis technique to estimate 5-HT release in the rat hippocampus, Martin and colleagues (1992) demonstrated that 5-HT₃ receptor activation enhances the release of 5-HT. Similarly, in slices of guinea pig hippocampus (and frontal cortex and hypothalamus), 5-HT₃ receptors enhance electrically evoked [³H]5-HT release (Blier et al., 1993). It is noteworthy, however, that at least with respect to the modulation of 5-HT release in the guinea pig hypothalamus, the 5-HT₃ receptor does not appear to be directly located on the 5-HT nerve terminal. Thus the 5-HT₃ receptor-mediated modulation of K⁺-stimulated [³H]5-HT release in slices is prevented by the inclusion of the sodium channel blocker tetrodotoxin (Blier et al., 1993) and furthermore, the response is not detected in synaptosomal preparations (Williams et al., 1992; Blier et al., 1993).

Another neurotransmitter that is prone to modulation via the 5-HT₃ receptor, which may be relevant to the anxiolytic-like action of 5-HT₃ receptor antagonists, is the peptide cholecystokinin (CCK). Increases in central CCK function in both laboratory animals and man manifests anxiety and panic (for reviews see Harro et al., 1993; Bradwejn and Koszycki, 1994) and therefore the ability of the 5-HT₃ receptor to increase CCK release may be relevant to the alteration in behaviour. The initial data implicating 5-HT/CCK interactions originated largely from Raiteri's laboratory. In their studies, 5-HT₃ receptor activation induces a concentration-dependent increase in K⁺-stimulated CCK-like immunoreactivity from synaptosomes prepared from

either the rat cerebral cortex or nucleus accumbens (Paudice and Raiteri, 1991). It should be noted, however, that the potency of agonists in this *in vitro* model is higher than that associated with most other responses mediated via the 5-HT₃ receptor. This may provide further evidence towards the presence of 5-HT₃ receptor subtypes. In addition to modulating CCK release *in vitro*, the response is detectable *in vivo* since 5-HT₃ receptor antagonists inhibit the veratridine-induced release of CCK-like immunoreactivity in the rat frontal cortex assessed by the microdialysis technique (Raiteri et al., 1993). The activity of antagonists in this latter model indicates that the tone on the 5-HT₃ receptor modulating CCK release is high, which may correlate with the relatively high potency of agonists to modulate CCK release *in vitro*. Given the ability of the 5-HT₃ receptor to modulate CCK release, it is relevant that studies performed by Morales and Bloom (1997) demonstrated the co-expression of 5-HT_{3A} receptor subunits and CCK by neurones in the cerebral cortex and hippocampus (Fig. 8).

12.7. Functional actions of the 5-HT₃ receptor relevant to cognition

It has long been established that the central 5-HT system is implicated in cognitive function. Much of the early work forwarded contradictory data (for review see Altman et al., 1987), although with hindsight, it is simple to interpret the conflicting data by the different functions associated with distinct 5-HT receptor subtypes.

The initial work assessing the ability of the 5-HT₃ receptor to modify cognitive processing was performed in Costall and Naylor's laboratory. This research group utilised three different species (mouse, rat and marmoset) in their attempts to influence cognitive performance via selective antagonism of the 5-HT₃ receptor. One of their earlier reports demonstrated that ondansetron not only enhanced the cognitive performance of normal laboratory animals, but, perhaps more significantly, was also able to overcome the cognitive deficit following lesion of the central cholinergic system (e.g. Barnes et al., 1990d; Carey et al., 1992). The degeneration of this latter system is widely believed to be associated with cognitive impairment in man (e.g. Bartus et al., 1982) although other neurotransmitter sys-

tems are also likely to contribute to the cognitive impairments associated with some neuropathological disorders (e.g. Alzheimer's disease, Parkinson's disease, Huntington's disease; e.g. Price et al., 1990).

Subsequently, Costall and Naylor's group extended their findings to other 5-HT₃ receptor antagonists and a number of other groups have similarly demonstrated that 5-HT₃ receptor antagonists facilitate cognitive processes (for review see Bentley and Barnes, 1995). Not all groups, however, report similar findings (e.g. see Riekkinen et al., 1991; for review see Bentley and Barnes, 1995) although the reasons for the apparently contrasting reports are difficult to rationalise since they often arise when different tests are employed. In addition, the bell-shaped dose response curve associated with some 5-HT₃ receptor antagonists clearly complicates dose selection and subsequent interpretation of negative data from studies when only a limited number of doses have been investigated.

As well as the behavioural data implicating that the 5-HT₃ receptor antagonists improve cognitive performance in animal models, additional findings using neurochemical and electrophysiological techniques may provide a mechanism underlying the modulation of this animal behaviour. For instance, a role of acetylcholine in learning and memory has been recognised for many years (e.g. Bartus et al., 1982), and it is therefore relevant that 5-HT₃ receptor activation inhibits the release of cortical acetylcholine (Barnes et al., 1989a,b; Bianchi et al., 1990; Maura et al., 1992; Ramírez et al., 1996; Crespi et al., 1997; Díez-Ariza et al., 1998; but see Johnson et al., 1993). However, some evidence is available to suggest that the inhibition of acetylcholine release mediated via the 5-HT₃ receptor is not a direct interaction (Bianchi et al., 1990; Ramírez et al., 1996; Díez-Ariza et al., 1998 for review see Peters et al., 1992; although see also Maura et al., 1992; Crespi et al., 1997), but mediated via GABA (Ramírez et al., 1996; Díez-Ariza et al., 1998). This latter neurochemical finding being consistent with the knowledge that GABAergic neurones express the 5-HT₃ receptor (Morales et al., 1996a; Morales and Bloom, 1997; Fig. 8) as well as electrophysiological data demonstrating a facilitation of GABA release following 5-HT₃ receptor activation (see below). The administration of a 5-HT₃ receptor antagonist, therefore, would remove a potential inhibitory tone on the cholinergic neurone resulting in a net

Fig. 8. Simultaneous detection of 5-HT₃ receptor mRNA and GABA immunoreactivity in layer II of parietal cortex (i) and CCK immunoreactivity in the hippocampus (ii). (i) (A) Observation of 5-HT₃ receptor-expressing cells under epiluminescence microscopy. (B) Observation of GABA-immunoreactive neurones under bright-field microscopy. In this brain section, all 5-HT₃ receptor expressing cells showed GABA immunoreactivity (filled arrows in A and B), but not all GABA immunoreactive neurones contained 5-HT₃ receptor transcripts (open arrows in B). Scale bar, 25 μ m. (ii) (A) Observation of a 5-HT₃ receptor/CCK double-labelled cell in the CA1 stratum radiatum (SR) extending into the stratum lacunosum moleculare (SLM). (B) Observation of a 5-HT₃ receptor/CCK double-labelled cell in the CA1 stratum pyramidale (SP) extending into the cell layer. (C) Two 5-HT₃ receptor/CCK double-labeled neurones immediately below the stratum granulare in the dentate gyrus (SG); one of them projects into the granule cell layer (arrow). Scale bar, 6 μ m. Reproduced from Morales and Bloom (1997), with permission.

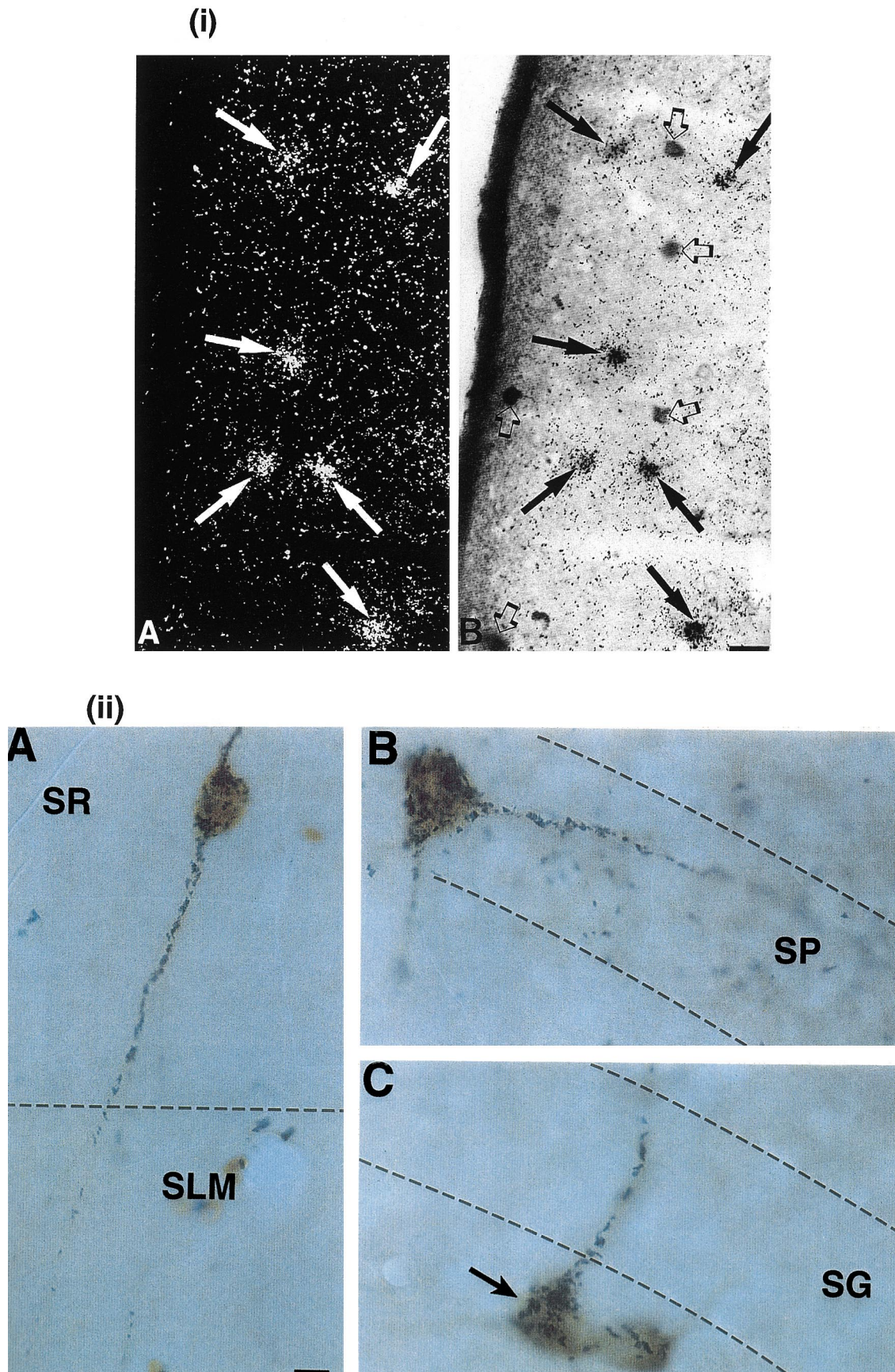


Fig. 8. (Caption on previous page)

increase in acetylcholine release-consistent with the action of a cognitive enhancer as defined by the cholinergic hypothesis of memory (Bartus et al., 1982). Indeed, such an action by 5-HT₃ receptor antagonists has been reported (e.g. Ramírez et al., 1996; Díez-Ariza et al., 1998). It should be noted, however, that a recent report demonstrated a facilitation of acetylcholine release in the rat hippocampus following 5-HT₃ receptor activation (Consolo et al., 1994a,b). This conflicting response, and the indirect mediation of the 5-HT₃ receptor-mediated inhibition of acetylcholine release may explain the apparently negative data that has been reported (Johnson et al., 1993).

Electrophysiological data also supports a role of the 5-HT₃ receptor in the modulation of learning processes. It is generally accepted that the phenomenon of long term potentiation (LTP) provides a cellular basis for memory (e.g. Collingridge and Singer, 1990), and it is therefore of interest that in the hippocampus, the induction of LTP is inhibited following activation of the 5-HT₃ receptor (Corradetti et al., 1992; Maeda et al., 1994; Passani et al., 1994; Staubli and Wu, 1995). This response may be mediated via activation of GABAergic inter-neurons (Corradetti et al., 1992; Maeda et al., 1994) which is again consistent with the studies demonstrating a direct association between GABA neurones and the 5-HT₃ receptor in the hippocampus (e.g. Ropert and Guy, 1991; Tecott et al., 1993; Kawa, 1994; Piguet and Galvan, 1994; Morales et al., 1996a,b; Morales and Bloom, 1997; Fig. 8). Additional evidence indicates that the 5-HT₃ receptor reduces glutamate-mediated synaptic neurotransmission (Zeise et al., 1994), which, given the primary role of glutamate in the expression of LTP (e.g. Collingridge and Singer, 1990), is consistent with the ability of the 5-HT₃ receptor to inhibit the induction of LTP.

12.8. Association of the 5-HT₃ receptor with dopamine function in the brain

Behavioural, neurochemical and electrophysiological investigations indicate that the 5-HT₃ receptor modulates dopamine neurone activity in the brain. Indeed, the reduction in central dopamine function following administration of 5-HT₃ receptor antagonists largely underpins the hypothesis that these agents possess antipsychotic potential and the ability to reduce the rewarding effects of certain drugs of abuse.

As an example of the ability of the 5-HT₃ receptor to modulate central dopamine function, 5-HT₃ receptor antagonists prevent the behavioural hyperactivity following an increase in extracellular dopamine levels in the nucleus accumbens, induced by a variety of pharmacological manipulations (e.g. intra-accumbens infusion of exogenous dopamine or amphetamine or stimulation of mesolimbic dopamine neurones follow-

ing intra-VTA injection of the neurokinin agonist, DiMe-C7; for review see Bentley and Barnes, 1995). Indeed, the nucleus accumbens is likely to provide a major site of action for 5-HT₃ receptor antagonists to reduce accumbens dopamine-mediated hyperactivity since direct injection of 5-HT₃ receptor antagonists into this brain region similarly prevents the hyperactivity (Costall et al., 1987). Furthermore, intra-accumbens administration of the 5-HT₃ receptor agonist, 2-methyl-5-HT, potentiated the hyperactivity resulting from intra-accumbens amphetamine; a response which was prevented by the combined administration of the 5-HT₃ receptor antagonist ondansetron (Costall et al., 1987).

Neurochemical studies also support a facilitatory role of the 5-HT₃ receptor with respect to central dopaminergic function. Thus dopamine release is increased from slices of rat nucleus accumbens (DeDeurwaerdere et al., 1998) and striatum (Blandina et al., 1988, 1989) following 5-HT₃ receptor activation. This latter response is consistent with some behavioural data where intra-striatal injection of 5-HT₃ receptor agonists induces contra-lateral turning (Bachy et al., 1993). It should also be noted, however, that other reports indicate that the 5-HT₃ receptor agonist phenylbiguanide (PBG) elevated extracellular dopamine levels in rat striatal slices via an interaction with the dopamine reuptake channel rather than the 5-HT₃ receptor (e.g. Schmidt and Black, 1989) and also 5-HT₃ receptor expression in the striatum is relatively low, and often undetectable (for review see Bentley and Barnes, 1995) although a subpopulation (approximately 5%) of striatal synaptosomes appear to express functional 5-HT₃ receptors (Nichols and Mollard, 1996; Rondé and Nichols, 1998; Fig. 9).

Electrophysiological evidence also indicates that the 5-HT₃ receptor modulates dopamine neurone activity. In an initial study, Sorensen et al. (1989) demonstrated that chronic (but not acute) administration of the 5-HT₃ receptor antagonist dolasetron induced a significant reduction in the number of spontaneously active dopamine neurones in the VTA and substantia nigra compacta. In addition, acute administration of the 5-HT₃ receptor antagonists LY277359 and granisetron potentiate the suppressant action of apomorphine on VTA but not substantia nigra compacta dopamine neurones (Minabe et al., 1991). A further study by the same group demonstrated that either acute or chronic administration of the selective 5-HT₃ receptor antagonist BRL46470 reduced the firing of VTA dopamine neurones although minor modifications in the dose of the antagonist resulted in a facilitation of the firing rate (Ashby et al., 1994a,b). In a separate study, however, acute administration of another 5-HT₃ receptor antagonist, DAU6215, did not modify dopamine neurone firing in either the VTA or substantia nigra compacta nor did it potentiate the suppressant action of apomorphine (Prisco et al., 1992). DAU6215 did, however,

selectively reduce the firing rate of VTA dopamine neurones following chronic administration (Prisco et al., 1992). These latter findings are consistent with evidence that 5-HT₃ receptor antagonists, given acutely, do not alter *basal* dopamine metabolism or release in the nigrostriatal or in the mesolimbic dopaminergic system (e.g. Imperato and Angelucci, 1989; Koulou et al., 1989). The functional effects associated with the 5-HT₃ receptor are summarised in Table 9.

13. 5-HT₄ receptor

The 5-HT₄ receptor was initially identified in cultured mouse colliculi neurones and guinea pig brain by Bockaert and co-workers using a functional assay-stimulation of adenylate cyclase activity (Dumuis et al., 1988; Bockaert et al., 1990). Similar and additional functional responses mediated via the 5-HT₄ receptor were subsequently demonstrated in various peripheral tissues (for review see Ford and Clarke, 1993). It should be noted, however, that the ability of 5-HT to stimulate adenylate cyclase in the brain had been appreciated for a number of years previous to the pharmacological definition of the 5-HT₄ receptor (e.g. von Hungren et al., 1975; Fillion et al., 1975); although the relatively high concentration of methysergide (and some other compounds inactive at 5-HT₄ receptors) needed to antagonise these responses casts doubt over the involvement of 5-HT₄ receptors (possible involvement of 5-HT₆/5-HT₇ receptors?).

13.1. 5-HT₄ receptor structure

The cDNA encoding the rat 5-HT₄ receptor was identified using degenerate PCR primers based on the

Table 9
Summary of the functional responses associated with activation of the brain 5-HT₃ receptor

Level	Response	Mechanism
Cellular	Cation channel (+)	Post
	Electrophysiological	Depolarisation
Behavioural	LTP (-)	Post
	Anxiolysis (antagonist)	Post
	Cognition (+ antagonist)	Post
	Locomotion (- antagonist)	Post
Neurochemical	Reward (- antagonist)	Post
	5-HT release (+)	Post (indirect?)
	Acetylcholine release (-)	Post indirect
	GABA release (+)	Post
	CCK release (+)	Post
	Dopamine release (+)	Post

highly conserved sequences of nucleotide bases encoding the putative III and V transmembrane domains of G-protein coupled 5-HT receptors. These degenerate oligonucleotides identified a novel cDNA fragment in a rat brain cDNA library which was used to identify two corresponding full length cDNA sequences (Gerald et al., 1995). Hydrophobicity analysis of the deduced polypeptide sequences (387 and 406 amino acids in length) indicated that they displayed the seven putative transmembrane domain topology of G-protein coupled receptors. In addition, since the amino acid sequences were identical up to position 360 (within the putative intracellular C-terminus), the two species were likely to arise due to alternative splicing of the mRNA. Hence the two species were designated 5-HT_{4S} and 5-HT_{4L} for the short and long form of the receptor, respectively (Gerald et al., 1995), although following recommendations from the IUPHAR receptor nomenclature committee, these alternatively spliced variants have now been re-named 5-HT_{4(a)} and 5-HT_{4(b)} for the short (5-HT_{4S}) and long (5-HT_{4L}) form of the receptor, respectively (Hoyer and Martin, 1997). It should be noted, however, that a recent report questions the length of the long form of the 5-HT₄ receptor since Van den Wyngaert et al. (1997) identified an open reading frame that corresponded to a polypeptide of 388 amino acids, rather than 406 amino acids (Gerald et al., 1995). This difference would appear due to a frame shift which introduces an additional cytosine and this portion of the sequence was subsequently confirmed in both rat and human genomic DNA (Van den Wyngaert et al., 1997).

Two additional splice variants of the 5-HT₄ receptor have been identified in tissues from mouse, rat and human, 5-HT_{4(c)} and 5-HT_{4(d)} (Blondel et al., 1998a; Bockaert et al., 1998). The 5-HT_{4(c)} and 5-HT_{4(d)} receptor variants encode polypeptide sequences of 380 and 360 amino acids, respectively, with all four isoforms diverging after Leu³⁵⁸ (Blondel et al., 1998a; Bockaert et al., 1998).

The rank order pharmacology of each of the four human receptor isoforms is similar, although renzapride appears to behave as a partial agonist (cAMP formation) in cells expressing the 5-HT_{4(c)} receptor despite acting as a full agonist at the other three receptor isoforms (Blondel et al., 1998a). Furthermore, the 5-HT_{4(c)} receptor was the only isoform to display constitutive activation of adenylate cyclase in a transient artificial expression system (Blondel et al., 1998a,b).

RT-PCR studies indicate that the 5-HT_{4(a)}, 5-HT_{4(b)} and 5-HT_{4(c)} receptor isoforms are expressed in the brain (and atrium and gut), whereas expression of the 5-HT_{4(d)} receptor isoform was only detected in the gut (Blondel et al., 1998a). Clearly it would be of

interest to determine if the receptor isoforms are differentially expressed in different regions of the brain and if a diversity of function can be attributed to the different products arising from the 5-HT₄ receptor gene; which has been mapped to the long arm of human chromosome 5 (5q31–q33; Claeysen et al., 1997a,b,c; Cichol et al., 1998). Whilst details concerning the structure of the 5-HT₄ receptor gene have yet to be reported, the gene would appear to be highly fragmented; comprising at least five introns (Bockaert et al., 1998).

A further deviation from the original report (Gerald

et al., 1995) is the now recognised presence of a consensus sequence (LVMP) within the 5-HT₄ receptor (Claeysen et al., 1996, 1997a,b,c; Blondel et al., 1997, 1998a; Van den Wyngaert et al., 1997) which is present within the putative second transmembrane region of all G-protein coupled 5-HT receptors further linking the origin of these receptors.

All the 5-HT₄ receptor isoforms contain consensus sequences indicating that they are subject to post-translational modification. Thus they contain a putative N-linked glycosylation site in the N-terminal putative

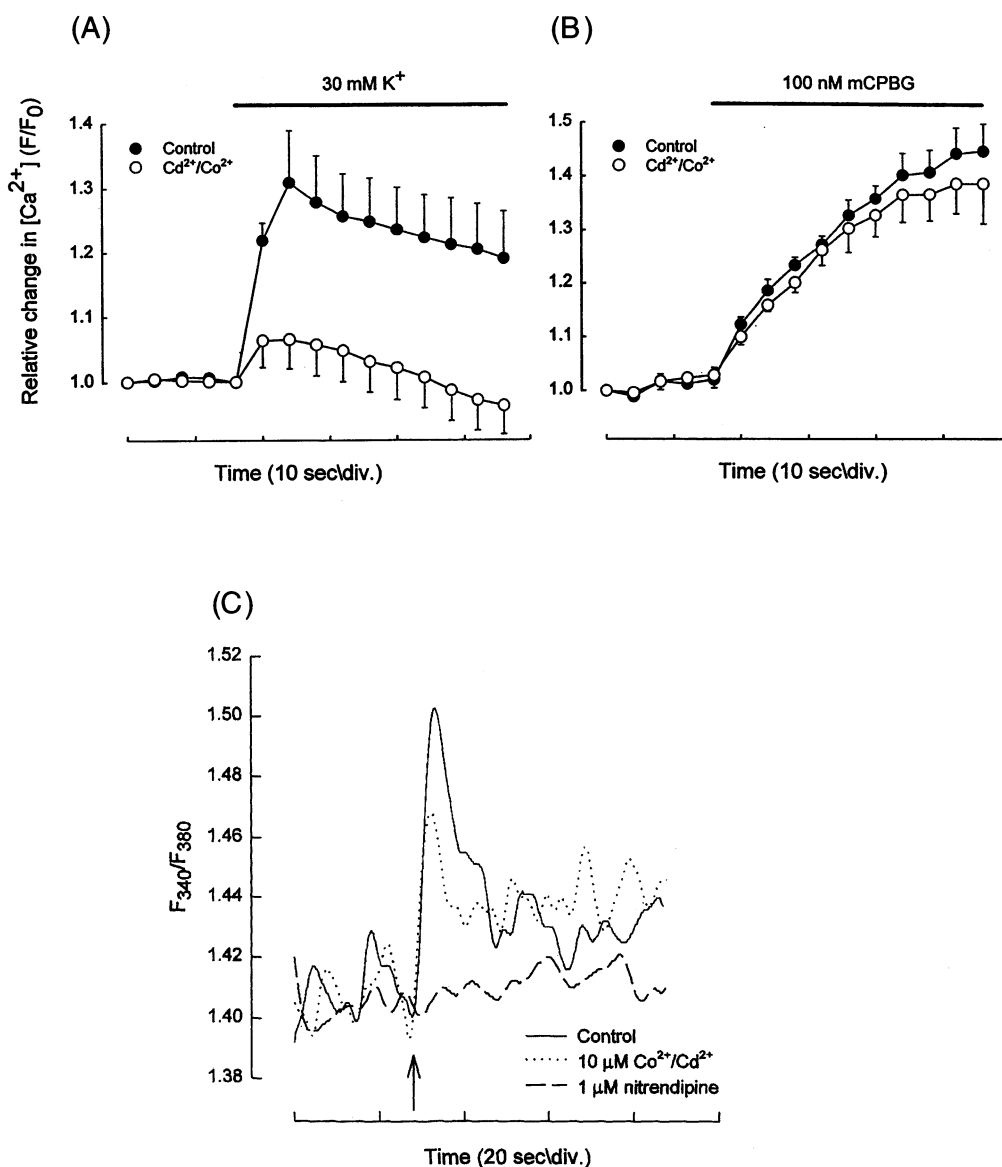


Fig. 9. Differential ability of the inorganic Ca^{2+} channel blockers, Cd^{2+} and Co^{2+} (both 10 μM), to block increases in $[Ca^{2+}]_i$ in individual striatal synaptosomes (rat) in response to K^+ -induced depolarisation (30 mM; A) or the 5-HT₃ receptor agonist *meta*-chlorophenylbiguanide (mCPBG, 100 nM; B) and their ability to attenuate the mCPBG (1 μM)-induced increase in $[Ca^{2+}]_i$ in undifferentiated NG108-15 cells (C). (A and B) Results from summarized data are mean \pm S.E.M., $n=4$ experiments (three to nine synaptosomes analysed per experiment). (C) Representative traces of changes in relative $[Ca^{2+}]_i$ levels, as the ratio of the fluorescence emitted at 510 nm in response to excitation at 340 and 380 nm (F_{340}/F_{380}). Also shown is the ability of the L-type Ca^{2+} channel antagonist, nitrendipine, to prevent the mCPBG-induced response. Bars (A and B) or arrow (C) indicates application of K^+ or mCPBG. Reproduced from Rondé and Nichols (1998), with permission.

extracellular domain (Gerald et al., 1995; Claeysen et al., 1996, 1997a,b,c; Blondel et al., 1997, 1998a; Van den Wyngaert et al., 1997), and a number of putative protein kinase C mediated phosphorylation sites located within the putative third intracellular loop and C-terminus. In addition, the C termini are rich in serine and threonine residues (Gerald et al., 1995; Claeysen et al., 1996, 1997a,b,c; Blondel et al., 1997, 1998a; Van den Wyngaert et al., 1997) which, consistent with the functional data (e.g. Ansanay et al., 1992), may provide targets for phosphorylation.

13.2. 5-HT₄ receptor distribution

Derivatives of a number of 5-HT₄ receptor ligands have made useful radioligands to map and pharmacologically characterise the 5-HT₄ receptor (e.g. [³H]GR113808, [¹²⁵I]SB207710, [³H]BIMU1). A consistent finding across the species investigated so far is the presence of relatively high levels of the 5-HT₄ receptor in the nigrostriatal and mesolimbic systems of the brain (rat, guinea pig, pig, cow, monkey, man e.g. Grossman et al., 1993; Waeber et al., 1993; Doménech et al., 1994; Jakeman et al., 1994; Schiavi et al., 1994; Patel et al., 1995; Mengod et al., 1996). A similar relative distribution of 5-HT₄ receptor mRNA in the rat CNS has been described (Gerald et al., 1995; Claeysen et al., 1996; Mengod et al., 1996).

In the initial report, there appeared to be a marked differential distribution of the two alternatively spliced 5-HT₄ receptor transcripts within the rat brain, determined by RT-PCR, following dissection of the brain areas; the 5-HT_{4(b)} receptor transcripts being expressed throughout the brain (including the striatum, but not the cerebellum), whereas the 5-HT_{4(a)} receptor transcripts were restricted to the striatum (Gerald et al., 1995). This anatomically distinct distribution may underlie functional differences of the expressed spliced variants of the 5-HT₄ receptor, however, a more recent study has failed to confirm this differential distribution of the alternatively spliced variants in either neonate mouse brain or adult mouse and rat brain (Claeysen et al., 1996).

13.3. 5-HT₄ receptor pharmacology

Research aimed at identifying 5-HT₄ receptor mediated responses has been greatly facilitated by the availability of a number of highly selective antagonists (e.g. GR113808, SB204070). These compounds, in addition to a range of less selective ligands, have allowed the pharmacological profile of the 5-HT₄ receptor in a number of species to be determined; such studies indicate that the pharmacology across species is well preserved.

13.4. Functional effects mediated via the 5-HT₄ receptor

13.4.1. Transduction system

In common with native 5-HT₄ receptors, heterologously expressed receptors couple positively to adenylate cyclase (Gerald et al., 1995; Claeysen et al., 1996; Van den Wyngaert et al., 1997). To date, no significant pharmacological differences between the spliced variants of the 5-HT₄ receptor have been reported, although differences in the efficiency with which the spliced variants couple to their transduction system might be inferred given that the divergence between the spliced variants is at the C-terminus (Gerald et al., 1995; Claeysen et al., 1996; Van den Wyngaert et al., 1997; Blondel et al., 1998a; Bockaert et al., 1998), which is known to be involved in the receptor coupling to the G-protein and modification in receptor function following phosphorylation. Indeed, phosphorylation of native 5-HT₄ receptors expressed by both neurones and smooth muscle would appear to be largely responsible for the desensitisation of the 5-HT₄ receptor (Ansanay et al., 1992; Rondé et al., 1995). The latter process is analogous to β -adrenoreceptor desensitisation, with prolonged agonist exposure (> 30 min) also inducing sequestration of the 5-HT₄ receptor (Ansanay et al., 1992, 1996).

Elegant studies have demonstrated that the 5-HT₄ receptor-mediated increase in cAMP levels lead to the phosphorylation of a range of target proteins by, for instance, cAMP-dependent protein kinase (e.g. phosphorylation of voltage-gated K⁺ channels leading to their closure, Fagni et al., 1992). Hence, for example, following activation of the neuronally located 5-HT₄ receptor, increased neuronal excitability and slowing of repolarisation can be detected electrophysiologically (Chaput et al., 1990; Andrade and Chaput, 1991; Roychowdhury et al., 1994)—consistent with the ability of this receptor to enhance neurotransmitter release (see below). In addition, it should be noted that at least in type 2 dorsal root ganglion cells, 5-HT₄ receptor activation is associated with an increase in tetrodotoxin-insensitive Na⁺ current (Cardenas et al., 1997). Whilst this latter response is likely to involve a diffusible cytosolic secondary messenger, it does not appear to be cAMP (Cardenas et al., 1997).

13.4.2. Modulation of neurotransmitter release following interaction with the 5-HT₄ receptor

There are now numerous reports demonstrating the ability of the 5-HT₄ receptor to modulate the activity of various neurones in the CNS. Initially the modulation of acetylcholine release via the 5-HT₄ receptor received most attention, probably because of the well documented ability of the 5-HT₄ receptor to facilitate acetylcholine release in the gastrointestinal tract (e.g. Tonini

et al., 1989, 1992; Craig and Clarke, 1990; Elswood et al., 1991; Kilbinger et al., 1995; Eglén et al., 1997). In microdialysis studies, Consolo and colleagues (1994) demonstrated an increase in acetylcholine release following i.c.v. administration of the 5-HT₄ receptor agonists BIMU1 and BIMU8. This response was reduced by co-administration of the 5-HT₄ receptor antagonists GR113808 and GR125487. Neither antagonist alone modified the release of acetylcholine (Consolo et al., 1994a,b), indicating a lack of endogenous tone on the 5-HT₄ receptor in this preparation which was subsequently verified in additional reports (Yamaguchi et al., 1997a,b). These latter reports used either 5-HT re-up-

take blockers (indeloxazine and citalopram) or the 5-HT releasing agent, *p*-chloroamphetamine, to raise extracellular levels of 5-HT which was associated with an increase in acetylcholine release in the rat frontal cortex that was attenuated by the selective 5-HT₄ receptor antagonists RS 23597 and GR113808 (Yamaguchi et al., 1997a,b). Interestingly, neither of the 5-HT₄ receptor agonists, BIMU1 nor BIMU8, modified acetylcholine release in the striatum or hippocampus (Consolo et al., 1994a,b). Previous reports, however, indicated that activation of central 5-HT₄ receptors (also following i.c.v. administration), increases total EEG-energy, including the cholinergic septal-hippocampal theta rhythm (Boddeke and Kalkman, 1990, 1992). These latter studies, however, were performed before the widespread availability of selective 5-HT₄ receptor ligands and therefore the actions of such compounds in this model would be worthy of investigation. It may be relevant, however, that additional evidence is available which indicates that the septal-hippocampal cholinergic neurones express 5-HT₄ receptors. Thus the septum (which contains the cell bodies of this projection) expresses moderate levels of 5-HT₄ receptor mRNA (Ullmer et al., 1996; although the transcripts were not detected with cellular resolution and therefore phenotypes of the expressing cells were not deduced). Moreover, postmortem brains of patients with Alzheimer's disease display a reduction in hippocampal 5-HT₄ receptor density (Reynolds et al., 1995), although it cannot be assumed that this reflects an association of the 5-HT₄ receptor with cholinergic neurones since a number of phenotypically different neurones are known to also degenerate in Alzheimer's disease (e.g. Price et al., 1990). In fact the presence of relatively high levels of 5-HT₄ receptor mRNA expressed by cells within the hippocampus indicates that this region also possesses 5-HT₄ receptors on non-cholinergic cells.

There is increasing evidence that the 5-HT₄ receptor also modulates dopamine release in the brain (Fig. 10). Thus, Benloucif et al. (1993) initially demonstrated, using the *in vivo* microdialysis technique with anaesthetised rats, that the non-selective 5-HT₄ receptor agonist 5-methoxytryptamine increased striatal dopamine release. This response was partially blocked by high concentrations of the weak 5-HT₄ receptor antagonist tropisetron. Subsequently these findings have been extended using a range of 5-HT₄ receptor ligands including the selective 5-HT₄ receptor antagonist, GR118 303 (Gale et al., 1994), and to also include both anaesthetised and awake animals (Bonhomme et al., 1995; Steward et al., 1996; Fig. 10), as well as *in vitro* preparations (Steward et al., 1996). In the latter model, the 5-HT₄ receptor-mediated response would appear prone to desensitisation, which is a characteristic of this receptor in other *in vitro* preparations (for review see Ford and Clarke, 1993).

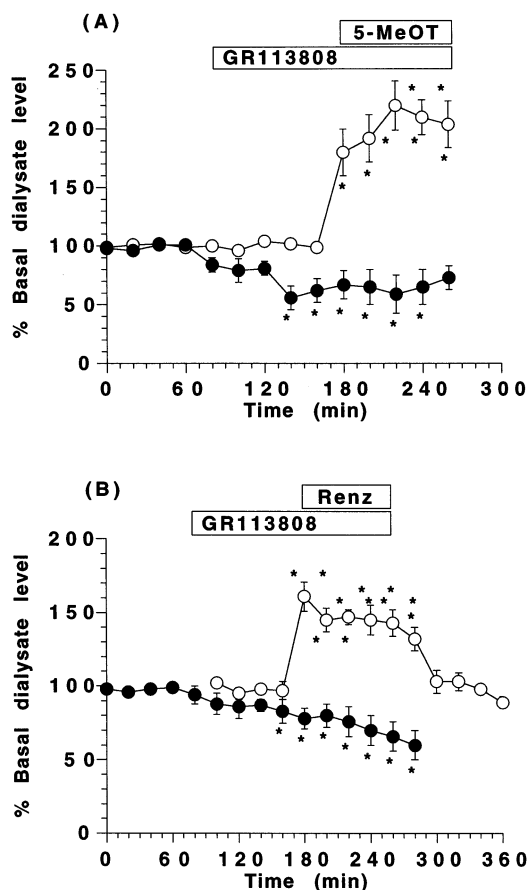


Fig. 10. Ability of 5-HT₄ receptor ligands to modulate dopamine release in the striatum of freely moving rats assessed using the *in vivo* microdialysis technique. (A) The non-selective 5-HT₄ receptor agonist 5-MeOT (○; 10 μM; in the presence of pindolol (10 μM) and methysergide (10 μM)) and 5-MeOT (10 μM; in the presence of pindolol (1 μM) and methysergide (1 μM)) plus the selective 5-HT₄ receptor antagonist GR113808 (●; 1 μM). (B) The 5-HT₄ receptor agonist renzapride (○; 100 μM; Renz) and renzapride (100 μM) plus GR113808 (●; 1 μM). Dopamine levels in dialysates are expressed as the percentage of the meaned absolute amount in the four collections preceeding the drug treatment. Data represents mean ± S.E.M., *n* = 4–6. Horizontal bars represent application of the indicated drug (corrected for the void volume). ANOVA < 0.05, * *P* < 0.05, ** *P* < 0.01 (Dunnett's *t*-test). Reproduced from Steward et al. (1996), with permission.

The 5-HT₄ receptor agonist-induced increase in striatal dopamine release *in vitro* and *in vivo*, is prevented by the inclusion of the Na⁺ channel blocker, tetrodotoxin (Steward et al., 1996; DeDeurwaerdere et al., 1997). This suggests that the response is indirect (i.e. the 5-HT₄ receptor is not located on dopamine neurone terminals in the striatum). It is of interest, therefore, that radioligand binding studies demonstrate that 5-HT₄ receptor levels are not altered in the striatum of rat brain following 6-hydroxydopamine lesion of the nigral-striatal dopamine system, whereas a substantial reduction in radiolabelled striatal 5-HT₄ receptors was detected following lesion of striatal neurones by kainic acid (Patel et al., 1995). This indicates that at least a major population of 5-HT₄ receptors in the striatum is not located on dopamine neurone terminals but is located on neurones that have their cell bodies in the striatum. Comparable findings have been demonstrated with human post mortem brain tissue from patients with Parkinson's disease and Huntington's disease (Reynolds et al., 1995). Indeed, growing evidence indicates that projection neurones from the striatum to the globus pallidus and substantia nigra (presumably GABAergic neurones; Gerfen, 1992; Kawaguchi et al., 1995) express 5-HT₄ receptors on their terminals. Thus high levels of 5-HT₄ receptor binding sites are detected in these latter regions in the relative absence of 5-HT₄ receptor mRNA (Mengod et al., 1996; Ullmer et al., 1996), whilst the striatum expresses relatively high levels of the mRNA. Furthermore, the release of GABA in the substantia nigra, under depolarising conditions (elevated [K⁺]), would appear to be facilitated via endogenous activation of the 5-HT₄ receptor (Zetterström et al., 1996). In addition, however, local activation of the 5-HT₄ receptor in the vicinity of the substantia nigra increases dopamine release from this region (Thorré et al., 1998), although it is yet to be determined whether this represents a direct or indirect activation of the dopamine neurones.

5-HT release in the hippocampus is also modulated via the 5-HT₄ receptor. Thus, 5-HT₄ receptor activation increases 5-HT release in the hippocampus (assessed using the *in vivo* microdialysis techniques; Ge and Barnes, 1996). It is noteworthy that in this latter study, 5-HT₄ receptor antagonists (GR113808 and GR125487) reduced the release of 5-HT, indicating the presence of an endogenous tone on the 5-HT₄ receptor in this preparation. A similar modulation of 5-HT release via the 5-HT₄ receptor is apparent within the substantia nigra (Thorré et al., 1998).

13.4.3. Modulation of behaviour via interaction with the 5-HT₄ receptor

In general, it would appear that administration of 5-HT₄ receptor ligands is not associated with any overt behavioural change (e.g. Fontana et al., 1997). Further-

more, in contrast to the numerous reports indicating that the 5-HT₄ receptor modulates striatal dopamine release (see above), administration of a brain penetrant 5-HT₄ receptor antagonist fails to modulate a number of behaviours resulting from enhanced dopamine release (Reavil et al., 1998). This may be explained by the low level of endogenous tone on the central 5-HT₄ receptor, which is supported by evidence that 5-HT₄ receptor antagonists induce at most, modest alterations in dopamine release (Bonhomme et al., 1995; Steward et al., 1996). However, the failure of the lipophilic 5-HT₄ receptor partial agonist RS67333, at centrally active doses, to modify locomotor activity (estimated by swim speed; Fontana et al., 1997) further complicates the issue. It should be noted, however, that whilst the 5-HT₄ receptor antagonist RS67532 did not modify swim speed, this compound reduced locomotor activity measured in activity boxes. Moreover, this was achieved at the same dose as used to antagonise the 5-HT₄ receptor-mediated facilitation of cognitive performance (Fontana et al., 1997; see below). Since central dopaminergic neurotransmission is clearly associated with locomotor activity (e.g. Eison et al., 1982), more work in this area is needed to further clarify the role of the 5-HT₄ receptor in the modulation of locomotor activity.

13.5. Cognitive performance

A growing number of reports have indicated that activation of central 5-HT₄ receptors facilitates cognitive performance (Fontana et al., 1997; Galeotti et al., 1997, 1998; Letty et al., 1997; Marchetti-Gauthier et al., 1997; Meneses and Hong, 1997; Terry et al., 1998). Thus, for instance, the 5-HT₄ receptor agonist (and 5-HT₃ receptor antagonist) BIMU1 has been shown to enhance the performance of rats in different behavioural models assessing both short-term (social olfactory memory assessing adult rat recognition of a juvenile rat; Letty et al., 1997; olfactory association memory; Marchetti-Gauthier et al., 1997) and long-term memory (olfactory association memory; Marchetti-Gauthier et al., 1997). These actions of BIMU1 are likely to be 5-HT₄ receptor mediated since they were prevented by the selective brain penetrant 5-HT₄ receptor antagonist, GR125487 (Letty et al., 1997; Marchetti-Gauthier et al., 1997). Similarly, in a further study, the lipophilic 5-HT₄ receptor partial agonist RS67333 facilitated the impaired performance of rats in the Morris water maze; the impairment being induced by the muscarinic receptor antagonist atropine (Fontana et al., 1997). This effect is likely to be mediated centrally since in the same study, a lipophobic 5-HT₄ receptor partial agonist (RS67506), with an otherwise comparable pharmacology to RS67333, failed to reverse the atropine-induced cognitive deficit (Fontana

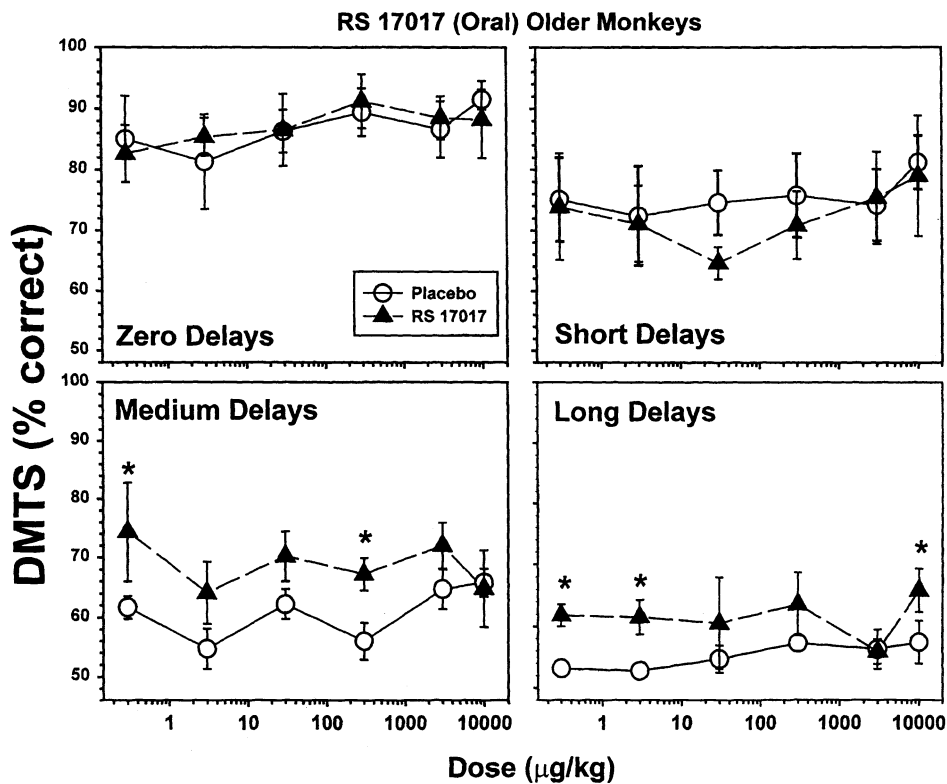


Fig. 11. Ability of the 5-HT₄ receptor agonist RS 17017 to modify cognitive performance of old *Macaque* monkeys assessed via a computer-assisted delayed matching-to-sample task (DMTS). Dose response curves generated with four aged macaques, 30 min following the oral administration of RS 17017 (▲) or placebo (○). Each point represents the average (percentage correct over 96 trials per session) of two or three replicates per dose (\pm S.E.M.). * $P < 0.05$, significantly different to placebo control. Reproduced from Terry et al. (1998), with permission.

et al., 1997). It is likely that the cognitive enhancing action of RS67333 is due to the intrinsic activity of this ligand since the behavioural response was prevented by prior administration of the selective 5-HT₄ receptor antagonist RS67532 (Fontana et al., 1997). The latter compound was without effect on the performance of both naive and atropine-treated rats in the Morris water maze (Fontana et al., 1997).

Results from a primate study further support the association between the 5-HT₄ receptor and cognition. Thus the 5-HT₄ receptor agonist, RS17017, improved the delayed matching performance of both young and old *Macaque* monkeys (Terry et al., 1998; Fig. 11). The results with the older monkeys is particularly significant since these animals displayed impairments in the behavioural paradigm relative to the younger monkeys and hence may represent a better model of the cognitive decline associated with age. It should be noted, however, that RS17017 displays some five-times higher affinity for the sigma-1 site (although at least an order of magnitude selectivity over 28 other neurotransmitter receptors and ion channels; Terry et al., 1998); the functional significance of this interaction was not explored in the study.

Given the well known association of acetylcholine and memory (e.g. Bartus et al., 1982), the proposed

ability of the 5-HT₄ receptor to facilitate cholinergic function within relevant regions of the brain (e.g. cerebral cortex, hippocampus; Boddeke and Kalkman, 1990, 1992; Consolo et al., 1994a,b) provides a plausible explanation for the facilitation of cognitive performance following 5-HT₄ receptor activation. However, the likely expression of 5-HT₄ receptors by non-cholinergic neurones in the hippocampus and cerebral cortex (as discussed above) suggests that additional mechanisms may also underlie the 5-HT₄ receptor-induced facilitation in cognitive performance. Indeed, considerable evidence indicates that hippocampal pyramidal neurones express 5-HT₄ receptors (e.g. Roychowdhury et al., 1994; Ullmer et al., 1996; Vilaró et al., 1996) which has led to speculation that 5-HT₄ receptor-mediated activation of these neurones would presumably facilitate the induction of long term potentiation (LTP), which is widely regarded as a cellular basis for memory (e.g. Bliss and Collingridge, 1993).

There are currently no reports concerning whether selective 5-HT₄ receptor ligands modify cognitive performance in man. Indeed a case report search on the Janssen-Cilag Ltd. International Pharmacovigilance Database has revealed no cases of enhanced alertness, awareness or cognitive function following administration of the 5-HT₄ receptor agonist cisapride (PREPUL-

SID[®]) despite the ability of this compound to cross the blood brain barrier (Tooley, personal communication). However, the results from clinical trials directly assessing this potential action of 5-HT₄ receptor agonists would be preferable to a lack of anecdotal reports, before the potential therapeutic benefit of this pharmacological approach can be evaluated. It is not surprising, however, that given the peripheral roles of the 5-HT₄ receptor (for review see Ford and Clarke, 1993), a number of side effects (e.g. cardiac arrhythmias, diarrhoea) have been reported following administration of the 5-HT₄ receptor agonist cisapride to patients (see McCallum et al., 1988; Verlinden et al., 1988; Kaumann et al., 1994). The side effect profile of a 5-HT₄ receptor *partial* agonist, however, may be more favourable. Alternatively, the combined administration of a 5-HT₄ receptor antagonist which fails to cross the blood-brain barrier, would presumably limit some of the side-effects mediated via peripheral 5-HT₄ receptors.

13.6. Anxiety

The 5-HT₄ receptor has also been implicated in anxiety. So far, however, all the data comes from animal models, and there is apparent disagreement regarding the precise role that the 5-HT₄ receptor plays. The first indication that the 5-HT₄ receptor may be involved with anxiety emerged from Costall and Naylor's laboratory. They initially demonstrated that the non-selective 5-HT_{3/4} receptor antagonists, tropisetron and SDZ 205-557, reduced the anxiolytic-like action of the benzodiazepine diazepam (and a number of other anxiolytic-like regimens; Costall and Naylor, 1992; Cheng et al., 1994). However, when either tropisetron or SDZ 205-557 were administered alone, at doses likely to occupy 5-HT₄ receptors, they were without effect (Costall and Naylor, 1992; Cheng et al., 1994). The reversal of disinhibition of animal behaviour was demonstrated using two behavioural paradigms; the mouse light/dark test and the rat social interaction test. The non-selective nature of tropisetron and SDZ 205-557 may have complicated interpretation (although these were the best compounds widely available at the time of these studies), however, the same group have more recently replicated the findings in the mouse light/dark test using the highly selective 5-HT₄ receptor antagonists GR113808, RS23957-190 and SB204070 (Costall and Naylor, 1996, 1997).

In contrast to the above, two groups have demonstrated that 5-HT₄ receptor antagonists have an anxiolytic-like action. First, Silvestre et al. (1996) demonstrated that GR113808 and SB204070 (albeit at higher doses than those used by Costall and Naylor, 1996, 1997) displayed modest anxiolytic-like action in the rat elevated X-maze (with comparable efficacy to

the 5-HT₃ receptor antagonist granisetron but lower efficacy than diazepam). GR113808 and SB204070 were only effective when administered 10 min prior to testing (rather than 30 min), which the authors reasonably attributed to the short plasma half-life of these compounds due to rapid hydrolysis (Silvestre et al., 1996). The finding that the anxiolytic-like action of both selective 5-HT₄ receptor antagonists was lost at a dose only three-fold higher than the effective dose (Silvestre et al., 1996) further complicates comparison of this study with those of Costall and Naylor's group. In addition, as with all studies investigating the actions of antagonists *in vivo*, the level of endogenous tone on the receptor is important and this may differ between species/strains in different environments.

In a more recent study, the anxiolytic-like actions of two selective 5-HT₄ receptor antagonists (SB204070 and SB207266) have been demonstrated in two animal models of anxiety (rat social interaction and elevated X-maze; Fig. 12), but not in another model (the rat Geller-Seifter conflict model; Kennett et al., 1997a,b). Indeed, this profile of anxiolytic-like action is reminiscent of that displayed by 5-HT₃ receptor antagonists which also fail to display anxiolytic-like actions in conflict models of anxiety (for review see Bentley and Barnes, 1995).

It is worth noting that neither SB204070 nor SB207266 were as effective as the benzodiazepine chlordiazepoxide in the rat elevated X-maze (Kennett et al., 1997a,b), which compares well with the studies of Silvestre and colleagues (1996). However, these selective 5-HT₄ receptor antagonists did display comparable levels of efficacy to chlordiazepoxide in the rat social interaction test (Kennett et al., 1997a,b), suggesting that 5-HT₄ receptor antagonists may be able to discriminate different forms of anxiety mimicked by different animal models. Also, as with the study of Silvestre and colleagues (1996), SB204070 displayed a bell-shaped dose response curve, although at least in the rat social interaction test, this was not apparent for SB207266 (Kennett et al., 1997a,b).

It may be noteworthy that the 5-HT₃ receptor antagonist/5-HT₄ receptor agonists, renzapride and S(-)zacopride, fail to display anxiolytic-like actions in a range of animal models (Morinan et al., 1989; Barnes et al., 1990, 1992). Given that a number of groups have now demonstrated that selective 5-HT₃ receptor antagonists display anxiolytic-like actions (see section on the 5-HT₃ receptor), the additional pharmacological action of renzapride and S(-)zacopride (i.e. 5-HT₄ receptor agonism) may prevent the anxiolytic-like action due to 5-HT₃ receptor antagonism. Experiments using combinations of selective 5-HT₃ receptor antagonists with selective 5-HT₄ receptor agonists may cast more light on the potential interaction of the 5-HT₃ and 5-HT₄ receptor with respect to the expression of anxiety-like behaviour in animals.

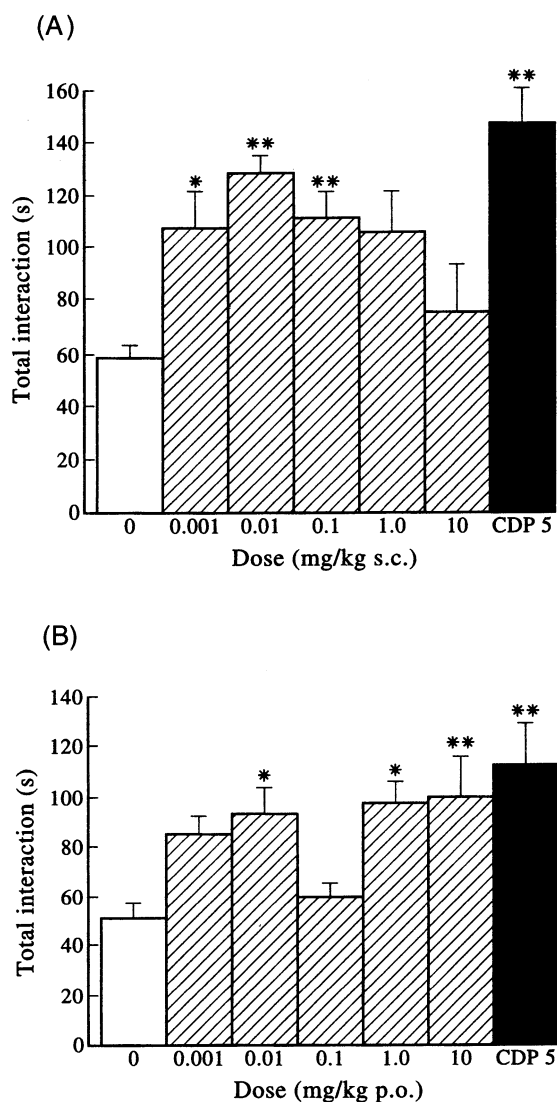


Fig. 12. Effects of the 5-HT₄ receptor antagonists SB 204070A (s.c.; A) and SB 207266A (p.o.; B) on rat behaviour in a 15 min social interaction test under high light unfamiliar conditions (an environment promoting anxiogenic-like behaviour). CDP, chlordiazepoxide. Data represents mean \pm S.E.M., $n = 10$ –12 per group. * $P < 0.05$, ** $P < 0.01$ by Dunnett's t -test and one-way ANOVA. Reproduced from Kennett et al. (1997a,b), with permission.

The ability of 5-HT₄ receptor agonists to increase (and 5-HT₄ receptor antagonists to decrease) 5-HT release in the dorsal hippocampus, provides a relevant neurochemical mechanism for 5-HT₄ receptor-mediated modulation of anxiety since previous studies have implicated an enhancement and a reduction in hippocampal 5-HT neurone function in anxiogenic and anxiolytic states, respectively (e.g. Andrews et al., 1994). Clearly, further studies will help clarify the situation concerning the apparent inconsistencies relating to the involvement of the 5-HT₄ receptor and anxiety (Table 10).

14. 5-HT₅ receptors

The 5-HT₅ receptor class is probably the least well understood of all the 5-HT receptor classes. The initial cDNA sequence (designated 5-HT_{5A}) was generated from a mouse brain library using degenerate oligonucleotides (Plassat et al., 1992a,b) corresponding to the regions encoding the highly conserved putative transmembrane domains III and VI of metabotropic 5-HT receptors (Hen, 1992). Subsequently, a related receptor was identified (5-HT_{5B}) by the same group, again derived from a mouse brain cDNA library (Matthes et al., 1993). At around the same time, both the rat 5-HT_{5A} and 5-HT_{5B} receptors were also identified by other researchers (Erlander et al., 1993; Wisden et al., 1993) using cDNA libraries derived from brain tissue, whilst report of the human 5-HT_{5A} receptor sequence followed shortly after (Rees et al., 1994). To date, no direct evidence is available concerning the existence of functional native 5-HT₅ receptors and hence, in accordance with recommendations from the IUPHAR receptor nomenclature committee, lower case appellation is used.

14.1. 5-HT_{5A} receptor structure

The mouse, rat and human 5-HT_{5A} receptor is predicted to be comprised of 357 amino acids (Plassat et al., 1992a,b; Erlander et al., 1993; Rees et al., 1994) and contains consensus sequences predictive of N-linked glycosylation sites in the putative N-terminal extracellular domain and a number of consensus sequences predictive of protein kinase C sensitive phosphorylation sites on putative cytoplasmic loops (Plassat et al., 1992a,b; Erlander et al., 1993; Rees et al., 1994). The recent demonstration that the 5-HT_{5A} receptor can be expressed at high levels using the *Pichia pastoris* expression system will aid further investigation of the structural nature of this receptor (Weiss et al., 1998).

14.2. 5-HT_{5B} receptor structure

The 5-HT_{5B} receptor of mouse and rat is predicted

Table 10
Summary of the functional responses associated with activation of the brain 5-HT₄ receptor

Level	Response	Mechanism
Cellular	Adenylate cyclase (+)	Post
Electrophysiological	Reduce after-hyperpolarisation	Post
Behavioural	Anxiolysis and anxiogenesis (antagonists)	Post
Neurochemical	Cognition (+)	Post
	5-HT release (+)	Post (indirect?)
	Acetylcholine release (+)	Post
	Dopamine release (+)	Post (indirect)

to comprise of 370–371 amino acids (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993) and contains a consensus sequence for a N-linked glycosylation site in the putative N-terminal extracellular domain and a number (three and four for mouse and rat, respectively) of consensus sequences for protein kinase C sensitive phosphorylation sites on putative intracellular domains (Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). The mouse 5-ht_{5B} receptor also possesses a consensus sequence for a protein kinase A (cAMP-dependent protein kinase) sensitive phosphorylation site in the putative third intracellular loop (Matthes et al., 1993).

14.3. Genomic structure of 5-ht_{5A} and 5-ht_{5B} receptor genes

The genomic structure of 5-ht_{5A} and 5-ht_{5B} genes was deduced by screening a mouse genomic library with probes corresponding to the mouse 5-ht_{5A} and 5-ht_{5B} receptor cDNAs. Both genes contain an intron at an identical position corresponding to the middle of the third cytoplasmic loop (between putative transmembrane domains V and VI; Matthes et al., 1993) and, therefore, potential shorter spliced variants are not likely to be functional (the human 5-ht_{5A} receptor gene also possesses an intron in an identical position; Rees et al., 1994). This finding further distinguishes the 5-ht₅ receptor family from the 5-HT₁ receptor family; genes encoding members of this latter family are intronless. Matthes and colleagues (1993) also demonstrated the chromosomal location of both 5-ht_{5A} and 5-ht_{5B} receptor genes; the 5-ht_{5A} gene was located on mouse chromosome 5 (position 5B) and human chromosome 7 (position 7q36), whereas the 5-HT_{5B} gene was located on mouse chromosome 1 (position 1F) and human chromosome 2 (position 2q11–13 with q13 displaying maximal hybridisation). However, there is doubt concerning the expression of 5-ht_{5B} receptors in human tissue due to the presence of a stop codon within the human 5-ht_{5B} receptor gene, which would result in the expression of a short, probably non-functional protein (Rees et al., 1994).

It has been speculated that mutations in the gene encoding the 5-ht_{5A} receptor may be detrimental to brain development given its close proximity of both the mouse reeler mutation and the human mutation for holoprosencephaly type III; both of these disorders result in abnormal brain development (Matthes et al., 1993).

14.4. 5-ht_{5A} receptor distribution

Northern blot analysis of poly (A)⁺ RNA demonstrated the presence of three 5-ht_{5A} mRNA species in extracts of both mouse cerebellum or whole brain (4.5, 5.0 and 5.8 kb; Plassat et al., 1992a,b), whereas two mRNA species were derived from extracts of various rat

brain regions (3.8 and 4.5 kb; hippocampus, hypothalamus, cerebral cortex, thalamus, pons, striatum and medulla but not kidney, heart and liver; Erlander et al., 1993). Use of PCR in an attempt to improve sensitivity detected 5-ht_{5A} mRNA from mouse and human fore-brain and cerebellum, and also from spinal cord, but still failed to detect transcripts from a range of peripheral tissues (Plassat et al., 1992a,b; Rees et al., 1994). In situ hybridisation studies confirmed the widespread distribution of 5-ht_{5A} receptor mRNA in both mouse and rat brain (Plassat et al., 1992a,b; Erlander et al., 1993). Furthermore, the cellular resolution achieved with this latter technique indicated that within mouse brain, 5-ht_{5A} receptor transcripts were associated with neurones within the cerebral cortex, the dentate gyrus and the pyramidal cell layer within hippocampal fields CA1–3, the granule cell layer of the cerebellum and tufted cells of the olfactory bulb (Plassat et al., 1992a,b).

14.5. 5-ht_{5B} receptor distribution

Northern blot analysis of poly (A)⁺ RNA extracted from brain and various peripheral tissues (heart, kidney, lung, liver and intestine) of the mouse failed to detect 5-ht_{5B} receptor transcripts (Matthes et al., 1993), although rat hippocampal poly (A)⁺ RNA displayed three 5-ht_{5B} transcripts (1.5, 1.8 and 3.0 kb). However, no transcripts were detected within poly (A)⁺ RNA derived from either other rat brain regions (hypothalamus, striatum, thalamus, cerebellum, pons, medulla) or peripheral tissues (heart, liver and kidney; Erlander et al., 1993). The failure to detect 5-ht_{5B} receptor transcripts extracted from rat hypothalamus was somewhat surprising given that the rat clone was derived from a hypothalamic cDNA library (Erlander et al., 1993). However, in situ hybridisation studies demonstrated a low specific signal in the supraoptic nucleus of the hypothalamus (Erlander et al., 1993) and some other rat brain regions (hippocampus; particularly the subiculum and the pyramidal cell layer in the CA1 field, medial and lateral habenula, dorsal raphe nucleus, olfactory bulb, entorhinal cortex and piriform cortex; Erlander et al., 1993; Wisden et al., 1993). The presence of 5-ht_{5B} receptor transcripts has also been demonstrated in mouse brain regions by in situ hybridisation (CA1 field of the hippocampus, medial and lateral habenula, dorsal raphe; Matthes et al., 1993).

14.6. 5-ht₅ receptor ontogeny

Northern blot analysis of poly (A)⁺ RNA extracted from whole rat brain indicated the presence of 5-ht_{5A} receptor mRNA as early as embryonic day 18 (E18). Comparable to adult rat, two 5-ht_{5A} receptor transcripts were evident (4.5 and 3.8 kb) and it is of interest

that the two mRNA species appeared to be regulated differentially during late embryonic and early postnatal development (E18–P15). However, by P20 the relative level of each species was comparable and, although at relatively low levels of expression, remained comparable in adulthood (Carson et al., 1996).

In addition to an increase in early postnatal levels of 5-ht_{5A} receptor mRNA, there was a corresponding increase in 5-ht_{5A}-like immunoreactivity in rat brain sections between P1 and P15, with levels being markedly lower in adulthood compared to their peak at P15 (Carson et al., 1996). A similar increase in expression in adult brain, relative to foetal brain, is evident in humans (Rees et al., 1994). Furthermore, the postnatal increase in rat 5-ht_{5A}-like immunoreactivity coincided with an increase in GFAP-like immunoreactivity and, at all times, the two antigens co-localised (Carson et al., 1996), indicating that astrocytes provide at least a major source of 5-ht_{5A} receptor expression during early postnatal development (as well as in adulthood).

In contrast with many other 5-HT receptor subtypes, rat 5-ht_{5B} receptor expression appears relatively absent during late embryonic development (both centrally and peripherally; Wisden et al., 1993), with 5-ht_{5B} receptor transcripts only being detected within a discrete region of the ventral medulla, possibly corresponding to the nucleus raphe pallidus, at E17 and E19, the earliest time points examined (Wisden et al., 1993). According to the classification of Dahlström and Fuxe (1964), 5-HT neurone cell bodies within this region comprise the B1 cluster which form a descending projection to the spinal cord.

At the day of birth (P0), faint 5-ht_{5B} receptor mRNA hybridisation signal was detectable within the entorhinal cortex and by P6, the pattern of 5-ht_{5B} receptor mRNA expression resembled that of adult rats (Wisden et al., 1993).

14.7. 5-ht₅ receptor pharmacology

Based on their relative lack of sequence homology to other 5-HT receptors (Table 1; Fig. 1), the 5-ht_{5A} and 5-ht_{5B} receptors clearly represent an additional subfamily. Radioligand binding studies have demonstrated that the two 5-ht₅ receptors display a comparable, although distinguishable, pharmacology (Erlander et al., 1993; Matthes et al., 1993) which displays some similarities to the pharmacology of the 5-HT_{1D}-like receptors provided the impetus to search for additional 5-ht receptors which resulted in the initial cloning of the 5-ht_{5A} receptor (Plassat et al., 1992a,b) and with hindsight, it remains a distinct possibility that a number of studies have misclassified 5-ht₅ receptor-mediated responses or binding sites.

14.8. Functional effects mediated via the 5-ht₅ receptor

The transduction system associated with the 5-ht₅ receptor remains to be defined unequivocally. Hydrophathy analysis of the predicted amino acid sequences of both the 5-ht_{5A} and 5-ht_{5B} receptors indicate that they are members of the seven putative transmembrane domain-G-protein coupled superfamily. Furthermore, radioligand binding studies with recombinant 5-ht_{5A} and 5-ht_{5B} receptors have provided some evidence to indicate that the receptors couple to G-proteins. Thus, a sub-population of putative agonist-preferring states can be demonstrated that is diminished by the presence of guanosine nucleotides (Plassat et al., 1992a,b; Matthes et al., 1993; Wisden et al., 1993). This being consistent with the recombinant receptor coupling to a G-protein to form the agonist preferring state which is uncoupled by guanosine nucleotides (e.g. DeLean et al., 1982). However, despite the use of heterologous expression systems that have allowed the activation of transduction systems by numerous other recombinant receptors (e.g. positive or negative modulation of adenylate cyclase or stimulation of phospholipase C; NS1 cells, NS4 cells, COS7 cells, HEK293 cells, CHO cells, HeLa cells, COS-M6 cells), neither the 5-ht_{5A} nor 5-ht_{5B} receptor has been found, by most investigators, to modify either levels of cAMP or inositol phosphates (Plassat et al., 1992a,b; Erlander et al., 1993; Matthes et al., 1993; Wisden et al., 1993). These cells, however, may be devoid of the appropriate G-protein subunit(s) to enable coupling of the recombinant 5-ht₅ receptors with sufficient efficiency to detect modifications in transduction processes or alternatively receptor activation may modify G-protein mediated ion channel kinetics. Indeed, it would be of interest to express 5-ht₅ receptors in cells which express more promiscuous G-proteins (e.g. G₁₆; for review see Milligan et al., 1996) in an attempt to demonstrate biochemically active 5-ht₅ receptors. A recent report, however, indicates that very high expression of human 5-ht_{5A} receptors by HEK293 cells (25 pmol/mg protein) allows the receptor to inhibit forskolin-stimulated adenylate cyclase (Francken et al., 1998). It should also be noted that one report has indicated that C6 glioma cells transfected with rat 5-ht_{5A} receptors displayed a 5-HT-induced attenuation of forskolin-stimulated cAMP accumulation which was absent in untransfected cells (Carson et al., 1996). These cells, however, also express a 5-HT-sensitive receptor which stimulates adenylate cyclase activity (Carson et al., 1996) which may complicate interpretation. Hence additional studies investigating the pharmacology of the receptor mediating the 5-HT-induced inhibition of forskolin-stimulated cAMP levels in this heterologous expression system are warranted.

The rationale of expressing 5-ht_{5A} receptors in a glial cell line stemmed from studies indicating that central

5-ht_{5A} receptors are predominantly expressed by astrocytes (Carson et al., 1996). Thus, rabbit polyclonal antibodies recognising distinct regions of the rat 5-ht_{5A} receptor (amino acids 239–257 of the third intracellular loop and amino acids 343–357 of the carboxyl terminus) were used in immunocytochemical studies to map the central distribution of the 5-ht_{5A} receptor. It was of interest that the distribution of 5-ht_{5A} receptor-like immunoreactivity concurred with the distribution of rat 5-ht_{5A} receptor mRNA (Erlander et al., 1993; Carson et al., 1996), indicating that at the cellular level, the receptors are located in close proximity to their site of synthesis. More detailed investigation indicated that the morphology and distribution of immunologically labelled cells resembled astrocytes (Carson et al., 1996). Furthermore, in both rat and mouse brain, and also in glial cell primary cultures (originating from rat cerebral cortex), 5-ht_{5A}-like immunoreactivity often co-localised with glial fibrillary acidic protein-like immunoreactivity (GFAP; a selective glial cell marker) whilst 5-ht_{5A}-like immunoreactivity was not found to co-localise with neurofilament-like immunoreactivity (a selective marker for neurones; Carson et al., 1996). Interestingly, 5-ht_{5A}-like immunoreactivity increased in parallel with GFAP-like immunoreactivity following reactive gliosis induced by a needle wound in 6 week old rats (Carson et al., 1996).

Little positive information has been published concerning the effects of knocking out the 5-ht_{5A} receptor (Grailhe et al., 1997), although mice lacking the receptor display increased locomotor activity and exploratory behaviour relative to wild-type animals (Dulawa et al., 1997; Grailhe et al., 1997; Hen et al., 1998), although this did not manifest as a difference in the anxiety-like behaviour of these animals in the elevated plus maze (Grailhe et al., 1997).

15. 5-ht₆ receptors

The 5-ht₆ receptor was initially detected by two groups following identification of a cDNA sequence which encoded a 5-HT-sensitive receptor with a novel pharmacology (Monsma et al., 1993; Ruat et al., 1993a,b). Both groups used the strategy of nucleotide sequence homology screening. Monsma and colleagues (1993) used highly degenerate primers derived from coding regions of the putative III and VI transmembrane domain regions of previously identified G-protein coupled receptors. In contrast, Ruat and colleagues (1993) first screened a rat genomic library under low stringency conditions with a probe corresponding to a coding region of the rat histamine H₂ receptor to identify a clone which allowed the development of a probe to identify the 5-ht₆ receptor cDNA in a rat striatal cDNA library. Some of the apparent differences

in the 5-ht₆ receptor cDNA sequences between the two initial reports were subsequently reconciled (Kohen et al., 1996; Boess et al., 1997) along with the reporting of the human 5-ht₆ receptor cDNA sequence (Kohen et al., 1996).

15.1. 5-ht₆ receptor structure

The rat and human 5-ht₆ receptor is predicted to be comprised of 436 or 438 and 440 amino acids, respectively (Ruat et al., 1993a,b; Kohen et al., 1996). Hydrophathy analysis of the deduced amino acid sequence indicates the presence of seven hydrophobic regions sufficient to span the membrane, which places the receptor in the G-protein-coupled, seven putative transmembrane domain, receptor superfamily (Monsma et al., 1993; Ruat et al., 1993a,b; Kohen et al., 1996). The 5-ht₆ receptor sequence contains a consensus sequence predictive of a N-linked glycosylation site in the putative N-terminal extracellular domain (Monsma et al., 1993; Ruat et al., 1993a,b; Kohen et al., 1996), and a number of consensus sequences indicating the presence of a number of phosphorylation sites in the presumed third cytoplasmic loop and the C-terminal (Monsma et al., 1993; Ruat et al., 1993a,b; Kohen et al., 1996). Consistent with the presence of these sites, the receptor is prone to agonist-induced desensitisation which appears to be due principally to receptor phosphorylation catalysed by cAMP-dependent protein kinase (see Sleight et al., 1997). In addition, the ability of a protein kinase C activator (phorbol-12-myristate 13-acetate) to induce a comparable desensitisation indicates that the receptor may be liable to both homologous and heterologous receptor desensitisation.

Genomic mapping identified the human 5-ht₆ receptor gene in the p35–36 portion of human chromosome 1. Although both the rat and human genes contain two introns in homologous positions, their occurrence within the putative third cytoplasmic loop and the third extracellular loop (Monsma et al., 1993; Ruat et al., 1993a,b; Kohen et al., 1996) renders it unlikely that any resulting spliced variants are functional.

15.2. 5-ht₆ receptor distribution

A number of reports have demonstrated the differential distribution of 5-ht₆ receptor mRNA. The transcripts appear to be largely confined to the central nervous system, although low levels have been detected in the stomach and adrenal glands (Ruat et al., 1993a,b; although see Monsma et al., 1993). Within the brain, high levels of 5-ht₆ receptor mRNA are consistently detected within the striatum (caudate nucleus) of rat, guinea pig and human by both Northern blot analysis of poly (A)⁺ RNA, RT-PCR and in situ hybridisation (Monsma et al., 1993; Ruat et al., 1993a,b; Ward et al.,

1995; Gérald et al., 1996; Kohen et al., 1996). Relatively high levels are also detected in the olfactory tubercles, nucleus accumbens and hippocampus (Monsma et al., 1993; Ruat et al., 1993a,b; Ward et al., 1995; Gérald et al., 1996; Kohen et al., 1996). Both rat and human brain would appear to contain two 5-HT₆ receptor mRNA species (4.0–4.1 and ~3.2 kb Ruat et al., 1993a,b; Kohen et al., 1996; Yau et al., 1997) although only one transcript species was detected in guinea pig brain (Ruat et al., 1993a,b), and Monsma and colleagues (1993) only detected a single mRNA species in rat brain (~4.2 kb).

Although the [³H]-derivative of the 5-HT₆ receptor antagonist, Ro 63-0563, labels selectively the 5-HT₆ receptor expressed in both artificial expression systems and brain tissue (Boess et al., 1998), the relatively high level of non-specific binding associated with brain tissue preparations (70–90% non-specific binding; Boess et al., 1998), along with the relatively low level of 5-HT₆ receptor expression within the brain, would make detailed investigation of the central localisation of the 5-HT₆ receptor difficult with this radioligand. In the absence of a suitable radioligand, the only detailed information concerning the distribution of expressed 5-HT₆ receptors stems from studies with antibodies. Thus, Gérald and co-workers (1997) raised polyclonal antibodies recognising a presumed unique portion of the C-terminus of the 5-HT₆ receptor. 5-HT₆ receptor-like immunoreactivity in rat brain was abundant in a number of regions (e.g. olfactory tubercle, striatum, nucleus accumbens, hippocampus, cerebral cortex; Gérald et al., 1997). In general this distribution is comparable to the distribution of 5-HT₆ receptor mRNA, suggesting that the receptor protein is expressed in close proximity to the site of synthesis. Light and electron microscopic studies exploiting the high levels of resolution achievable using immunocytochemistry demonstrated that, in both the hippocampus and striatum, the 5-HT₆ receptor-like immunoreactivity was associated with dendritic processes making synapses with unlabelled axon terminals (Gérald et al., 1997). These data indicate that the 5-HT₆ receptors in these regions are predominantly post-synaptic to 5-HT neurones. This is consistent with a previous report from the same group demonstrating that lesion of central 5-HT neurones is not accompanied by a loss of 5-HT₆ receptor mRNA within the raphe nuclei (despite an approximately 90% loss of 5-HT transporter mRNA within this region; Gérald et al., 1996). Furthermore, the absence of 5-HT₆ receptor-like immunoreactivity associated with the soma of pyramidal and granule cell neurones in the hippocampus, despite relatively high levels of 5-HT₆ receptor mRNA expression by these cells (Ward et al., 1995; Yau et al., 1997), led Gérald and co-workers (1997) to speculate that 5-HT₆ receptors are located on the dendrites of excitatory pyramidal and granule cell neurones in the hippocampus. (Fig. 13)

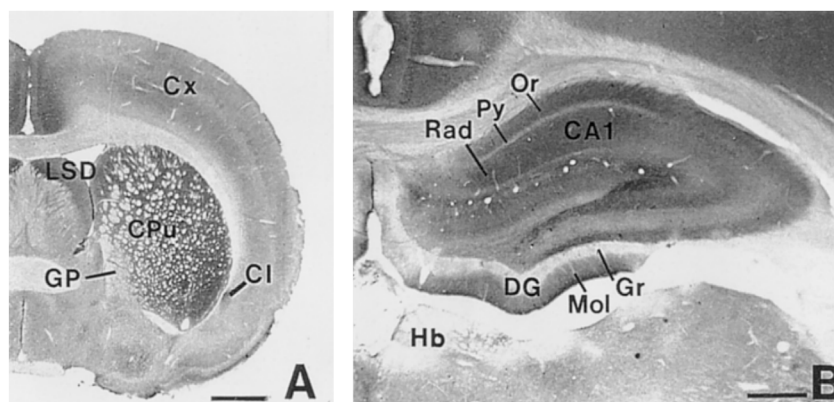
The localisation of 5-HT₆ receptor-like immunoreactivity to dendritic processes within the striatum (Gérald et al., 1997) is also of interest given the study of Ward and Dorsa (1995). The latter researchers demonstrated extensive co-localisation of 5-HT₆ receptor transcripts with mRNA encoding precursors of the neuropeptides enkephalin, substance P and dynorphin. The corollary of these studies is that 5-HT₆ receptors are expressed on the dendritic processes of GABAergic medium spiny projection neurones which terminate in the substantia nigra (these neurones predominantly co-localise either dynorphin or substance P; for review see Gerfen, 1992) and globus pallidus (these neurones predominantly co-localise enkephalin; for review see Gerfen, 1992). These striatonigral and striatopallidal neurones exert a differential modulation (inhibition and disinhibition, respectively) of output neurones of the basal ganglia in the substantia nigra (for review see Gerfen, 1992).

15.3. 5-HT₆ receptor pharmacology

Until very recently, no reports concerning a selective 5-HT₆ receptor ligand had been published such that a detailed pharmacological profile needed to be established before a response or binding site can be ascribed to the 5-HT₆ receptor. However, two compounds have now been identified as selective 5-HT₆ receptor antagonists (Ro 04-6790 and Ro 63-0563; Sleight et al., 1998) which will aid considerably the pharmacological definition of 5-HT₆ receptor mediated responses. Furthermore, Ro 04-6790, unlike Ro 63-0563, crosses the blood-brain barrier (Sleight et al., 1998), which will benefit investigation of the central 5-HT₆ receptor, *in vivo*. Prior to the availability of these latter compounds, the pharmacology of the 5-HT₆ receptor had already received considerable attention due to the interaction of a number of anti-psychotic (both typical and atypical including clozapine) and anti-depressant agents with this receptor, at clinically relevant concentrations (Monsma et al., 1993; Roth et al., 1994; Kohen et al., 1996), suggesting that interaction with this receptor may contribute to the clinical efficacy and/or side effects associated with these agents (e.g. see Monsma et al., 1993; Roth et al., 1994; Kohen et al., 1996). However, the failure of Ro 04-6790 to prevent haloperidol- or SCH23390-induced catalepsy (Bourson et al., 1998), indicates that 5-HT₆ receptor antagonism is not (solely?) responsible for the relative lack of extrapyramidal side effects associated with atypical anti-psychotic compounds such as clozapine.

In the vast majority of studies to date, non-selective radioligands have been employed to label the recombinant 5-HT₆ receptor although their lack of selectivity hinders characterisation in native tissue (e.g. Bourson et al., 1995). Snyder and co-workers (Glatt et al., 1995) attempted to use [³H]clozapine to label the 5-HT₆ recep-

(i)



(ii)

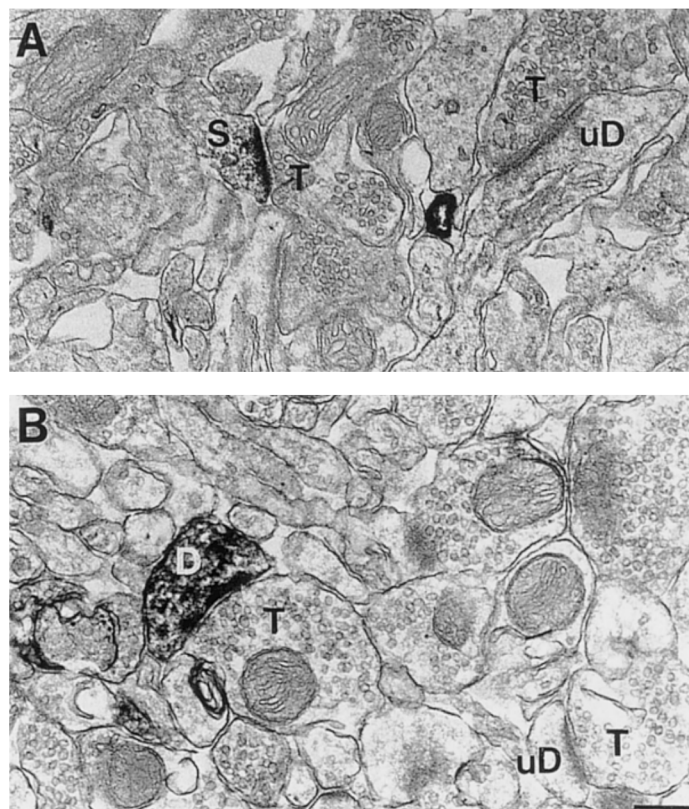


Fig. 13. Immunochemical labelling of the 5-ht₆ receptor in rat brain. (i) Immunoperoxidase staining with purified anti-5-ht₆ receptor antibody at the level of the striatum (A) and hippocampus (B). CA1, CA1 field of the hippocampus; Cl, claustrum; CPU, caudate-putamen (striatum); Cx, cerebral cortex; DG, dentate gyrus; LSD, dorsal lateral septum; GP, globus pallidus; Gr, granule cell layer; Hb, habenula; Mol, molecular layer of the dentate gyrus; Or, strata oriens of the CA1; Py, pyramidal cell layer; Rad, strata radiatum of the CA1. Scale bars represent 1.5 mm (A) and 0.5 mm (B). (ii) Electron micrographs of immunostaining by purified anti-5-ht₆ receptor antibody in the caudate-putamen (A) and the dentate gyrus of the hippocampus (B). (A) A dendritic spine (probably of a medium-sized spiny neurone) in contact with an unlabeled axon terminal shows a dense immunostaining at the level of the synaptic differentiation. (B) An immunoreactive dendrite filled with the enzymatic reaction product receives a synaptic contact from an unlabeled nerve terminal. A dense staining is observed at the postsynaptic side of the synapse. Axodendritic synapses between unlabeled terminals and dendrites are also observed in both areas. D, dendrite; S, dendritic spine; T, axon terminal; uD, unlabeled dendrite. Scale bar represents 0.25 μ m. Reproduced from Gérald et al. (1997), with permission.

tor. They demonstrated the labelling of at least two sites, one of which would appear to be the muscarinic receptor (Glatt et al., 1995). However, in the presence

of muscarinic receptor blockade, the remaining [³H]clozapine binding site in rat brain displayed considerable pharmacological similarity to the recombinant

rat 5-HT₆ receptor, although the apparent weak affinity of 5-HT in this study ($K_i = 0.2$ mM; Glatt et al., 1995) has yet to be explained.

With the identification of selective 5-HT₆ receptor ligands, it is likely that their [³H]-derivatives will find use as radioligands. Indeed, the [³H]-derivative of Ro 63-0563 is able to label the heterologously expressed 5-HT₆ receptor, although the relatively high level of non-specific binding associated with this radioligand will limit its use to label 5-HT₆ sites in brain tissue (Boess et al., 1998). Nevertheless, this compound does label a saturable population of apparently homogenous sites in the striatal membranes prepared from rat and pig which display the pharmacology of the 5-HT₆ receptor (Boess et al., 1998).

15.4. Functional effects mediated via the 5-HT₆ receptor

Consistent with the deduced 5-HT₆ receptor structure (i.e. seven putative transmembrane domains, relatively short presumed third cytoplasmic loop, relatively long presumed cytoplasmic C-terminus), the recombinant 5-HT₆ receptor expressed in various artificial expression systems couples to a metabotropic transduction system which enhances adenylate cyclase activity (Monsma et al., 1993; Ruat et al., 1993a,b; Kohen et al., 1996; Boess et al., 1997). Furthermore, subsequent studies using striatal tissues (mouse striatal neurones in primary culture and pig striatal homogenate; Schoeffter and Waerber, 1994; Sebben et al., 1994) and mouse neuroblastoma N18TG2 cells (Unsworth and Molinoff, 1993), together with retrospective re-analysis of previous work (e.g. NCB20 cells; Conner and Mansour, 1990), indicates that native 5-HT₆ receptors are also positively coupled to adenylate cyclase.

15.5. Behavioural consequences following interaction with the 5-HT₆ receptor

In the absence of selective 5-HT₆ receptor ligands, early studies attempted to reduce the expression of the receptor using antisense oligonucleotides directed against 5-HT₆ receptor mRNA (Bourson et al., 1995; Yoshioka et al., 1998).

Thus in one study, central administration (i.c.v.) of both a 5-HT₆ receptor antisense probe (18 bases complementary to the first 18 nucleotide bases of the rat 5-HT₆ receptor cDNA) and a control scrambled probe (comprising the same nucleotide bases) unfortunately induced some non-specific behavioural changes possibly as a consequence of the toxic nature of these compounds. Nevertheless, it was noted that rats receiving the antisense probe demonstrated a higher incidence of yawning and stretching. Furthermore, the increase in the numbers of yawns and stretches were dose-related (although so were the non-specific effects) and, unlike

the non-specific effects, they were almost completely prevented by the muscarinic receptor antagonist, atropine (Bourson et al., 1995). It was further noted that animals that had received the antisense probe had an ~20% reduction in the density of non-D₂, non-5-HT₂ receptor [³H]LSD-labelled sites in the frontal lobes (regions rostral to the optic chiasma) relative to animals treated with the scrambled probe. As pointed out by the authors, even under the conditions employed to minimise non-5-HT₆ receptor interaction, [³H]LSD is still likely to label a number of 5-HT receptors in addition to 5-HT₆ receptors. However, the decrease in binding levels is consistent with the predicted result. In support of the authors' conclusions from their antisense studies, a more recent study by the same group has demonstrated a similar behavioural syndrome in rats following peripheral administration of the selective 5-HT₆ receptor antagonist Ro 04-6790 at a sufficient dose to occupy 5-HT₆ receptors in the brain (Sleight et al., 1998). In addition, the same group has further demonstrated an interaction between the 5-HT₆ receptor and the central acetylcholine system. Thus, muscarinic receptor antagonist-induced ipsilateral rotation in unilateral 6-hydroxydopamine-lesioned rats was attenuated by the 5-HT₆ receptor antagonist, Ro 04-6790 (Bourson et al., 1998).

A further study utilising an antisense oligonucleotide directed against 5-HT₆ receptor mRNA also demonstrated a reduction (~30%) in the density of non-D₂, non-5-HT₂ receptor [³H]LSD-labelled sites in whole brain homogenates from rats treated with the antisense probe relative to a scrambled probe (Yoshioka et al., 1998). Whilst the antisense probe treatment failed to alter behaviour in an animal model of anxiety (conditioned fear stress), the elevation in 5-HT release within the prefrontal cortex induced by the conditioned fear stress was attenuated considerably by the antisense probe treatment (Yoshioka et al., 1998; Fig. 14). The results from such experiments add further interest in the 5-HT₆ receptor as a potential target to manipulate 5-HT function in the brain and consensus recognition of an involvement of the 5-HT₆ receptor in brain function is eagerly awaited. The recent preliminary report relating to the production of a 5-HT₆ receptor knockout mouse (Brennan and Tecott, 1997) plus further experimentation using selective 5-HT₆ receptor ligands will increase our understanding of the physiological and potential pathological roles of this receptor. However, the 5-HT₆ receptor knockout mouse would not appear to display any marked phenotypic abnormalities (Tecott et al., 1998), although these mice tend to display increased anxiety-like behaviour in the elevated zero-maze.

It may be relevant to the potential association of the 5-HT₆ receptor with depression that pharmacological adrenalectomy increases expression of 5-HT₆ receptor mRNA within the CA1 field of the hippocampus (by

approximately 30%; although not in other hippocampal regions). Furthermore, this response is not apparent in animals where physiological levels of corticosterone had been maintained by corticosterone implants (Yau et al., 1997). Interestingly, in addition to 5-HT₆ receptor mRNA, adrenalectomy also elevates expression of 5-HT_{1A} and 5-HT₇ receptor mRNA in hippocampus whereas expression of other 5-HT-related genes (5-HT_{2A/2C} and 5-HT transporter) are unaltered (c.f. Le Corre et al., 1997). Therefore, the gene expression of three distinct 5-HT receptor subtypes appears to be under a common inhibitory influence of corticosteroids. Since high corticosteroids and stress are implicated as vulnerability factors in major depression, abnormalities in the functioning of all three receptors may be a feature of the illness. However, the ability of currently utilised anti-depressants to antagonise 5-HT₆ receptor-mediated responses (e.g. mianserin; Boess et al., 1997) adds further complexity to the potential relationship between the 5-HT₆ receptor and depression.

16. 5-HT₇ receptors

Notwithstanding the 5-HT₇ receptor being the most recently identified 5-HT receptor, functional responses now attributed to this receptor have been documented for a number of years (for review see Eglen et al., 1997).

5-HT₇ receptor cDNAs have now been identified from a number of species (e.g. *Xenopus laevis* (toad), mouse, rat, guinea pig, human; Bard et al., 1993; Lovenberg et al., 1993a,b; Meyerhof et al., 1993; Plassat et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Tsou et al., 1994; Nelson et al., 1995) using the well trodden approach of screening cDNA libraries with degenerate oligonucleotides corresponding to conserved sequences amongst receptor families.

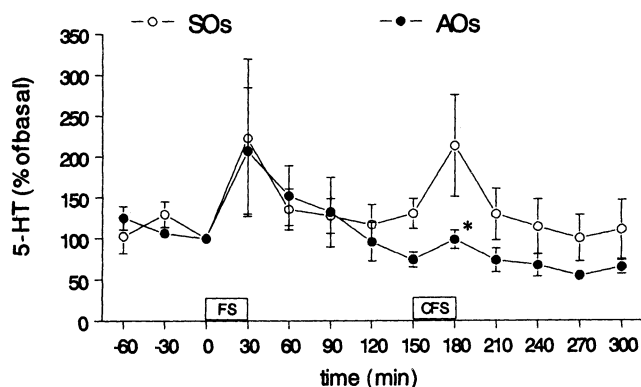


Fig. 14. Effects of treatment with antisense and sense oligonucleotides (AOs and SOs, respectively) directed against 5-HT₆ receptor mRNA (i.c.v. 14 µg/day for 7 days) on the increase in 5-HT release in the rat prefrontal cortex induced by foot-shock (FS) and conditioned fear stress (CFS). * $P < 0.05$, significantly different from SOs group (Student's *t*-test). Reproduced from Yoshioka et al. (1998), with permission.

16.1. 5-HT₇ receptor structure

The 5-HT₇ receptor appears to be the mammalian homologue of the 5-HT_{dro1} receptor identified in the fruitfly, *Drosophila melanogaster* (Witz et al., 1990). The full length mammalian 5-HT₇ receptor is predicted to be 445–448 amino acids in length (*Xenopus laevis* 425 amino acids; Nelson et al., 1995; e.g. Lovenberg et al., 1993a,b; Heidmann et al., 1997; Jasper et al., 1997). The 5-HT₇ receptor gene is located on human chromosome 10 (10q21–q24; Gelernter et al., 1995) and contains two introns (Ruat et al., 1993a,b; Erdmann et al., 1996; Heidmann et al., 1997). The presence of one of these introns corresponds to the predicted second intracellular loop and, therefore, any alternatively spliced variants arising at this site are probably ineffectual (Shen et al., 1993; Erdmann et al., 1996; Heidmann et al., 1997). The second intron corresponds to the C-terminus and results in the generation of a number of alternatively spliced variants including a longer isoform due to the retention of an exon cassette (Lovenberg et al., 1993a,b; Heidmann et al., 1997; Jasper et al., 1997).

Despite the presence of at least four splice variants of the 5-HT₇ receptor (5-HT_{7(a)}, 5-HT_{7(b)}, 5-HT_{7(c)}, 5-HT_{7(d)}), rat and human tissues appear to each express only three of the variants. Thus, only the 5-HT_{7(a)}, 5-HT_{7(b)} and 5-HT_{7(c)} receptor isoforms are expressed in rat tissues, due to the absence of the exon responsible for the 5-HT_{7(d)} receptor isoform in the rat gene; Whilst the human gene would appear capable of generating the 5-HT_{7(c)} receptor isoform, it has yet to be detected in human native tissue (Heidmann et al., 1997).

The predicted amino acid sequences of the 5-HT₇ receptor isoforms display the characteristic seven putative membrane spanning regions (Bard et al., 1993; Lovenberg et al., 1993a,b; Meyerhof et al., 1993; Plassat et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Tsou et al., 1994; Nelson et al., 1995; Heidmann et al., 1997, 1998; Stam et al., 1997) of the G-protein coupled receptor superfamily. The receptor contains consensus sequences for two N-linked glycosylation sites (three for the *Xenopus* receptor; Nelson et al., 1995) in the predicted extracellular N-terminal (Bard et al., 1993; Lovenberg et al., 1993a,b; Meyerhof et al., 1993; Plassat et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Tsou et al., 1994) and a number of consensus sequence phosphorylation sites for both protein kinase A and C in the putative third intracellular loop and the cytoplasmic C-terminus (Bard et al., 1993; Lovenberg et al., 1993a,b; Meyerhof et al., 1993; Plassat et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Tsou et al., 1994; Nelson et al., 1995; Heidmann et al., 1997). Moreover, since the alternatively spliced variants differ in the number of phosphorylation sites and the lengths of their C-termini, this may have functional consequences (e.g. differential susceptibility to desensitisation and G-protein coupling efficiency).

16.2. 5-HT₇ receptor distribution

The 5-HT₇ receptor exhibits a distinct distribution in the CNS. In rat and guinea pig brain, both the mRNA and receptor binding sites display a similar distribution (Gustafson et al., 1996; Stowe and Barnes, 1998b), indicating that the receptor is expressed close to the site of synthesis. 5-HT₇ receptor expression is relatively high within regions of the thalamus, hypothalamus and hippocampus with generally lower levels in areas such as the cerebral cortex and amygdala (To et al., 1995; Gustafson et al., 1996; Stowe and Barnes, 1998b).

With respect to the distribution of the isoforms of the 5-HT₇ receptor, large tissue-specific differences in the splicing of pre-mRNA within a species are not apparent (Heidmann et al., 1997, 1998; Stam et al., 1997). However, the relative abundance of the 5-HT_{7(b)} receptor isoform displays marked differences between rat (low) and human (high) tissues (Heidmann et al., 1997, 1998; Stam et al., 1997).

16.3. 5-HT₇ receptor pharmacology

Although one report of a selective 5-HT₇ receptor antagonist has reached the literature (Forbes et al., 1998), this compound is not widely available at present which still therefore necessitates generation of a broad pharmacological profile to attribute responses to the 5-HT₇ receptor. However, the ability of a range of clinically utilised psychoactive agents to interact with the 5-HT₇ receptor at relevant concentrations (typical and atypical antipsychotics and antidepressants including clozapine; e.g. Roth et al., 1994; To et al., 1995; Stowe and Barnes, 1998a), similar to the 5-HT₆ receptor, suggests that this receptor may be important as a target in psychiatric conditions, although genetic variation within the 5-HT₇ receptor gene does not appear to be associated with either schizophrenia or bipolar affective disorder (Erdmann et al., 1996).

To date, no major pharmacological differences have been identified between the 5-HT₇ receptor isoforms.

16.4. Functional responses mediated via the 5-HT₇ receptor

16.4.1. Transduction system

Both the recombinant and the native 5-HT₇ receptor stimulate adenylate cyclase in accordance with its putative seven transmembrane motif (Bard et al., 1993; Lovenberg et al., 1993a,b; Plassat et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Tsou et al., 1994; Hirst et al., 1997; Heidmann et al., 1997, 1998; Stam et al., 1997). Consistent with other members of the G-protein coupled receptor superfamily, amino acid residues within the third intracellular loop of the 5-HT₇ receptor are likely to be involved in the coupling to G α_s (Obosi et al., 1997).

It should be noted, however, that artificial expression of the 5-HT_{7(a)} receptor has identified that the receptor activates the G β -insensitive isoforms of adenylate cyclase, AC1 and AC8, as well as the G β -sensitive isoform AC5 (Baker et al., 1998). A rise in intracellular [Ca²⁺] appeared responsible for the 5-HT_{7(a)} receptor-mediated activation of AC1 and AC8, consistent with the Ca²⁺/calmodulin sensitivity of these isoforms, although the response was independent of phosphoinositide turnover and protein kinase C activity as well as G γ activation (Baker et al., 1998). Furthermore, this transduction system may be relevant in native tissue (e.g. see Mork and Geisler, 1990; Miller et al., 1996; Baker et al., 1998).

16.4.2. Circadian rhythms

A number of reports now implicate a role for the 5-HT₇ receptor in the regulation of circadian rhythms. Thus, 5-HT has been known for some time to induce phase shifts in behavioural circadian rhythms (e.g. Edgar et al., 1993) and neuronal activity in the suprachiasmatic nucleus (e.g. Medanic and Gillette, 1992; Prosser et al., 1993), the likely site of the mammalian circadian clock (for review see Turek, 1985). Until recently, the 5-HT receptor mediating this response was generally considered to be the 5-HT_{1A} receptor largely based on the ability of 8-OHDPAT to mimic the response to 5-HT (e.g. Prosser et al., 1993; Cutrera et al., 1996). However, 8-OHDPAT is now recognised additionally to be a 5-HT₇ receptor agonist, albeit at relatively high concentrations (Plassat et al., 1993; Tsou et al., 1994; Nelson et al., 1995). Furthermore, the finding that the phase shift in neuronal activity induced by 8-OHDPAT was blocked by ritanserin but not by selective 5-HT_{1A} receptor antagonists (e.g. WAY 100 635; Ying and Rusak, 1997) makes it more likely that the 5-HT₇ receptor mediates the response (Lovenberg et al., 1993a,b; Ying and Rusak, 1997). This hypothesis is strengthened by the fact that 5-HT₇ receptor mRNA is expressed by cells in the suprachiasmatic nucleus (Stowe and Barnes, 1998b) and also since treatments which either promote the levels of cAMP or mimic the effects of cAMP, reproduce 5-HT's ability to induce a phase shift in neurone activity in these neurones (Prosser and Gillette, 1989; Prosser et al., 1993). Furthermore, inhibitors/blockers of enzymes and ion channels which are activated by cAMP prevent the 5-HT receptor agonist-induced response (Prosser et al., 1993).

16.4.3. Modulation of neuronal activity

Growing evidence generated by Beck and Bacon (1998a,b) indicates that the 5-HT₇ receptor inhibits the slow afterhyperpolarisation in CA3 hippocampal pyramidal neurones analogous to the 5-HT₄ receptor mediated response in CA1 hippocampal neurones.

Table 11
Summary of the functional responses associated with activation of the brain 5-HT₇ receptor

Level	Response	Mechanism
Cellular	Adenylate cyclase (+)	Post
Electrophysiological	Phase shift advance suprachiasmatic nucleus	Post

16.4.4. Seizures

A recent study reported that the ability of a number of non-selective 5-HT receptor antagonists to prevent the 5-HT-induced activation of adenylate cyclase by heterologously expressed 5-HT₇ receptors, correlated significantly with their ability to prevent audiogenic seizures in DBA/2J mice (Bourson et al., 1998). Whilst highly speculative, such studies may indicate a role for 5-HT₇ receptor antagonists in the treatment of epilepsy.

16.4.5. Manipulation of receptor expression

Intra-cerebroventricular administration of antisense oligonucleotides directed against 5-HT₇ receptor mRNA reduced (by ~45%) [³H]5-HT binding associated with the 5-HT₇ receptor in the rat hypothalamus (but not cerebral cortex), although this treatment did not modify either locomotor or exploratory behaviour nor behaviour in an animal model of anxiety; the elevated plus-maze (Clemett et al., 1998). Furthermore, the antisense treatment did not alter plasma corticosterone or prolactin levels nor central 5-HT turnover. However, as is common with central administration of antisense oligonucleotides, non-specific effects were apparent such as a reduction in food intake (Clemett et al., 1998).

It has been reported that chronic administration of the SSRI fluoxetine (which itself displays micromolar affinity for the 5-HT₇ receptor; Clemett et al., 1997), induces a downregulation of a population of binding sites that includes the 5-HT₇ receptor in the hypothalamus (density reduced by approximately 30%; Sleight et al., 1995; see also Gobbi et al., 1996). However, chronic exposure of rat frontocortical astrocytes in primary culture to either of the SSRIs, paroxetine or citalopram, did not modify 5-HT₇ receptor-mediated stimulation of cAMP levels (Shimizu et al., 1996), which may simply reflect a lack of 5-hydroxytryptaminergic innervation in the latter preparation. Indeed, direct 5-HT₇ receptor agonist exposure in this preparation induces homologous receptor desensitisation (Shimizu et al., 1998). However, chronic exposure of the astrocytes to the antidepressant compounds amitriptyline, chlomipramine, mianserin, maprotiline and setiptiline (but not imipramine) enhanced 5-HT-stimulated cAMP production which appeared to be most likely via interaction with the 5-HT₇ receptor (Shimizu et al., 1996).

The mechanism underlying this latter finding remains to be determined. The functional effects associated with 5-HT₇ receptor activation are summarised in (Table 11).

17. Summary and main conclusions

Since 1986, the number of recognised mammalian 5-HT receptor subtypes in the CNS has more than doubled to 14, and these have been classified into seven receptor families (5-HT₁₋₇) on the basis of their structural, functional and to some extent pharmacological characteristics. Recent findings suggest that even this level of complexity will escalate given the existence in the brain of as yet unclassified novel 5-HT binding sites, but particularly new evidence that specific 5-HT receptor subtypes (so far, 5-HT_{2C}, 5-HT₃, 5-HT₄ and 5-HT₇ receptors) can occur as multiple isoforms due to gene splicing or post-transcriptional RNA editing. It should also be noted that there is emerging evidence that many 5-HT receptor subtypes have naturally occurring polymorphic variants, and these could be a major source of biological variation within the 5-HT system.

Much new information about the CNS distribution and function of 5-HT receptor subtypes has accrued as a result of the development by the pharmaceutical industry of novel compounds with high selectivity for individual 5-HT receptor subtypes. Indeed, at the current time selective ligands have been identified for all but a few of the 5-HT receptor subtypes known (5-HT_{1E} and 5-HT_{5A/B}).

A striking feature of the 5-HT receptor subtypes revealed by autoradiographic studies is that each has a highly distinct pattern of distribution in the CNS, such that individual brain regions contain their own complement of 5-HT receptor subtypes. All of the receptors are located postsynaptically where some are known to modulate ion flux to cause neuronal depolarisation (5-HT_{2A}, 5-HT_{2C}, 5-HT₃ and 5-HT₄ receptors) or hyperpolarisation (5-HT_{1A} receptor). Certain 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{1B} and possibly 5-HT_{1D} receptors) are located on the 5-HT neurones themselves where they serve as 5-HT autoreceptors at the somatodendritic or nerve terminal level. It is becoming clear that some 5-HT receptors (5-HT_{1B,D}, 5-HT_{2A,C}, 5-HT₃ and 5-HT₄ receptors) are also located on the nerve terminals of non-5-HT neurones where they appear to function as heteroreceptors, regulating neurotransmitter release.

Most recently, evidence has emerged that certain 5-HT receptor subtypes (5-HT_{2A} and possibly 5-HT_{2C}, 5-HT₄ and 5-HT₆ receptors) mediate postsynaptic effects which extend beyond a short-term influence on neurotransmission to the triggering of a cascade of intracellular mechanisms, resulting in altered gene ex-

pression. It has been speculated that some of the genes activated are involved in the modulation of trophic mechanisms and neural connectivity. Moreover, it seems highly likely that 5-HT receptor-mediated changes in gene expression have a significant role to play in the neuroadaptive processes that are thought to be fundamental to the mechanisms of psychotropic drug therapy and abuse.

It is now clear that in the whole animal model, activation of specific 5-HT receptor subtypes can be linked with the modulation of specific behaviours. The 5-HT_{1A}, 5-HT_{2A/C}, 5-HT_{2c} receptors currently stand out in terms of the wide range of behaviours and physiological responses that agonists for these receptors can evoke. Selective antagonists have established a key role for the 5-HT_{2c} receptor in feeding and (along with the 5-HT₃ and 5-HT₄ receptors) anxiety. However, much more will be learned about the behavioural sequelae accompanying interaction with these and other 5-HT receptor subtypes with the development of selective, brain penetrant ligands as well as the increased application of genetically engineered (5-HT receptor knockout) animals.

Finally, the clinical utility of certain 5-HT receptor selective ligands has been established in various neuropsychiatric disorders including major depression and anxiety (buspirone) and migraine (sumatriptan). Ongoing clinical trials investigating the therapeutic usefulness of selective 5-HT receptor ligands, including 5-HT_{1A} (auto)receptor antagonists (depression), 5-HT_{2A} antagonists (schizophrenia) and 5-HT_{2C} antagonists (anxiety), underpin the common belief that the full potential clinical benefits of discoveries in 5-HT neuropharmacology have yet to be realised.

18. Note added in proof

Recently, an additional 5-HT₃ receptor has been identified, the human (h) 5-HT_{3B} receptor subunit (Davies et al., 1999), would appear to be the 'missing' structural component of native 5-HT₃ receptors. Thus, this subunit when expressed alone fails to form functional 5-HT₃ receptors, although when co-expressed with the 5-HT_{3A} receptor subunit, the resultant

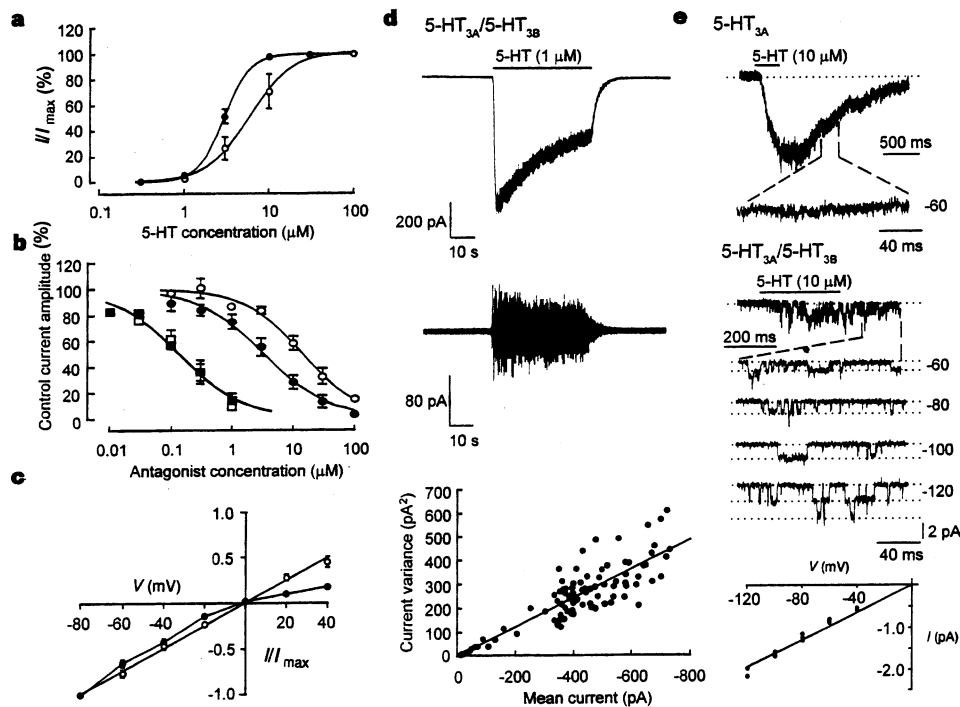


Fig. 15. The pharmacological and biophysical properties of homomeric and heteromeric 5-HT₃ receptors. (a) Concentration-dependent activation of currents by 5-HT recorded from HEK-293 cells transfected with 5-HT_{3A} cDNA alone (filled circles) or in combination with 5-HT_{3B} cDNA (open circles). Data points represent mean current amplitudes recorded from at least four cells normalized to the maximum current amplitude. (b) Concentration-dependent inhibition by metoclopramide (squares) and tubocurarine (circles) of currents mediated by 5-HT_{3A} (filled symbols) and heteromeric (open symbols) receptors. (c) Current-voltage relationships for responses evoked by 10 μM 5-HT, recorded from cells expressing 5-HT_{3A} (filled circles) and heteromeric (open circles) receptors. Data points represent mean current amplitudes recorded from at least four cells and normalised to the amplitude of the current recorded at -80 mV. Data points for heteromeric receptors were fitted with a linear function. (d) Representative low-gain d.c. and high-gain a.c.-coupled records of an inward current response to 5-HT (1 μM) recorded at a holding potential of -60 mV from a HEK-293 cell expressing heteromeric 5-HT₃ receptors. The relationship between membrane current variance and mean current amplitude (1 s periods) was fitted by linear regression for five cells, to yield a single-channel amplitude (i) of 0.65 ± 0.02 pA and an elementary conductance (γ) of 11.7 ± 0.3 pS. (e) Single-channel recordings from out-side-out patches containing 5-HT_{3A} (top panel) and heteromeric (lower panel) 5-HT₃ receptors. The conductance (16 pS) of channels mediated by heteromeric receptors was derived from the linear fit to the current-voltage relationship obtained from three excised patches. Reproduced from Davies et al., (1999), with permission.

presumably heteromeric 5-HT₃ receptor complex more fully replicates the biophysical characteristics of native neuronal 5-HT₃ receptors (e.g. high channel conductance ~ 16 pS; Davies et al., 1999; Figure 15).

Despite the h5-HT_{3B} receptor subunit displaying 41% amino acid identity with the h5-HT_{3A} receptor, it is of considerable interest that the 5-HT_{3B} receptor subunit displays a number of unusual characteristics in its primary structure which distinguishes it from the other members of the ligand-gated ion channel superfamily e.g. lack of negatively charged amino acids in the M2 domain which comprise the cytoplasmic, intermediate and extracellular rings, and also the polar amino acids which form the central polar ring (Davies et al., 1999). Indeed, amongst other members of the cys–cys loop ligand gated ion channel family, these ‘rings’ are believed to control ion conductance through the channel and hence their absence in the 5-HT_{3B} receptor subunit, which conveys increased channel conductance, poses questions concerning the structure-function relationship within the superfamily.

In addition to differences between the M2 regions of the 5-HT_{3A} and 5-HT_{3B} receptor subunits, the putative large intracellular loops also display considerable differences in their amino acid sequences; thus this loop is shorter in the 5-HT_{3B} receptor subunit (134 and 100 amino acids for the h5-HT_{3A} and 5-HT_{3B} receptor subunit, respectively) with only 29% amino acid identity (% maximised by aligning analogous regions). Hence this portion of the h5-HT_{3B} receptor subunit may provide polypeptide sequences to generate selective polyclonal antibodies (this is also the region of the 5-HT_{3A} receptor subunit for which a selective antibody has been generated; e.g. see Fletcher and Barnes, 1997; Fletcher et al., 1998).

Since both homomeric 5-HT_{3A} and heteromeric 5-HT_{3A/3B} receptor complexes are likely to exist in native tissue (e.g. see data reported in Yang et al., 1992; Hussy et al., 1994; for review see Fletcher and Barnes, 1998), this may provide an opportunity to pharmacologically manipulate different populations of 5-HT₃ receptors based on their subunit compositions. However, only minimal pharmacological differences have been identified so far (e.g. heteromeric 5-HT_{3A/3B} receptors being ~ 5-fold less sensitive to the non-selective antagonist d-tubocurarine relative to homomeric 5-HT_{3A} receptors; Davies et al., 1999). In addition to potential pharmacological differences with respect to the 5-HT recognition site, it would be of interest to investigate the potential differences with respect to the allosteric sites on homomeric versus heteromeric 5-HT₃ receptors since this approach may offer an alternative means of pharmacologically differentiating sub-populations of 5-HT₃ receptors.

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