The 5-HT_3 Receptor in Mammalian Brain: A New Target for the Development of Psychotropic Drugs?

José Antonio Apud, M.D., Ph.D.

KEY WORDS: *Serotonin; Serotonin receptors; 5-HT_3 binding sites; 5-HT_3 receptor antagonists; Ligand-gated Na^+-K^+ channel*

Serotonin (5-HT) is a neurotransmitter in the central nervous system (CNS) of vertebrates and invertebrates. In vertebrates, 5-HT participates in the regulation of various physiologic functions, including pain perception, blood pressure, sleep, homeothermia, and sexual activity. It is also believed that 5-HT may participate in the expression of symptoms of certain psychiatric disorders, such as depression and anxiety. In this context, most of our knowledge concerning the participation of 5-HT and 5-HT receptors in psychopathology has come from the characterization of the mechanisms of action of various drugs that are effective in relieving the symptoms of psychiatric disorders.

That the 5-HT receptor population in the periphery might be heterogeneous was first suggested by the early pioneering work of two independent groups (Rocha e Silva et al. 1953; Gaddum and Hameed 1954). It was not until the late 1970s, however, that Peroutka and Snyder (1979) described, in the CNS, the existence of two different 5-HT recognition sites labeled by lysergic acid diethylamine, the serotonin-1 (5-HT_1) and the serotonin-2 (5-HT_2) binding sites.

The 5-HT_1 site, which can also be labeled by [^{3}H]5-HT, was characterized by a dissociation constant for 5-HT in the nanomolar range. The combination of appropriate specific ligands and computer-assisted analysis of [^{3}H]5-HT binding isotherms in the CNS revealed five different subtypes of 5-HT_1 binding sites: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1E} (Pedigo et al. 1981; Pazos et al. 1984; Peroutka 1986; Heuring and Peroutka 1987; Leonhardt et al. 1989). The receptor status of the 5-HT_{1E} subtype, however, is still a pending question. In contrast, the 5-HT_2 site was characterized as a 5-HT receptor with low (micromolar) affinity for 5-HT but high (nanomolar) affinity for a series of compounds (ketanserin, ritanserin, cinanserin, and spiperone) (Bradley et al. 1985; Glennon 1987) that antagonize certain behavioral responses elicited by the administration of 5-HT-mimetic drugs (Peroutka et al. 1981).

A third type of 5-HT receptor, the 5-HT_3 recognition site, has also been identified (Fozard et al. 1979; Fozard 1984; Richardson et al. 1985; Hoyer and Neijt 1987; Kilpatrick et al. 1987; Resier and Hamprecht 1989; Peters and Lambert 1989; Barnes et al. 1989a). The 5-HT_3 receptor, which represents the so-called "M" receptor identified by Gaddum and Piccarelli (1957) in the guinea pig intestine, was initially described in peripheral neurons in which it mediates depolarization of neurotransmitter release (Bradley et al. 1985). More recently, 5-HT_3 binding sites were also identified in neuronal cell lines (Hoyer and Neijt 1987; Resier and Hamprecht 1989; Peters and Lambert 1989), in discrete brain areas (Kilpatrick et al. 1987; Barnes et al. 1988; Peroutka and Hamik 1988; Barnes et al. 1989a; Waeber et al. 1989), and in the spinal cord (Glaum and Anderson 1988; Hamon et al. 1989). Whether these binding

From the Fidia-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington D.C. (On leave from the Catedra de Farmacologia y Toxicologia, Facultad de Medicina, Universidad de Buenos Aires and ININFA, CONICET, Buenos Aires, Argentina.)

Address reprint requests to: Jose Antonio Apud, Fidia-Georgetown Institute for the Neurosciences, Georgetown University Medical School, 3900 Reservoir Road NW, Washington, D.C. 20007.

Received August 1, 1991; revised January 30, 1992; accepted February 12, 1992.

Published by Elsevier Science Publishing Co., Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/93/$6.00

sites represent functional receptors and meet the criteria for 5-HT_3 receptors (Bradley et al. 1985) has been addressed by a series of studies showing behavioral, biochemical, and electrophysiologic responses to both 5-HT_3 agonist and antagonist drugs (Costall et al. 1987, 1990; Blandina et al. 1988; Yakel and Jackson 1988; Barnes et al. 1989b; Blandina et al. 1989; Imperato et al. 1989; Peters and Lambert 1989; Hagan et al. 1990).

A 5-HT receptor that does not meet the criteria for a 5-HT_1, 5-HT_2, or 5-HT_3 receptor has also been recently described. This receptor, which is found in mouse embryo colliculi neurons and in the guinea pig hippocampus, has been classified as 5-HT_4 (Dumuis et al. 1988). A series of different putative 5-HT receptors (5-HT_{1p}, drosophila 5-HT receptor, the tryptamine-preferring receptors of the invertebrates, the stomach fundus receptor) have also been identified in peripheral tissues of vertebrates and invertebrates.

The 5-HT_1, 5-HT_2, and 5-HT_4 receptors are coupled to second messenger systems. Activation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors inhibits, whereas activation of the 5-HT_4 receptor stimulates, adenylate cyclase (Bradley et al. 1985; Dumuis et al. 1988). On the other hand, activation of both 5-HT_{1C} and 5-HT_2 receptors increases phosphoinositide (PI) metabolism (Conn and Sanders-Bush 1984; Conn et al. 1986; Nakaki et al. 1984). It was also shown recently that 5-HT_3 receptor activation stimulates PI hydrolysis in the rat cortex (Edwards et al. 1991). Whether the increase of PI turnover is the result of a direct 5-HT_3 receptor-mediated mechanism or else an indirect effect mediated through a still unknown pathway is not yet clearly understood.

In the last several years, the 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_2 receptors have been cloned (Fargin et al. 1988; Julius et al. 1988; Pritchett et al. 1988; Hamblin and Metcalf 1991). More recently, the 5-HT_3 receptor has also been cloned (Maricq et al. 1991). The characteristics of the cloned 5-HT_3 receptor are largely consistent with the properties of the purified receptor obtained from brain tissue and neural tumor cell lines (McKernan et al. 1990a, 1990b; Miquel et al. 1990) (see below).

DEVELOPMENT OF SELECTIVE DRUGS ACTIVE AT THE 5-HT_3 RECEPTOR

It has long been known that both morphine and cocaine block the excitatory action of 5-HT on peripheral neurons (Rocha e Silva et al. 1953; Gaddum and Hameed 1954). Only recently, however, was evidence presented indicating that both drugs induce their effects via a specific competitive antagonistic action on 5-HT_3 receptors (Fozard et al. 1983). Further studies demonstrated that metoclopramide and a series of substituted benzamides (Fozard et al. 1978; Fozard 1984) also behave as competitive antagonists of 5-HT action on peripheral tissues possessing 5-HT_3 receptors.

The broad pharmacologic profiles of compounds with affinity for the neural 5-HT_3 receptor hampered the definition of this receptor. By chemically substituting compounds that bind with rather low specificity to the 5-HT_3 site, it became possible to synthesize selective 5-HT_3 receptor agonists and antagonists (Fozard and Gittos 1983; Richardson et al. 1985; Fake et al. 1987). On the basis of the assumption that different conformations of the 5-HT molecule are required for activation of the so-called "D" (5-HT_2) and "M" (5-HT_3) receptors, Richardson et al. (1985) produced both specific agonists and antagonists for the 5-HT_3 receptor with 5-HT as the starting compound. By systematic methyl substitutions in the indole nucleus and ethylamine side chain, the conformational freedom of the 5-HT molecule was reduced. This strategy provided somewhat specific 5-HT_2 (α-methyl-5-HT) and highly specific 5-HT_3 (2-methyl-5-HT) receptor agonists. Moreover, by extending the ethylamine chain of 5-HT and producing rigid analogues in which the terminal nitrogen is included in a tropine or homeotropine ring system, the selective 5-HT_3 antagonist (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930) was obtained (Richardson et al. 1985).

The synthesis of a series of compounds structurally related to (−)-cocaine led to the discovery of another selective 5-HT_3 receptor antagonist. Substitution by chlorine in the benzene ring of a number of substituted benzoic acid esters of tropine led to the synthesis of 1-α-H,3-α,5-α-H-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), a selective and competitive 5-HT_3 receptor antagonist (Fozard and Gittos 1983; Fozard 1984a).

By restricting the conformational freedom of the diethylaminoethyl side chain and changing the aromatic nucleus of the metoclopramide molecule, a series of 3-indazole carboxamides was obtained. Among these compounds, endo-N-(9-methyl-9-azabicyclo[3,3,1]non-3-yl)-1-methyl-imidazole-carboxamide (BRL 43694) was found to possess potent and selective 5-HT_3 antagonistic properties (Fake et al. 1987).

Other compounds with selective activity at the 5-HT_3 receptor have also been identified. Among them, 1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-l-yl)methyl]-4H-carbazol-4-1 hydrochloride hydrate (GR38032) (Butler et al. 1988) and 4-amino-N-(1-azabicyclo[2.2.2.]oct-yl)-5-chloro-2-methoxybenzamide hydrochloride hydrate (zacopride) (Smith et al. 1988) also proved to be potent 5-HT_3 receptor antagonists. Quipazine possesses high affinity for the 5-HT_3 binding site in nervous tissue (Peroutka and Hamik 1988) but also binds with relatively high affinity to other 5-HT receptors (Glennon et al. 1986).

Recently a computer-aided molecular modeling

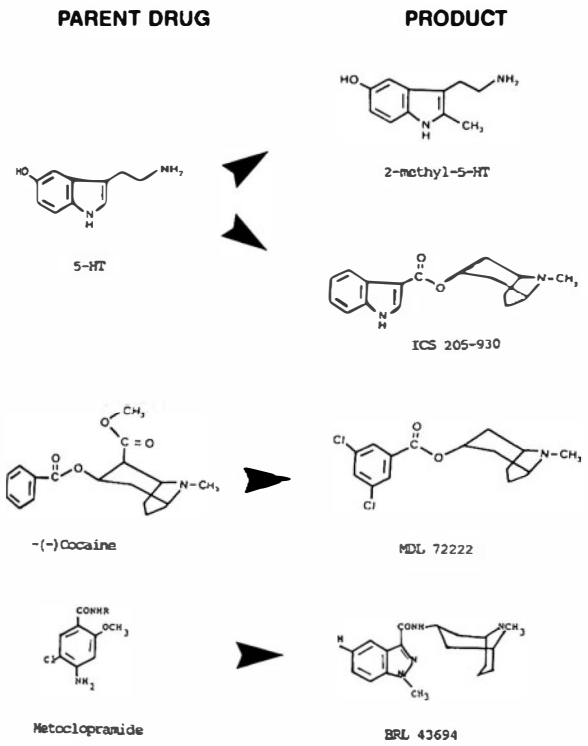

Figure 1. Synthesis of potent and specific drugs [PRODUCT] with affinity for the 5-HT_3 receptor derived from different compounds [PARENT DRUGS] that bind the 5-HT_3 site with low specificity.

(CAMM) system has been used to identify an initial three-component pharmacophore for specific 5-HT_3 receptor ligands such as ICS 205-930, GR38032F, zacopride, and the novel compound 3-[2-(guanidylmethyl)-4-thiazolyl]indole (Rizzi et al. 1990). The CAMM represents a powerful tool in the drug-design field because it helps to explain or predict a variety of molecular properties.

5-HT_3 RECOGNITION SITES IN NERVOUS SYSTEM TISSUE: BIOCHEMICAL CHARACTERIZATION AND DISTRIBUTION

During the last 3 years, various 5-HT_3 receptor antagonists have been labeled with radioactive isotopes. The first radioligand used to identify 5-HT_3 receptors in nervous system tissue was [^{3}H]ICS 205-930. Scatchard analysis with cell membranes of either NG108-15 neuroblastoma-glioma cells (Hoyer and Neijt 1987) or N1E-115 neuroblastoma cells (Hoyer and Neijt 1988) indicated that [^{3}H]ICS 205-930 labeled a single population of binding sites, with a B_{max} of 60.4 and 40.5 fentomole per milligram of protein and a pK_d of 8.91 and 9.2 mol/L, respectively. The binding was competitive and stereoselective (Hoyer and Neijt 1988), and the inhibition profiles of various potent 5-HT_3 receptor agonists and antagonists on [^{3}H]ICS 205-930 binding were consistent with those expected for a 5-HT_3 receptor (Bradley et al. 1985). Moreover, the inability of adenine and guanine nucleotides to affect agonist binding suggests that 5-HT_3 recognition sites are not coupled to G-proteins (Hoyer and Neijt 1988).

The identification and characterization of 5-HT_3 binding sites in the rat brain support the notion that the behavioral effects observed in rats after the administration of drugs selective for 5-HT_3 receptors might be mediated by a central rather than a peripheral 5-HT_3 receptor. After Kilpatrick et al. (1987) first demonstrated the presence of [^{3}H]GR65630 binding sites in rat brain, the putative rat brain 5-HT_3 receptor was characterized with the use of other different radioligands (Kilpatrick et al. 1990; Pratt and Bowery 1989; Reynolds et al. 1989; Schmidt et al. 1988; Watling 1988; Waeber et al. 1989; Watling et al. 1988; Barnes et al. 1988; Barnes et al. 1990; Peroutka and Hamik 1988). The highest affinity for the 5-HT_3 site (K_d = 0.066 nmol/L) was shown by [^{3}H]GR67330 when compared to other labeled 5-HT_3 receptor ligands (K_d values of 0.24 to 1.2 nmol/L). The B_{max} values for these ligands ranged between 32 and 145 fentomole per milligram of protein (Barnes et al. 1990; Kilpatrick et al. 1987, 1989, 1990; Peroutka 1988; Barnes et al. 1988; Watling et al. 1988). Equilibrium saturation analysis for the radioligands revealed that binding was to a single saturable high-affinity site. Binding was also reversible, stereospecific, and unevenly distributed throughout the rat CNS (see below) (Kilpatrick et al. 1987, 1990; Barnes et al. 1988; Watling et al. 1988; Milburn and Peroutka 1989; Barnes et al. 1990). The affinity of serotonergic drugs for the 5-HT_3 binding site labeled with any of the 5-HT_3 receptor ligands clearly indicates that the various compounds bind to an identical 5-HT_3 binding site.

The ability of various 5-HT uptake blockers that possess antidepressant action to displace with nanomolar affinity [^{3}H]quipazine from rat cortical membranes (Schmidt and Peroutka 1989) led to the suggestion that the 5-HT_3 receptor might be involved in the pharmacologic action of antidepressants. However, in membranes of the rat entorhinal cortex labeled with [^{3}H]zacopride or of N1E-115 cells labeled with [^{3}H]ICS 205-930, 5-HT uptake blockers display very low affinity for the 5-HT_3 binding site (Hoyer et al. 1989). These results support the idea that [^{3}H]quipazine might also bind to a site unrelated to the 5-HT_3 recognition site in nervous system tissue (for example, the 5-HT transporter in the presynaptic terminal). In these experiments, it was also shown that potent atypical antipsychotic drugs (loxapine, clozapine, and clotiapine) show higher affinity ($pK_d > 6$) for the 5-HT_3 binding site than metoclopramide, 5-HT, and 2-methyl-5-HT, drugs currently used in the characterization of 5-HT_3 receptors in nervous system tissue. If the antipsychotic action of atypical neuroleptics involves an interaction with brain

Table 1. 5-HT_3 Receptors in the Central Nervous System

Agonists	Antagonists	Radioligands	Transducer Mechanism	Neurophysiology	Biochemical Characteristics
5-HT 2-methyl-5HT 1-phenyl biguanide	MDL72222 ICS 205 930 GR38032 F GR65630 MDL 73,147F Metoclopramide Zacopride	^{3}H-GR67330 ^{3}H-GR65630 ^{3}H-ICS 205-930 ^{3}H-BRL43694 ^{3}H-5-HT ^{3}H-zacopride ^{3}H-S-zacopride ^{3}H-quipazine	Ligand-gated Na^+-K^+ channel	Fast depolarization	Heterooligomeric complex of 250 kD MW

Biochemical Regulation	Molecular Characteristics	Distribution			
		Very High	High	Low	Low or Absent
DA release or turnover (nigrostriatal & mesocortical systems) ACh release (cortex) NA release?	1462 Nucleotides encoding 487 amino acids 27% Homology with α subunit of nicotine ACh receptor; 22% homology with $GABA_A$ receptor and with the 48 kD subunit of the glycine receptor Hydrophobicity analysis predict a protein containing four hydrophobic transmembrane regions Ligand-gated ion channel receptor superfamily characteristics	Area postrema	Cortex Hippocampus Amygdala Nuclei solitary tract Spinal trigeminal nucleus Substantia gelatinosa	Septum Nucleus Accumbens Hypothalamus	Striatum Cerebellum Thalamus

5-HT_3 binding sites, then 5-HT_3 receptors may represent a new target for the development of atypical neuroleptics. This assumption, however, is not supported by preliminary studies, which showed that zacopride failed to modify positive symptoms in schizophrenic patients (Newcomer et al. 1990).

The 5-HT_3 binding sites initially described in the rat brain and tumor cell lines were also found in the brains of other animal species, including humans (Peroutka 1988; Kilpatrick et al. 1989; Barnes et al. 1989a). Specific binding of [^{3}H]quipazine was detected in whole brain membranes of pig but not of cow, dog, turtle, mouse, guinea pig, chicken, or rabbit (Peroutka 1988). The ability of various serotonergic compounds to displace [^{3}H]quipazine with a similar affinity pattern in rat and pig cortical membranes suggests the presence of a similar 5-HT_3 receptor in the brains of these two species (Peroutka 1988).

Binding sites of [^{3}H]zacopride (Barnes et al. 1989a), [^{3}H]GR65630 (Kilpatrick et al. 1989), and [^{3}H]ICS 205-930 (Waeber et al. 1989) have also been identified in human brain. Binding of [^{3}H]zacopride in hippocampus and amygdala was shown to be saturable, specific, and of high affinity. Scatchard transformation of the data revealed K_d values of 2.64 and 2.93 nmol/L and B_{max} values of 55 and 44 fentomole per milligram of protein in amygdala and hippocampus, respectively (Barnes et al. 1989a). The 5-HT_3 recognition sites in human brain meet the criteria for 5-HT_3 receptors (Bradley et al. 1985). However, characterization of 5-HT_3 binding sites with various radioligands will be necessary to elucidate the biochemical processes that mediate the central actions of 5-HT_3 antagonists in human brain.

In all species studied, the area postrema, which is the site of the chemoreceptor trigger zone, possesses the highest concentration of 5-HT_3 binding sites (Pratt et al. 1990). In rat and mouse brain, high densities of [^{3}H]GR65630, [^{3}H]GR67330, [^{3}H](S)-zacopride, or [^{3}H]quipazine specific binding sites are present in the entorhinal, frontal, retrosplenic, cingulate, temporal, parietal, and occipital cortex and in the amygdala and hippocampus. In these same species, 5-HT_3 specific binding sites are very low in number or undetectable in the nucleus accumbens, striatum, thalamus, hypothalamus, and cerebellum (Kilpatrick et al. 1987, 1988, 1989, 1990; Milburn and Peroutka 1989; Barnes et al. 1990). In rat spinal cord, 5-HT_3 binding sites seem to be located presynaptically on capsaicin-sensitive primary afferent fibers (Glaum and Anderson 1988; Hamon et al. 1989). Interestingly, the ferret possesses high concentrations of [^{3}H]GR65630 binding sites in the nucleus accumbens, olfactory tubercle, amygdala, and striatum (Kilpatrick et al. 1989).

Biochemical and autoradiographic localization of

5-HT$_3$ binding sites with [^{3}H]GR65630 (Kilpatrick et al. 1989), [^{3}H]zacopride (Barnes et al. 1989a), or [^{3}H]ICS 205-930 (Waeber et al. 1989) in human brain membranes or slices has revealed a 5-HT$_3$ receptor distribution similar to that of rodent brain. However, in contrast with other mammalian species, human cortical areas possess low concentrations of 5-HT$_3$ binding sites (Waeber et al. 1989). Relatively high concentrations of 5-HT$_3$ recognition sites are observed particularly in the nuclei of the solitary tract, the spinal trigeminal nucleus, and the substantia gelatinosa of the spinal cord (Waeber et al. 1989).

SOLUBILIZATION AND PURIFICATION OF THE 5-HT$_3$ RECEPTOR

Two groups have recently solubilized from rat brain (McKernan et al. 1990a) and NG108-15 neuroblastoma-glioma cells (Miquel et al. 1990) a 5-HT$_3$ receptor with biochemical characteristics similar to those of the 5-HT$_3$ receptor identified in the corresponding membrane fractions (Kilpatrick et al. 1987, 1989; Hoyer and Neijt 1988; Barnes et al. 1990). Further studies led to the purification of the solubilized receptor from the NCB20 neuronal-glioma hybrid cell line, in which the number of 5-HT$_3$ recognition sites per milligram of protein is 20 to 30 times the number in rat brain (McKernan 1990b). A 1700-fold purification of the 5-HT$_3$ receptor was achieved by affinity chromatography, with the selective 5-HT$_3$ receptor antagonist L-685,603 immobilized in agarose as the ligand. Scatchard analysis and competition studies with the radioligand [^{3}H]Q ICS 205-930 confirmed the presence of a single population of 5-HT$_3$ binding sites in the purified preparation (McKernan et al. 1990b).

Polyacrylamide gel electrophoresis of the purified receptor revealed two bands that migrated with apparent molecular masses of 54 and 38 kD (McKernan et al. 1990b). Recent radiation inactivation studies have shown that, in rat brain, the binding sites labeled by [^{3}H]zacopride and by [^{3}H]GR65630 possess molecular masses of 35 kD (Bolanos et al. 1990) and of 49 kD (Lummis el al. 1990), respectively. Gel-exclusion chromatography and sucrose-density gradient centrifugation of the solubilized receptor and gel filtration of the purified material suggested that the 5-HT$_3$ receptor has an apparent molecular mass of 250 kD.

The apparent molecular mass of the purified 5-HT$_3$ receptor (250 kD) is in the same range as that determined for three ligand-gated ion channels: the gamma-aminobutyric acid type A (GABA$_A$) receptor (Sigel et al. 1983; Stephenson 1988; Mamalaki et al. 1989), the glycine receptor (Pfeiffer et al. 1982), and the nicotonic acetylcholine (ACh) receptor (Whiting and Lindstrom 1986). Considering the physicochemical similarities between the 5-HT$_3$ receptor and these three receptors, it was predicted that the 5-HT$_3$ receptor belongs to the superfamily of ligand-gated ion channels. The receptors in this family possess a heterooligomeric structure, probably comprising four or five subunits, which together form the hydrophilic pore of the ion-specific channel.

CLONING OF THE 5-HT$_3$ RECEPTOR

Molecular biological studies further supported the concept that the 5-HT$_3$ receptor belongs to the superfamily of ligand-gated ion channels. Maricq et al. (1991) isolated two messenger ribonucleic acid (mRNA) fractions from polyadenylated mRNA of NCB-20 cells that gave rise to functional expression of 5-HT-gated currents after injection into Xenopus oocytes. Construction of a complementary deoxyribonucleic acid (cDNA) library in an RNA expression vector for the two positive mRNA fractions led to the identification of a single positive clone (p5HT3R-A). Oocytes injected with transcripts from this clone produced a fast inward current in response to 5-HT that was similar to that observed in clonal cell lines and brain tissues possessing native 5-H$_3$ receptors (Yakel et al. 1988; Neijt et al. 1988a).

Sequence analysis of the clone for the 5-HT$_3$ receptor revealed a 2131-bp cDNA, which encodes a protein of 487 amino acids. Interestingly, the molecular mass of the predicted protein (56 kD) is similar to that of one of the fragments of the purified receptor (see above) (Miquel et al. 1990; McKernan et al. 1990a). The hydropathy profile of the cloned 5-HT$_3$ receptor predicts a structure consisting of four hydrophobic transmembrane regions, a large extracellular aminoterminal domain, and a long intracellular loop connecting the third and fourth transmembrane regions (Maricq et al. 1991).

The cloned 5-HT$_3$ receptor shows 27% identity with the α subunit of the Torpedo californica nicotinic ACh receptor and 22% identity to both the β_1 subunit of the bovine GABA$_A$ receptor and the 48-kD subunit of the rat glycine receptor (Maricq et al. 1991).

Crude membranes of COS-7 cells transfected with the 5-HT$_3$ receptor cDNA specifically bound the selective 5-HT$_3$ receptor antagonist [^{3}H]GR63650; moreover, kinetic studies showed that the affinity and Hill coefficient values for [^{3}H]GR63650 binding to these membranes (Maricq et al. 1991) were identical to those observed in NCB-20 cells or rat brain tissue bearing native 5-HT$_3$ receptors (Kilpatrick et al. 1987; McKernan et al. 1990b). The expression of specific 5-HT$_3$ binding sites in COS-7 cells and the generation of currents in oocytes (see below) with a single cDNA clone indicate that functional homomeric 5-HT$_3$ receptors can be expressed in different biological systems (Maricq et al. 1991).

The distribution of the 5-HT_3 receptor mRNA was determined with the use of polymerase chain reaction (PCR). With oligonucleotide primers corresponding to positions 84–114 and 682–712 of the cloned 5-HT_3 receptor sequence, a single PCR product was identified in the mouse cortex, brainstem, midbrain, spinal cord, and heart (Maricq et al. 1991). The observed distribution of the mRNA is consistent with ligand-binding studies, with the exception that mRNA was not detected in the intestine. The absence of a PCR product in the intestine, which is rich in 5-HT_3 binding sites (Kilpatrick et al. 1987), suggests that a distinct 5-HT_3 receptor subtype encoded by a separate gene may exist in this organ.

ELECTROPHYSIOLOGIC RESPONSES TO 5-HT_3 RECEPTOR ACTIVATION IN THE BRAIN

Serotonin has been shown to produce three different types of electrophysiologic response in cultured mouse hippocampal and striatal neurons (Yakel et al. 1988), the most frequent of which is an inhibitory response that results from receptor-mediated activation of an inwardly rectifying K^+ conductance. A fast excitatory response (present in 6% to 10% of cells tested), which was apparent after delivery of 5-HT by pressure ejection, was evident after 35 msec, peaked at 200 msec, and lasted approximately 2 to 4 seconds (Yakel et al. 1988). A similar fast depolarizing response to 5-HT is also observed in clonal cell lines (Yakel and Jackson 1988; Neijt et al. 1988a; Yang 1990) and in Xenopus oocytes injected with RNA transcripts obtained from the 5-HT_3 receptor cDNA (Maricq et al. 1991). The fast depolarizing current evoked by 5-HT is reproduced by the 5-HT_3 receptor agonists 2-methyl-5-HT, 1-phenylbiguanide and 1-(m-chlorophenyl) biguanide, and is antagonized by selective 5-HT_3 receptor antagonists (Neijt et al. 1986, 1988a; Yakel et al. 1988; Yakel and Jackson 1988; Maricq et al. 1991). Both voltage-clamp studies and radioligand-binding analysis indicate that 5-HT binds to the 5-HT_3 receptor in a cooperative manner (Neijt et al. 1988a; Kilpatrick et al. 1987; Hoyer et al. 1988; Maricq et al. 1991).

Thus, biochemical, molecular biological (see above), and electrophysiologic evidence (Hoyer and Neijt 1988; McKernan et al. 1990; Maricq et al. 1991) indicates that the 5-HT_3 receptor belongs to the family of ligand-gated ion channel receptors: 1) the 5-HT_3 receptor is activated by 5-HT even in the absence of adenosine triphosphate or guanosine triphosphate (GTP) (Yang 1990); 2) the response to 5-HT persists even after long periods of internal dialysis with CsF, AlF^{4-}, or GTP in order to activate G-proteins (Yang 1990); 3) the response to 5-HT remains unaffected by treatment of cells with pertussis toxin to prevent G-protein-mediated responses (Derkach et al. 1989); and 4) application of 5-HT results in a fast excitatory 5-HT_3 receptor-mediated response (Yakel and Jackson 1988; Derkach et al. 1989, Yang 1990; Maricq et al. 1991).

Unlike other receptors for biogenic amines, both native and cloned 5-HT_3 receptors are directly coupled to an ion channel that is permeable to the monovalent cations Na^+ and K^+ (Derkach et al. 1989; Yakel et al. 1990; Maricq et al. 1991). Activation of 5-HT_3 receptors in voltage-clamped membranes results in an opening of ligand-gated Na^+-K^+ channels with an increase in the inward current that flows through these channels (Peters and Usherwood 1983). The large inward driving force for Na^+ results in a greater Na^+ influx and a reduced K^+ efflux through the channel, with the consequence that the resting membrane potential (−55 mV) is progressively driven toward the Na^+ equilibrium potential (+55 mV); however, when the membrane potential reaches around 0 mV (the reversal potential for the Na^+-K^+ ion channel), the inward Na^+ current is reduced and the outward K^+ current is increased (Yakel and Jackson 1988; Lambert et al. 1989). The increase in the membrane potential triggers the opening of Ca^{2+} channels, which leads to an influx of extracellular Ca^{2+} into the cell. The influx of Ca^{2+} does not modify the membrane potential, but Ca^{2+} functions as an intracellular messenger to couple the activation of the 5-HT_3 receptor-linked ion channel to transmitter release. Transmitter release is modulated by Ca^{2+} probably through facilitation of a transient fusion of the synaptic vesicle membrane with the presynaptic terminal membrane. Whether Ca^{2+} promotes vesicular fusion directly or through a Ca^{2+}-binding protein that promotes fusion is not yet known.

In clonal cell lines, extracellular Ca^{2+} and Mg^{2+} exert a regulatory influence on 5-HT_3 receptor-mediated responses by modulating the amplitude and duration of 5-HT-induced currents (Peters et al. 1988). Both cations reduce the conductance of the native 5-HT_3 receptor channel without affecting the current-voltage (I/V) ratio. However, the homomeric cloned 5-HT_3 receptor expressed in Xenopus oocytes differs from the native 5-HT_3 receptor (Maricq et al. 1991); in the presence of Ca^{2+} or Mg^{2+}, the I/V relation of the cloned receptor has a region of negative slope-conductance (the current increases in amplitude as the cell progressively depolarizes). In this context, the 5-HT_3 receptor shares some properties with the N-methyl-D-aspartate (NMDA)-sensitive glutamate receptor, the conductance of which is blocked by Mg^{2+} (Nowak et al. 1984).

The Ca^{2+} permeability and single-channel conductance of the 5-HT_3 receptor-linked Na^+-K^+ channel in NG108-15 cells are similar to those of the non-NMDA type of excitatory amino-acid receptor but lower than those of the nicotinic ACh receptor (Yakel et al. 1990). In neuroblastoma N18 cells, however, the permeability ratio of the 5-HT_3 receptor channel for Ca^{2+} is simi-

lar to that of the nicotinic cholinergic receptor (Adams et al. 1980; Yang 1990).

Two types of desensitization kinetics after 5-HT application have been observed for the 5-HT$_3$ receptor. In N1E-115 cells and in oocytes expressing cloned 5-HT$_3$ receptors, desensitization of the receptor is rapid (Neijt et al. 1988a; Maricq et al. 1991) and both the onset and recovery from desensitization can be identified with a single exponential function (Neijt et al. 1988a). In NG108-15 cells and rat hippocampal neurons, a complex biphasic desensitizing response to 5-HT is observed (Yakel and Jackson 1988). Classically, the channels activated by excitatory neurotransmitters have been described as voltage independent (Kandel 1985); however, in NG108-15 cells, but not in N1E-115 cells, the time course of desensitization after 5-HT application is voltage dependent (Yakel and Jackson 1988).

A pharmacologically induced increase in the concentration of cyclic adenosine monophosphate (cAMP) in NG108-15 cells or in hippocampal neurons enhances the rate of desensitization of the 5-HT$_3$ receptor induced by 5-HT (Yakel and Jackson 1988). Although the mechanism by which 5-HT induces 5-HT$_3$ receptor desensitization is unknown, it can be speculated that modulation of 5-HT$_3$ receptor desensitization by cAMP may be mediated by phosphorylation of the receptor by a cAMP-dependent protein kinase (Sibley and Lefkowitz 1985).

BIOCHEMICAL RESPONSES TO 5-HT$_3$ RECEPTOR ACTIVATION

Neurotransmitter release and metabolism in the CNS is modified by 5-HT$_3$ receptor-selective drugs (Blandina et al. 1988, 1989; Barnes et al. 1989b; Imperato and Angelucci 1989; Hagan et al. 1990). In superfused rat striatal slices, Blandina et al. (1988, 1989) demonstrated that 5-HT and the selective 5-HT$_3$ agonist 2-methyl-5-HT stimulated both basal and K^+-induced dopamine (DA) release. The DA release induced by 5-HT$_3$ receptor stimulation was shown to be Ca^{2+}-dependent and partially sensitive to tetrodotoxin (Blandina et al. 1989). The selective 5-HT$_3$ receptor antagonist ICS 205-930, but not the 5-HT$_1$/5-HT$_2$ receptor antagonists methiothepin and methysergide, inhibited the effect of both 5-HT and 2-methyl-5-HT on DA release; the K_i value was similar to that expected for an effect mediated by 5-HT$_3$ receptors (Blandina et al. 1989).

The in vitro experiments performed with superfused striatal slices (Blandina et al. 1989) suggest that 5-HT$_3$ receptor antagonists either block postsynaptic receptors located on intrastriatal neurons involved in the regulation of DA release or block presynaptic 5-HT$_3$ receptors located on the nigrostriatal dopaminergic terminals. Recent experiments, however, have shown that injection of 6-hydroxydopamine in the substantia nigra fails to reduce the number of 5-HT$_3$ recognition sites in the striatum, suggesting a lack of 5-HT$_3$ binding sites in the nerve terminals of the nigrostriatal dopaminergic system (Hamon, personal communication).

Acute in vivo administration of 5-HT$_3$ receptor selective antagonists failed to modify dopaminergic neuronal activity (Koulu et al. 1989; Imperato and Angelucci 1989; Hagan et al. 1990). However, the activation of dopaminergic turnover in the mesolimbic dopaminergic pathway by the administration of morphine (Imperato and Angelucci 1989), nicotine and ethanol (Carboni et al. 1989), or the neurokinin receptor agonist DiMe-C 7 (Hagan et al. 1990), but not that induced by haloperidol (a DA receptor blocker) (Koulu et al. 1989), is blocked by selective 5-HT$_3$ receptor antagonists, including ICS 205-930, GR38032F, GR65630, and MDL 72222.

The cellular site at which 5-HT$_3$ antagonists act to block the stimulation of DA turnover in vivo is not known. The increase in DA turnover in the nucleus accumbens induced by either systemic administration of morphine (Imperato and Angelucci 1989) or by injection of DiMe-C 7 in the ventral tegmental area (VTA) (Hagan et al. 1990) can be selectively blocked by systemic or intra-VTA injection of various 5-HT$_3$ receptor antagonists. However, when ICS 205-930 is injected in the nucleus accumbens, it fails to counteract the increase in DA release induced by morphine administration (Imperato and Angelucci 1989). In contrast, the increase in DA release in the nucleus accumbens induced either by intracerebroventricular administration of 2-methyl-5-HT (Jiang et al. 1990) or by direct perfusion of 1-phenylbiguanide, a purported 5-HT$_3$ receptor agonist, in the nucleus accumbens (Chen et al. 1991), is effectively blocked by selective 5-HT$_3$ receptor antagonists infused directly into the nucleus accumbens (Chen et al. 1991). These results suggest that, according to the mechanism of activation of the mesolimbic dopaminergic neurons, 5-HT$_3$ receptor antagonists modulate DA release through an action either on the VTA, the area in which the cell bodies of the mesolimbic dopaminergic system are located, or at the level of the nucleus accumbens, where the dopaminergic nerve terminals are present. Whether the action of 5-HT$_3$ receptor antagonists within the VTA is exerted on a postsynaptic 5-HT$_3$ receptor located either on the dopaminergic cell perikarya or on intra-VTA interneurons, which in turn, regulate the activity of the dopaminergic cells, is not clear. In the nucleus accumbens, the 5-HT$_3$ receptors that regulate DA release may be located in presynaptic dopaminergic nerve terminals.

The 5-HT$_3$ receptors also seem to participate in the regulation of noradrenaline release from nerve terminals. In fact, both MDL 72222 and ICS 205-930 selec-

tively block the stimulatory effect of 5-HT on [^{3}H]noradrenaline release. This action of the 5-HT_3 receptor antagonists is only apparent, however, at concentrations that are two to three orders of magnitude higher than those required to block 5-HT_3 receptors (Feuerstein and Hertting 1986), and so the effect may not be specific.

The K^+-induced increase in [^{3}H]ACh release from the rat entorhinal cortex can be effectively reduced by activation of 5-HT_3 receptors with 5-HT or 2-methyl-5-HT (Barnes et al. 1989b). Both GR38032F and zacopride fail to modify basal and evoked ACh release, but these agents antagonize in a concentration-dependent manner the inhibitory action of 2-methyl-5-HT (Barnes et al. 1989b). Whether this facilitatory action of 5-HT_3 receptor antagonists is due to a direct blockade of the presynaptic inhibitory effect of 5-HT_3 receptor agonists on ACh release or to a blockade of 5-HT_3 receptors located on inhibitory afferent fibers or interneurons impinging on cortical cholinergic neurons is not yet clear.

Thus biochemical, pharmacologic, and electrophysiologic evidence indicates that 5-HT_3 receptors in nervous system tissue may participate in the modulation of both neuronal electrical activity and neurotransmitter release from nerve terminals. Apparently, 5-HT_3 receptors are not simply restricted to presynaptic terminals (where they modulate transmitter release) but are also distributed more widely over the cell surface (where they modulate membrane potential).

The modulation of neurotransmitter release by 5-HT seems to be mediated by a 5-HT_3 receptor-gated Na^+-K^+ channel that is functionally linked to a selective Ca^{2+} channel. Whether the same molecular mechanisms apply to both the stimulation of DA release in the nigrostriatal and mesolimbic dopaminergic systems and the inhibition of ACh release in cortical tissue by 5-HT and 5-HT_3 receptor agonists is not clear. In frontocingulate and entorhinal cortices, 5-HT_3 receptor agonists activate PI metabolism (Edwards et al. 1991). Moreover, in medial prefrontal cortex, the 5-HT_3 receptor agonist 2-methyl-5-HT selectively depresses, rather than increases, the firing rate of both spontaneously active and glutamate-activated cells (Ashby et al. 1989). If, as suggested by the latter experimental evidence, more than one functional mechanism is linked to 5-HT_3 receptor action, DA and ACh release may be differentially regulated by distinct subtypes of 5-HT_3 receptors or by different intracellular mechanisms linked to Ca^{2+} function.

5-HT_3 RECEPTORS AND CENTRAL NERVOUS SYSTEM DISORDERS

The development of drugs that are selective for the 5-HT_3 receptor, together with the availability of behavioral tests designed to evaluate the effect of CNS-active drugs on the performance of rodents and primates, has allowed an assessment of the potential role of 5-HT_3 receptors in brain function. The 5-HT_3 receptor antagonists are highly effective in various models of anxiety, psychosis, cognitive impairment, and drug dependence and withdrawal and have minimal side effects (Costall et al. 1990).

A great deal of information on the potential of 5-HT_3 receptor antagonists for modulating brain function has been obtained from both experimental and clinical studies on cytotoxic drug-induced and radiation-induced emesis (Cunningham et al. 1987; Leibundgut and Lancranjan 1987; Andrews et al. 1988; Dubois et al. 1988). Historically, the ability of metoclopramide to inhibit both apomorphine- and cytotoxic drug-induced emesis was ascribed to the ability of the drug to block D_2 DA receptors in the trigger zone of the hindbrain. More recently, the development of a series of substituted benzamides and other drugs with activity on radiation- and cytotoxic drug-induced emesis, but not on apomorphine-induced vomiting, led to the suggestion that the new compounds were acting through mechanisms other than those linked to the D_2 receptor. In fact, the newly developed drugs with activity on emesis proved to be potent antagonists of the 5-HT_3 receptor (Fozard and Gittos 1983; Fake et al. 1987; Smith et al. 1988). The inability of 5-HT_3 receptor antagonists to block apomorphine-induced emesis showed that these compounds could not be exerting their effects through postsynaptic D_2 receptors. Because 5-HT increases the release of DA in various brain areas through a 5-HT_3 receptor-mediated mechanism, 5-HT_3 receptor antagonists may exert their antiemetic action by blocking the release of DA induced by radiation or cytotoxic drugs in the trigger zone of the hindbrain. The development of 5-HT_3 receptor antagonists therefore offered an alternative to conventional antiemetic therapy, especially in view of the fact that these drugs have significantly fewer side effects than D_2 receptor blockers.

It has been postulated that classic DA receptor antagonists exert their antipsychotic action by blocking the hyperactivity of mesolimbic dopaminergic neurons that was presumed, but never demonstrated, to occur in psychosis. Interestingly, GR38032 and ICS 205-930 are highly effective in blocking the increase in mesolimbic DA turnover induced by administration of DiMe-C 7 and morphine, respectively (Imperato and Angelucci 1989; Hagan et al. 1990). Furthermore, behavioral studies have shown that the locomotor hyperactivity induced by selective pharmacologic stimulation of the mesolimbic system and the rebound hyperactivity that follows chronic fluphenazine administration in rats is selectively reduced by specific 5-HT_3 receptor antagonists (Costall et al. 1987, 1990; Butler et al. 1988; Hagan et al. 1990).

In normal rats, the failure of a single injection of 5-HT$_3$ receptor antagonist to modify either locomotor activity (Costall et al. 1990) or mesolimbic and nigrostriatal DA turnover (Imperato and Angelucci 1989; Hagan et al. 1990) indicates the lack of a tonic serotonergic stimulation of 5-HT$_3$ receptors. In agreement with these results, intracerebroventricular administration of 5,7-dihydroxytryptamine, a selective neurotoxin for serotonergic neurons, fails to modify 5-HT$_3$ receptors in rat brain (Leysen, personal communication). The 5-HT$_3$ antagonist GR38032 is also ineffective at reducing the increase in dihydroxyphenylacetic acid concentrations, a reliable index of DA metabolism, in the mesolimbic and nigrostriatal dopaminergic pathways induced by a single haloperidol injection (Koulu et al. 1989).

Long-term administration of both MDL 73,147EF, a selective 5-HT$_3$ receptor antagonist, and haloperidol produces a significant reduction in the number of spontaneously active dopaminergic neurons in the VTA and substantia nigra (Sorensen et al. 1989). These results clearly indicate that 5-HT$_3$ receptor antagonists exert a neuroleptic-like activity and support the view that 5-HT$_3$ receptors regulate central dopaminergic function (Tricklebank 1989).

The pharmacologic profile of 5-HT$_3$ receptor blockers in the mesolimbic system suggests a therapeutic potential of these compounds as antipsychotic agents. Moreover, the ability of these drugs to reduce the rebound hyperactivation after long-term treatment with classic neuroleptics suggests that 5-HT$_3$ receptor antagonists ameliorate the undesirable side effects observed after chronic administration of DA receptor blockers. In this line, although preliminary clinical studies do not support a role for 5-HT$_3$ antagonists as potential antipsychotic drugs, zacopride seemed to be effective in reducing abnormal motor movements in a patient with a moderate degree of tardive dyskinesia (Newcomer et al. 1990).

The mesolimbic system also seems to be important in the mechanisms by which substances with abuse potential produce their pharmacologic effects. The increase in DA turnover in the nucleus accumbens after administration of morphine (Costa et al. 1973; Imperato and Angelucci 1989), amphetamine (Costa and Gropetti 1970; Costa et al. 1972; Costall et al. 1980), or cocaine (Costa et al. 1973) may contribute to the rewarding mechanisms (Wise and Bogharth 1988) and to the maintenance of psychologic dependency after administration of these drugs of abuse (Costall et al. 1990). Interestingly, 5-HT$_3$ receptor antagonists proved to be highly effective in reducing the consequences of withdrawal after long-term treatment with a number of these drugs, including alcohol, anxiolytics, cocaine, amphetamine, and nicotine (Oakley et al. 1988a, 1988b; Costall et al. 1990). Whether the effects of 5-HT$_3$ receptor antagonists are mediated through a reduction in the hyperactivation of the dopaminergic system induced by drugs of abuse is unknown.

A common feature of many experimental models that are responsive to 5-HT$_3$ receptor antagonists is an increased activity of presynaptic dopaminergic neurons (Costall et al. 1987a, 1987c, 1990; Carboni et al. 1989b; Imperato and Angelucci 1989; Hagan et al. 1990). This common characteristic suggests that the activation of a serotonergic neuron that impinges on a dopaminergic neuron (either at the presynaptic terminal, or on the cell body or a dendrite) represents a necessary step for the subsequent depolarization of the dopaminergic cell. Thus, 5-HT$_3$ receptor antagonists, by blocking the action of 5-HT at the 5-HT$_3$ receptor-gated Na^+-K^+ channel, would reduce Na^+ influx through the channel and prevent changes in the resting membrane potential of the dopaminergic cell. The resultant blockade of Ca^{2+} channel activation by the 5-HT$_3$ receptor antagonists would prevent the activation of intracellular mechanisms that mediate DA release.

In most tests that assess anxiolytic activity (with the exception of the Vogel test [Costall et al. 1990]), 5-HT$_3$ receptor blockers have pharmacologic profiles similar to those of anxiolytic benzodiazepines, even though the 5-HT$_3$ receptor antagonists fail to bind to GABA$_A$ receptor-associated benzodiazepine recognition sites (Jones et al. 1988). In contrast to benzodiazepines, however, 5-HT$_3$ receptor antagonists lack sleep-inducing, muscle-relaxant, and anticonvulsant effects. Moreover, 5-HT$_3$ receptor blockers fail to induce dependence after long-term treatment (Jones et al. 1988). Interestingly, the 5-HT$_3$ receptor antagonist GR38032F, but not the anxiolytic nonbenzodiazepine drug buspirone, exerts its anxiolytic effect even in rodents with an underlying withdrawal syndrome (Costall et al. 1990). The mechanism by which 5-HT$_3$ receptor antagonists exert their anxiolytic action in rodents is still unclear. In most tests that evaluate anxiety-related pharmacologic profiles, 5-HT$_3$ receptor antagonists show a dose-response relation within the range of those observed in other in vivo studies that evaluate 5-HT$_3$ receptor function (Butler et al. 1988). Moreover, the fact that structurally different 5-HT$_3$ receptor antagonists are endowed with anxiolytic activity strongly supports a role for 5-HT$_3$ receptors in the mediation of this activity in experimental animals. In this line, preliminary clinical studies have shown that zacopride exerts a mild anxiolytic effect without sedation and reduces the distress associated with psychotic symptoms (Newcomer et al. 1990).

The ability of GR38032F to facilitate ACh release in cortical tissue (Barnes et al. 1989b) may indicate the biochemical basis by which 5-HT$_3$ receptor antagonists improve cognitive performance in behavioral tests that evaluate cognitive function (Costall et al. 1989). Consistent with the observation that 5-HT$_3$ receptor antagonists reverse scopolamine-induced cognitive deficits (Barnes et al. 1989c) is a preliminary report showing that

zacopride partially reverses the cognitive impairment induced by scopolamine in normal human volunteers.

CONCLUSIONS

Progress in the understanding of the functional role of 5-HT_3 receptors in the brain will evolve from the pioneering efforts of different research teams. With the use of various structure-related predictive strategies, researchers have successfully developed potent and specific 5-HT_3 receptor agonists and antagonists. Moreover, the availability of labeled receptor ligands with relatively high specific activity has allowed the identification of 5-HT_3 binding sites in mammalian brain. The 5-HT_3 recognition site in the brain is similar to that in the periphery and is probably located on neurons. Distribution studies have indicated that the area postrema possesses the highest concentration of 5-HT_3 binding sites, although high concentrations are also present in cortical and limbic brain structures.

Biochemical, molecular biological, and electrophysiologic studies have suggested that the 5-HT_3 receptor belongs to the superfamily of ligand-gated ion channels with molecular masses of 250 to 300 kD, which includes $GABA_A$, glycine, and neuronal nicotinic ACh receptors. The 5-HT_3 receptor thus appears to be unlike other 5-HT receptors in that it does not belong to the G-protein-linked receptor superfamily but appears to comprise an ion channel that is permeable to Na^+ and K^+. The 5-HT_3 receptor has a heterooligomeric structure composed of four or five subunits, which together form a transmembrane ion channel complex that is susceptible to transmitter gating. In oocytes expressing the cloned 5-HT_3 receptor as well as in primary cultures and cell lines of neuronal origin, 5-HT_3 receptor activation results in a fast depolarizing excitatory response that can be selectively blocked by 5-HT_3 receptor antagonists.

A growing body of evidence suggests the presence of distinct 5-HT_3 receptor subpopulations in the nervous system. Thus, neurophysiologic studies have shown a 35- to 40-fold difference in single-channel conductance and at least a twofold difference in ion selectivity among clonal cell line and enteric neuron 5-HT_3 receptors (Derkach et al. 1989; Lamberts et al. 1989; Yang 1990). In addition, at odds with the failure of the 5-HT_3 receptor antagonist MDL 72222 to counteract the effect of 5-HT in enteric neurons, this compound reversibly blocks the 5-HT-evoked current in Xenopus oocytes expressing the cloned 5-HT_3 receptor (Maricq et al. 1991). These data are consistent with the apparent absence of 5-HT_3 receptor mRNA in the mouse intestine (Maricq et al. 1991). Further evidence for the existence of multiple 5-HT_3 receptors has been provided by studies showing that the recovery of the 5-HT_3 receptor-gated Na^+-K^+ channel from rapid desensitization follows either monophasic or complex biphasic kinetics depending on the tissue involved (Yakel and Jackson 1988). Moreover, in NG108-15 cells, but not in N1E-115 cells, the time course of desensitization is voltage dependent (Yakel and Jackson 1988).

Studies of 5-HT_3 receptor function also support the concept of 5-HT_3 receptor heterogeneity. Thus, in the striatum and the nucleus accumbens, activation of 5-HT_3 receptors stimulates the release of DA (Blandina et al. 1988, 1989; Imperato and Angelucci 1989), whereas in cortical tissue the stimulation of 5-HT_3 receptors inhibits ACh release (Barnes et al. 1989b). The recent identification of a second transducing system linked to 5-HT_3 receptor function, namely PI hydrolysis (Edwards et al. 1991), further supports the existence of different 5-HT_3 receptor subpopulations in neural tissues. The possibility, however, that the increase of PI metabolism after 5-HT_3 receptor stimulation is due to a still unknown indirect effect cannot be totally ruled out.

Biochemical and behavioral studies have provided data on the therapeutic potential of 5-HT_3 receptor antagonists. The high concentration of 5-HT_3 binding sites in the area postrema, a brain area related to the control of emesis, suggests a potential role for 5-HT_3 receptor antagonists as potent antiemetics during tumor chemotherapy or radiotherapy. On the other hand, the ability of 5-HT_3 receptor antagonists to reduce drug-induced hyperactivity of mesolimbic and nigrostriatal dopaminergic neurons implicates these compounds as prospective antipsychotic agents and as potential alternatives for the treatment of neuroleptic-induced side effects. The ability of various 5-HT_3 receptor antagonists to exert anxiolytic effects in animal models and to effectively limit the severity of symptoms caused by morphine withdrawal in dependent individuals point to the potential use of 5-HT_3 receptor antagonists in a wide variety of psychiatric disorders.

A significant number of issues still need to be addressed to reach a better understanding of the role of the 5-HT_3 receptor in mammalian physiology and pathology. Clinically useful selective agonists with less polar properties are still required for pharmacologic studies in humans. Identification of the cellular locations of 5-HT_3 receptors, as well as studies on their pharmacologic and biochemical regulation in brain, should provide valuable information to permit the targeting of specific neuronal synapses that contribute to the genesis of symptoms of specific psychiatric disorders. The generation of currents with a single 5-HT_3 receptor clone demonstrates that a functional single unit can be expressed in Xenopus oocytes. Some of the functional properties of this receptor, however, seem to differ from the native multimeric receptor; thus, it will be crucial to determine the additional subunit compo-

sition of the native 5-HT$_3$ receptor to understand the molecular mechanisms of receptor regulation. Moreover, further molecular biological studies should help elucidate whether there is only one 5-HT$_3$ receptor or whether receptor subtypes exist.

REFERENCES

Adams JD, Dwyer TM, Hille B (1980): The permeability of endplate channels to monovalent and divalent metal cations. J Gen Physiol 75:493–510

Andrews PLR, Rapeport WG, Sanger GJ (1988): Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol Sci 9:334–341

Barnes JM, Barnes NM, Costall B, Ironside JW, Naylor RJ (1989a): Identification and characterization of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue. J Neurochem 53:1787–1793

Barnes JM, Barnes NM, Costall B, Naylor RJ, Tyers MB (1989b): 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. Nature 338:762–763

Barnes JM, Costall B, Kelly ME, Naylor RJ, Onaivi ES, Tomkins DM, Tyers MB (1989c): GR38032 improves performance in a mouse habituation test, and inhibits cholinergic-linked defects. Br J Pharmacol 98:693P (abstract)

Barnes JM, Barnes NM, Champaneria S, Costall B, Naylor RJ (1990): Characterization and autoradiographic localization of 5-HT$_3$ receptor recognition sites identified with [^{3}H]-(S)-zacopride in the forebrain of the rat. Neuropharmacology 29:1037–1045

Barnes NM, Costall B, Naylor RJ (1988): [^{3}H]Zacopride: ligand for the identification of 5-HT$_3$ recognition sites. J Pharm Pharmacol 40:548–551

Blandina P, Goldfarb J, Green JP (1988): Activation of a 5-HT$_3$ receptor releases dopamine from striatal slices. Eur J Pharmacol 155:349–350

Blandina P, Goldfarb J, Craddock-Royal B, Green JP (1989): Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptor in rat striatum. J Pharmacol Exp Ther 251:803–809

Bolanos FJ, Schechter LE, Miquel MC, Emerit MB, Rumigny JF, Hamon M (1990): Common pharmacological and physico-chemical properties of 5-HT$_3$ binding sites in the rat cerebral cortex and NG 108-15 cells. Biochem Pharmacol 40:1541–1550

Bradley PB, Engel G, Feniuk W, Fozard J, Humphrey P, Middlemiss DN, Mylecharane E, Richardson B, Saxena P (1985): Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25:563–576

Butler A, Hill JM, Ireland SJ, Jordan CC, Tyers MB (1988): Pharmacological properties of GR38032F, a novel antagonist at 5-HT$_3$ receptors. Br J Pharmacol 94:397–412

Carboni E, Acquas E, Frau R, DiChiara GD (1989): Differential inhibitory effects of a 5-HT$_3$ antagonist on drug-induced stimulation of dopamine release. Eur J Pharmacol 164: 514–520

Chen J, van Pragg HM, Gardner EL (1991): Activation of 5-HT$_3$ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res 543:354–357

Conn PJ, Sanders-Bush E (1984): Selective 5-HT$_2$ antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacology 23:993–996

Conn PJ, Sanders-Bush E, Hoffman BJ, Hartig PR (1986): A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. Proc Natl Acad Sci USA 83:4086–4088

Costa E, Gropetti A (1970): Biosynthesis and storage of catecholamines in tissues of rats injected with various doses of d-amphetamine. In Costa E, Garattini S (eds), Amphetamines and Related Compounds. New York, Raven Press, pp 231–255

Costa E, Gropetti A, Naimzada MK (1972): Effects of amphetamine on the turnover rate of brain catecholamines and motor activity. Br J Pharmacol 44:742–751

Costa E, Carenzi A, Guidotti A, Revuelta A (1973): Narcotic analgesics and the regulation of neuronal catecholamine stores. In Usdin E, Snyder SH (eds), Frontiers of Catecholamine Research. London, Pergamon Press, pp 1003–1010

Costall B, Fortune DH, Hui SCG, Naylor RJ (1980): Neuroleptic antagonism of the motor inhibitory effects of apomorphine within the nucleus accumbens: drug interaction at presynaptic receptors? Eur J Pharmacol 63:347–358

Costall B, Domeney AM, Naylor RJ, Tyers MB (1987): Effects of the 5-HT$_3$ receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br J Pharmacol 92:881–894

Costall B, Domeney AM, Kelly ME, Naylor RJ, Onaivi ES, Tomkins DM, Tyers MB (1989): Effects of GR38032F on performance of common marmosets in an object discrimination reversal task. Br J Pharmacol 98:637P (abstract)

Costall B, Naylor RJ, Tyers MB (1990): The psychopharmacology of 5-HT$_3$ receptors. Pharmacol Ther 47:181–202

Cunningham D, Pople A, Grazet J-C, Ford HT, Challoner T, Coombes RC (1987): Prevention of emesis in patients receiving cytotoxic drugs by GR38032F, a selective 5-HT$_3$ receptor antagonist. Lancet 1:1461–1463

Derkach V, Suprenant A, North RA (1989): 5-HT$_3$ receptors are membrane ion channels. Nature 339:706–709

Dubois A, Fiala N, Boward GA, Bogo V (1988): Prevention and treatment of the gastric symptoms of radiation sickness. Radiat Res 115:595–604

Dumuis A, Bouhelal R, Sebben M, Cory R, Bockaert J (1988): A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol Pharmacol 34:880–887

Edwards E, Harkins H, Ashby CR, Wang RY (1991): Effect of 5-hydroxytryptamine$_3$ receptor agonists on phosphoinositides hydrolysis in the rat fronto-cingulate and entorhinal cortices. J Pharmacol Exp Ther 256:1025–1032

Fake CS, King FD, Sanger GJ (1987): BRL 43694: a potent and novel 5-HT$_3$ antagonist. Br J Pharmacol 91:335P (abstract)

Fargin A, Raymond JR, Lohse MJ, Kobilka BK, Caron MG, Lefkowitz RJ (1988): The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes a 5-HT$_{1a}$ receptor. Nature 335:358–360

Feuerstein TJ, Hertting G (1986): Serotonin (5-HT) enhances hippocampal noradrenaline release: Evidence for facilitatory 5-HT receptors within the CNS. Naunyn Schmiedebergs Arch Pharmacol 333:191–198

Fozard JR (1983): Differences between receptors for 5-HT on autonomic neurons revealed by nor-(−)-cocaine. J Auton Pharmacol 3:21–26

Fozard JR (1984): Neuronal 5-HT receptors in the periphery. Neuropharmacology 23:1473–1486

Fozard JR (1984a): MDL72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn Schmiedebergs Arch Pharmacol 326:36–44

Fozard JR, Gittos MW (1983): Selective blockade of 5-hydroxytryptamine neuronal receptors by benzoic acid esters of tropine. Br J Pharmacol 80:511P (abstract)

Fozard JR, Mobarok Ali ATM (1978): Blockade of neuronal tryptamine receptors by metoclopramide. Eur J Pharmacol 49:109–112

Fozard JR, Mobarok Ali AT, Newgrosh G (1979): Blockade of serotonin receptors on autonomic neurons by (−)-cocaine and some related compounds. Eur J Pharmacol 59:195–210

Gaddum JH, Hameed KA (1954): Drugs which antagonize 5-hydroxytryptamine. Br J Pharmacol 9:240–248

Gaddum JH, Piccarelli ZP (1957): Two kinds of tryptamine receptors. Br J Pharmacol Chemother 12:323–328

Glaum SR, Anderson E (1988): Identification of 5-HT_3 binding sites in rat spinal cord synaptosomal membranes. Eur J Pharmacol 156:287–290

Glennon RA (1987): Central serotonin receptors as targets for drug research. J Med Chem 30:1–9

Glennon RA, Slusher MR, Lyon RA, Titeler M, McKenney JD (1986): 5-HT_1 and 5-HT_2 binding characteristics of some quipazine analogs. J Med Chem 29:2375–2379

Hagan RM, Jones BJ, Jordan CC, Tyers MB (1990): Effect of 5-HT_3 receptor antagonists on responses to selective activation of mesolimbic dopaminergic pathways in the rat. Br J Pharmacol 99:227–232

Hamblin MW, Metcalf MA (1991): Primary structure and functional characterization of a human 5-HT_{1D}-type serotonin receptor. Mol Pharmacol 40:143–148

Hamon M, Gallissot MC, Menard F, Gozlan H, Bourgoin S, Verge D (1989): 5-HT_3 receptor binding sites are on capsaicin-sensitive fibers in the rat spinal cord. Eur J Pharmacol 164:315–322

Heuring RE, Peroutka SJ (1987): Characterization of a novel ^{3}H-5-HT binding subtype in bovine brain membranes. J Neurosci 7:894–903

Hoyer D, Neijt H (1987): Identification of 5-HT_3 recognition sites by radioligand binding in NG108-15 neuroblastoma-glioma cells. Eur J Pharmacol 143:291–293

Hoyer D, Neijt HC (1988): Identification of serotonin 5-HT_3 recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. Mol Pharmacol 33: 303–309

Hoyer D, Gozlan H, Bolanos F, Schechter LE, Hamon M (1989): Interaction of psychotropic drugs with central 5-HT_3 recognition sites: Fact or artifact? Eur J Pharmacol 171:137–139

Imperato A, Angelucci L (1989): 5-HT_3 receptors control dopamine release in the nucleus accumbens of freely moving rats. Neurosci Lett 101:214–217

Jiang LH, Ashby CR, Kasser RJ, Wang RY (1990): The effect of intraventricular administration of the 5-HT_3 receptor agonist 2-methylserotonin on the release of dopamine in the nucleus accumbens: An in vivo chronocoulometric study. Brain Res 513:156–160

Jones BJ, Costall B, Domeney A, Kelly AM, Naylor RJ, Oakley NR, Tyers MB (1988): The potential anxiolytic activity of GR38032F, a 5-HT_3 receptor antagonist. Br J Pharmacol 93:985–993

Julius D, MacDermott A, Axel R, Jessel TM (1988): Molecular characterization of a functional cDNA encoding the serotonin$_{1c}$ receptor. Science 241:558–564

Kandel ER (1985): Chemically gated ion channels at central synapses. In Kandel ER, Schwartz JH (eds), Principles of Neural Science, ed 2. New York, Elsevier, pp 108–119

Kilpatrick GJ, Jones BJ, Tyers MB (1987): Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748

Kilpatrick GJ, Jones BJ, Tyers MB (1989): Binding of the ligand, [^{3}H]GR65630, to rat area postrema, vagus nerve and the brains of several species. Eur J Pharmacol 159:157–164

Kilpatrick GJ, Butler A, Hagan RM, Jones BJ, Tyers MB (1990): [^{3}H]GR67330, a very high affinity ligand for 5-HT_3 receptors. Naunyn Schmiedebergs Arch Pharmacol 342:22–30

Koulu M, Sjoholm B, Lappalainen J, Virtanen R (1989): Effects of acute GR38032F (odansetron), a 5-HT_3 receptor antagonist, on dopamine and serotonin metabolism in mesolimbic and nigrostriatal dopaminergic neurons. Eur J Pharmacol 169:321–324

Lambert J, Peters JA, Hales TG, Dempster J (1989): The properties of 5-HT receptors in clonal cell lines studied by patch-clamp techniques. Br J Pharmacol 97:27–40

Leibundgut U, Lancranjan I (1987): First results with ICS 205-930 (5-HT_3 receptor antagonist) in prevention of chemotherapy induced emesis. Lancet 1:1198 (letter)

Leonhardt S, Herrick-Davis K, Titeler M (1989): Detection of a novel serotonin receptor subtype (5-HT_{1E}) in human brain: interaction with a GTP-binding protein. J Neurochem 53:465–471

Lummis SCR, Nielsen M, Kilpatrick GJ, Martin IL (1990): Target size of 5-HT_3 receptors in N1E-115 neuroblastoma cells and rat brain. Eur J Pharmacol 189:229–232

Mamalaki C, Barnard EA, Stephenson FA (1989): Molecular size of the gamma-aminobutyric acid A receptor purified from mammalian cerebral cortex. J Neurochem 52:124-134

Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991): Primary structure and functional expression of the 5-HT_3 receptor, a serotonin-gated ion channel. Science 254: 432–437.

McKernan RM, Quirk K, Jackson RG, Ian Ragan C (1990a): Solubilization of the 5-hydroxytryptamine 3 receptor from pooled rat cortical and hippocampal membranes. J Neurochem 54:924–930

McKernan RM, Gilard NP, Quirk K, Kneen CO, Stevenson GI, Swain CJ, Ian Ragan C (1990b): Purification of the 5-hydroxytryptamine 5-HT_3 receptor from NCB20 cells. J Biol Chem 265:13572–13577

Milburn CM, Peroutka SJ (1989): Characterization of [^{3}H]quipazine binding to 5-hydroxytryptamine$_3$ receptors in rat brain membranes. J Neurochem 52:1787–1792

Miquel MC, Emerit MB, Bolanos FJ, Schechter LE, Gozlan H, Hamon M (1990): Physicochemical properties of serotonin 5-HT$_3$ binding sites solubilized from membranes of NG 108-15 neuroblastoma-glioma cells. J Neurochem 55:1526–1536

Nakaki T, Roth BL, Chuang D-M, Costa E (1984): Phasic and tonic components in 5-HT$_2$ receptor-mediated rat aorta contraction: participation of Ca^{2+} channels and phospholipase C. J Pharmacol Exp Ther 243:442–446

Neijt HC, Vijverberg HPM, van der Bercken J (1986): The dopamine response in mouse neuroblastoma cells is mediated by serotonin 5-HT$_3$ receptors. Eur J Pharmacol 127:271–274

Neijt HC, Te Duits IJ, Vijverberg HPM (1988a): Pharmacological characterization of serotonin 5-HT$_3$ receptor-mediated electrical response in cultured mouse neuroblastoma cells. Neuropharmacology 27:301–307

Neijt HC, Karpf A, Schoeffter P, Engel G, Hoyer D (1988b): Characterization of 5-HT$_3$ recognition sites in membranes of NG-108-15 neuroblastoma-glioma cells with [^{3}H]ICS 205-930. Naunyn Schmiedebergs Arch Pharmacol 337:493–499

Newcomer JW, Faustman WO, Roth B, Bierley RA, Moses JA, Csernansky JG (1990): Zacopride in schizophrenia: a single-blind 5-HT$_3$ antagonist trial. Proc Soc Neurosci 16:752 (Abstract)

Nowak L, Bregestovski P, Ascher P, Herbert A, Prochiantz A (1984): Magnesium gates glutamate activated channels in mouse central neurons. Nature 307:462–465

Oakley NR, Jones BJ, Tyers MB (1988a): Tolerance and withdrawal studies with diazepam and GR38032F in the rat. Br J Pharmacol 95:764P (abstract)

Oakley NR, Jones BJ, Tyers MB, Costall B, Domeney AM (1988b): The effect of GR38032F on alcohol consumption in the marmoset. Br J Pharmacol 95:870P (abstract)

Pazos A, Hoyer D, Palacios TM (1984): Mesulergine, a selective serotonin 2 ligand in the rat cortex, does not label receptors in porcine and human cortex: evidence for species differences on brain serotonin 2 receptors. Eur J Pharmacol 106:531–538

Pedigo NW, Yamamura DL, Nelson J (1981): Discrimination of multiple ^{3}H-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J Neurochem 36: 220–226

Peroutka SJ (1986): Pharmacological differentiation and characterization of 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites in rat frontal cortex. J Neurochem 47:529–540

Peroutka SJ (1988): Species variations in 5-HT$_3$ recognition sites labeled by ^{3}H-quipazine in the central nervous system. Naunyn Schmiedebergs Arch Pharmacol 338: 472–475

Peroutka SJ, Snyder SH (1979): Multiple serotonin receptors: differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamine and [^{3}H]-spiroperidol. Mol Pharmacol 16:687–699

Peroutka SJ, Hamik A (1988): [^{3}H]Quipazine labels 5-HT$_3$ recognition sites in rat cortical membranes. Eur J Pharmacol 148:297–299

Peroutka SJ, Lebovitz RM, Snyder SH (1981): Two distinct central serotonin receptors with different physiological functions. Science 212:827–829

Peters JA, Usherwood PNR (1983): 5-Hydroxytryptamine responses of murine neuroblastoma cells: ions and putative antagonists. Br J Pharmacol 80:523P (abstract)

Peters JA, Lambert JJ (1989): Electrophysiology of 5-HT$_3$ receptors in neuronal cell lines. Trends Pharmacol Sci 10:172–174

Peters JA, Hales TG, Lambert JJ (1988): Divalent cations modulate 5-HT$_3$ receptor-induced currents in N1E115-neuroblastoma cells. Eur J Pharmacol 151:491–495

Pfeiffer F, Graham D, Betz H (1982): Purification by affinity chromatography of the glycine receptor of the rat spinal cord. J Biol Chem 257:9389–9393

Pratt GD, Bowery NG (1989): The 5-HT$_3$ receptor ligand [^{3}H]BRL 43694 binds to presynaptic sites in the nucleus tractus solitarius of the rat. Br J Pharmacol 97:414P (abstract)

Pratt GD, Bowery NG, Kilpatrick JG, Leslie RA, Barnes NM, Naylor R, Jones BJ, Nelson DR, Palacios JM, Slater P, Reynolds JM (1990): Consensus meeting agrees distribution of 5-HT$_3$ receptors in mammalian hindbrain. Trends Pharmacol Sci 11:135–137

Pritchett DB, Bach AWJ, Wozny AWJ, Taleb O, Dal Toso R, Shih JC, Seeburg PH (1988): Structure and functional expression of cloned rat serotonin 5-HT$_2$ receptor. EMBO J 7:4135–4140

Resier G, Hamprecht B (1989): Serotonin raises the cyclic GMP levels in a neuronal cell line via 5-HT$_3$ receptors. Eur J Pharmacol 172:195–198

Reynolds DJM, Andrews PLR, Leslie RA, Harvey JM, Grasby PM, Grahame-Smith DC (1989): Bilateral abdominal vagotomy abolishes binding of ^{3}H BRL 43694 in ferret dorsovagal complex. Br J Pharmacol 98:692P (abstract)

Richardson BP, Engel G, Donatsch P, Stadler PA (1985): Identification of serotonin-M receptor subtypes and their specific blockade by a new class of drugs. Nature 316: 126–131

Rizzi JP, Nagel AA, Rosen T, McLean S, Seeger T (1990): An initial three-component pharmacophore for specific serotonin-3 receptor ligands. J Med Chem 33:2721–2725

Rocha e Silva M, Valle JR, Piccarelli ZP (1953): A pharmacological analysis of the mode of action of serotonin upon the guinea pig ileum. Br J Pharmacol 8:378–388

Schmidt AW, Peroutka SJ (1989): Antidepressant interactions with 5-hydroxytryptamine$_3$ receptor binding sites. Eur J Pharmacol 163:397–398

Schmidt AW, Siok CJ, Seeger TF (1988): 5-HT$_3$ binding sites identified in rat brain using [^{3}H]ICS 205-930. Soc Neurosci Abstr 14:846

Sibley DR, Lefkowitz RJ (1985): Molecular mechanisms of receptor desensitization using the beta-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317:124–129

Sigel E, Stephenson FA, Mamalaki C, Barnard EA (1983): A gamma-aminobutyric acid/benzodiazepine receptor complex of bovine cerebral cortex. J Biol Chem 258:6965–6971

Smith WL, Sancilio LF, Owera-Atepo JB, Naylor JR, Lambert

L (1988): Zacopride: a potent 5-HT_3 antagonist. J Pharm Pharmacol 40:301–302

Sorensen SM, Humphreys TM, Palfreyman MG (1989): Effect of acute and chronic MDL 73,147EF, a 5-HT_3 receptor antagonist, on A9 and A10 dopamine neurons. Eur J Pharmacol 163:115–118

Stephenson FA (1988): Understanding the $GABA_A$ receptor: a chemically gated ion channel. Biochem J 249:21–32

Tricklebank MD (1989): Interactions between dopamine and 5-HT_3 receptors suggest new treatments for psychosis and drug addiction. Trends Pharmacol Sci 10:127–129

Waeber C, Hoyer D, Palacios JM (1989): 5-Hydroxytryptamine 3 receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205-930. Neuroscience 31:393–400

Watling KJ (1988): Radioligand binding studies identify 5-HT_3 recognition sites in neuroblastoma cell lines and mammalian CNS. Trends Pharmacol Sci 9:227–229

Watling KJ, Aspley S, Swain CJ, Saunders J (1988): [^{3}H]-Quaternised ICS 205-930 labels 5-HT_3 receptor binding sites in rat brain. Eur J Pharmacol 149:397–398

Whiting PJ, Lindstrom JM (1986): Purification and characterization of a nicotinic acetylcholine receptor from chick brain. Biochemistry 25:2082–2093

Wise RA, Bogarth MA (1982): Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. Pharmacol Biochem Behav 17:239–243

Yakel JL, Jackson MB (1988): 5-HT_3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuron 1:615–621

Yakel JL, Trussell LO, Jackson MB (1988): Three serotonin responses in cultured mouse hippocampus and striatal neurons. J Neurosci 8:1273–1285

Yakel JL, Shao XM, Jackson MB (1990): The selectivity of the channel coupled to the 5-HT_3 receptor. Brain Res 533: 46–52

Yang J (1990): Ion permeation through 5-hydroxytryptamine-gated channels in neuroblastoma N18 cells. J Gen Physiol 96:1177–1198