

The 5-HT₃ Receptor in Mammalian Brain: A New Target for the Development of Psychotropic Drugs?

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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-HT₁) and the serotonin-2 (5-HT₂) binding sites.

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The 5-HT₁ site, which can also be labeled by [³H]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 [³H]5-HT binding isotherms in the CNS revealed five different subtypes of 5-HT₁ 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-HT₂ 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-HT₃ 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-HT₃ 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-HT₃ 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

sites represent functional receptors and meet the criteria for 5-HT₃ receptors (Bradley et al. 1985) has been addressed by a series of studies showing behavioral, biochemical, and electrophysiologic responses to both 5-HT₃ 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-HT₁, 5-HT₂, or 5-HT₃ 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-HT₄ (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-HT₁, 5-HT₂, and 5-HT₄ 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-HT₄ receptor stimulates, adenylate cyclase (Bradley et al. 1985; Dumuis et al. 1988). On the other hand, activation of both 5-HT_{1C} and 5-HT₂ 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-HT₃ 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-HT₃ 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-HT₂ receptors have been cloned (Fargin et al. 1988; Julius et al. 1988; Pritchett et al. 1988; Hamblin and Metcalf 1991). More recently, the 5-HT₃ receptor has also been cloned (Maricq et al. 1991). The characteristics of the cloned 5-HT₃ 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-HT₃ 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-HT₃ receptors (Fozard et al. 1983). Further studies demonstrated that metoclopramide and a series of substituted benzamides (Fozard et al. 1978; Fozard 1984) also be-

have as competitive antagonists of 5-HT action on peripheral tissues possessing 5-HT₃ receptors.

The broad pharmacologic profiles of compounds with affinity for the neural 5-HT₃ receptor hampered the definition of this receptor. By chemically substituting compounds that bind with rather low specificity to the 5-HT₃ site, it became possible to synthesize selective 5-HT₃ 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-HT₂) and "M" (5-HT₃) receptors, Richardson et al. (1985) produced both specific agonists and antagonists for the 5-HT₃ 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-HT₂ (α -methyl-5-HT) and highly specific 5-HT₃ (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-HT₃ antagonist (3 α -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-HT₃ 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-HT₃ 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-HT₃ antagonistic properties (Fake et al. 1987).

Other compounds with selective activity at the 5-HT₃ receptor have also been identified. Among them, 1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-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-HT₃ receptor antagonists. Quipazine possesses high affinity for the 5-HT₃ 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

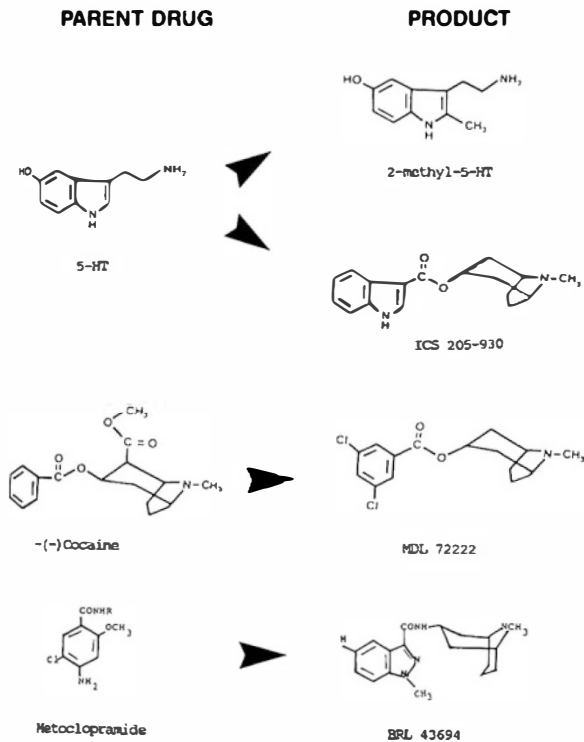


Figure 1. Synthesis of potent and specific drugs [PRODUCT] with affinity for the 5-HT₃ receptor derived from different compounds [PARENT DRUGS] that bind the 5-HT₃ site with low specificity.

(CAMP) system has been used to identify an initial three-component pharmacophore for specific 5-HT₃ receptor ligands such as ICS 205-930, GR38032F, zacopride, and the novel compound 3-[2-(guanidylmethyl)-4-thiazolyl]indole (Rizzi et al. 1990). The CAMP represents a powerful tool in the drug-design field because it helps to explain or predict a variety of molecular properties.

5-HT₃ RECOGNITION SITES IN NERVOUS SYSTEM TISSUE: BIOCHEMICAL CHARACTERIZATION AND DISTRIBUTION

During the last 3 years, various 5-HT₃ receptor antagonists have been labeled with radioactive isotopes. The first radioligand used to identify 5-HT₃ receptors in nervous system tissue was [³H]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 [³H]ICS 205-930 labeled a single population of binding sites, with a B_{max} of 60.4 and 40.5 femtomole 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-HT₃ receptor agonists and antagonists on [³H]ICS 205-930 binding

were consistent with those expected for a 5-HT₃ receptor (Bradley et al. 1985). Moreover, the inability of adenine and guanine nucleotides to affect agonist binding suggests that 5-HT₃ recognition sites are not coupled to G-proteins (Hoyer and Neijt 1988).

The identification and characterization of 5-HT₃ binding sites in the rat brain support the notion that the behavioral effects observed in rats after the administration of drugs selective for 5-HT₃ receptors might be mediated by a central rather than a peripheral 5-HT₃ receptor. After Kilpatrick et al. (1987) first demonstrated the presence of [³H]GR65630 binding sites in rat brain, the putative rat brain 5-HT₃ 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-HT₃ site (K_d = 0.066 nmol/L) was shown by [³H]GR67330 when compared to other labeled 5-HT₃ 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 femtomole 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-HT₃ binding site labeled with any of the 5-HT₃ receptor ligands clearly indicates that the various compounds bind to an identical 5-HT₃ binding site.

The ability of various 5-HT uptake blockers that possess antidepressant action to displace with nanomolar affinity [³H]quipazine from rat cortical membranes (Schmidt and Peroutka 1989) led to the suggestion that the 5-HT₃ receptor might be involved in the pharmacologic action of antidepressants. However, in membranes of the rat entorhinal cortex labeled with [³H]zacopride or of N1E-115 cells labeled with [³H]ICS 205-930, 5-HT uptake blockers display very low affinity for the 5-HT₃ binding site (Hoyer et al. 1989). These results support the idea that [³H]quipazine might also bind to a site unrelated to the 5-HT₃ 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-HT₃ binding site than metoclopramide, 5-HT, and 2-methyl-5-HT, drugs currently used in the characterization of 5-HT₃ receptors in nervous system tissue. If the antipsychotic action of atypical neuroleptics involves an interaction with brain

Table 1. 5-HT₃ 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 a 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 hydro- phobic 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-HT₃ binding sites, then 5-HT₃ 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-HT₃ 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 [³H]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 [³H]quipazine with a similar affinity pattern in rat and pig cortical membranes suggests the presence of a similar 5-HT₃ receptor in the brains of these two species (Peroutka 1988).

Binding sites of [³H]zacopride (Barnes et al. 1989a), [³H]GR65630 (Kilpatrick et al. 1989), and [³H]ICS 205-930 (Waeber et al. 1989) have also been identified in human brain. Binding of [³H]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 femtomole per milligram of protein in amygdala and hippocampus, respectively (Barnes et

al. 1989a). The 5-HT₃ recognition sites in human brain meet the criteria for 5-HT₃ receptors (Bradley et al. 1985). However, characterization of 5-HT₃ binding sites with various radioligands will be necessary to elucidate the biochemical processes that mediate the central actions of 5-HT₃ 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-HT₃ binding sites (Pratt et al. 1990). In rat and mouse brain, high densities of [³H]GR65630, [³H]GR67330, [³H](S)-zacopride, or [³H]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-HT₃ 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-HT₃ binding sites seem to be located presynaptically on capsaicin-sensitive primary afferent fibers (Glau and Anderson 1988; Hamon et al. 1989). Interestingly, the ferret possesses high concentrations of [³H]GR65630 binding sites in the nucleus accumbens, olfactory tubercle, amygdala, and striatum (Kilpatrick et al. 1989).

Biochemical and autoradiographic localization of

5-HT₃ binding sites with [³H]GR65630 (Kilpatrick et al. 1989), [³H]zacopride (Barnes et al. 1989a), or [³H]ICS 205-930 (Waeber et al. 1989) in human brain membranes or slices has revealed a 5-HT₃ receptor distribution similar to that of rodent brain. However, in contrast with other mammalian species, human cortical areas possess low concentrations of 5-HT₃ binding sites (Waeber et al. 1989). Relatively high concentrations of 5-HT₃ 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-HT₃ 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-HT₃ receptor with biochemical characteristics similar to those of the 5-HT₃ 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-HT₃ 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-HT₃ receptor was achieved by affinity chromatography, with the selective 5-HT₃ receptor antagonist L-685,603 immobilized in agarose as the ligand. Scatchard analysis and competition studies with the radioligand [³H]Q ICS 205-930 confirmed the presence of a single population of 5-HT₃ 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 [³H]zacopride and by [³H]GR65630 possess molecular masses of 35 kD (Bolanos et al. 1990) and of 49 kD (Lummis et 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-HT₃ receptor has an apparent molecular mass of 250 kD.

The apparent molecular mass of the purified 5-HT₃ 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 nicotinic acetylcholine (ACh) receptor (Whiting and Lindstrom 1986). Considering the physicochemical similarities be-

tween the 5-HT₃ receptor and these three receptors, it was predicted that the 5-HT₃ 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-HT₃ RECEPTOR

Molecular biological studies further supported the concept that the 5-HT₃ 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-HT₃ receptors (Yakel et al. 1988; Neijt et al. 1988a).

Sequence analysis of the clone for the 5-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ receptor cDNA specifically bound the selective 5-HT₃ receptor antagonist [³H]GR63650; moreover, kinetic studies showed that the affinity and Hill coefficient values for [³H]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-HT₃ receptors (Kilpatrick et al. 1987; McKernan et al. 1990b). The expression of specific 5-HT₃ 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-HT₃ receptors can be expressed in different biological systems (Maricq et al. 1991).

The distribution of the 5-HT₃ 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-HT₃ 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-HT₃ binding sites (Kilpatrick et al. 1987), suggests that a distinct 5-HT₃ receptor subtype encoded by a separate gene may exist in this organ.

ELECTROPHYSIOLOGIC RESPONSES TO 5-HT₃ 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-HT₃ receptor cDNA (Maricq et al. 1991). The fast depolarizing current evoked by 5-HT is reproduced by the 5-HT₃ receptor agonists 2-methyl-5-HT, 1-phenylbiguanide and 1-(*m*-chlorophenyl) biguanide, and is antagonized by selective 5-HT₃ 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-HT₃ 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-HT₃ receptor belongs to the family of ligand-gated ion channel receptors: 1) the 5-HT₃ 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, AlF₄⁻, 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-HT₃ 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-HT₃ 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-HT₃ 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 Ca²⁺ channels, which leads to an influx of extracellular Ca²⁺ into the cell. The influx of Ca²⁺ does not modify the membrane potential, but Ca²⁺ functions as an intracellular messenger to couple the activation of the 5-HT₃ receptor-linked ion channel to transmitter release. Transmitter release is modulated by Ca²⁺ probably through facilitation of a transient fusion of the synaptic vesicle membrane with the presynaptic terminal membrane. Whether Ca²⁺ promotes vesicular fusion directly or through a Ca²⁺-binding protein that promotes fusion is not yet known.

In clonal cell lines, extracellular Ca²⁺ and Mg²⁺ exert a regulatory influence on 5-HT₃ 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-HT₃ receptor channel without affecting the current-voltage (I/V) ratio. However, the homomeric cloned 5-HT₃ receptor expressed in *Xenopus* oocytes differs from the native 5-HT₃ receptor (Maricq et al. 1991); in the presence of Ca²⁺ or Mg²⁺, 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-HT₃ receptor shares some properties with the N-methyl-D-aspartate (NMDA)-sensitive glutamate receptor, the conductance of which is blocked by Mg²⁺ (Nowak et al. 1984).

The Ca²⁺ permeability and single-channel conductance of the 5-HT₃ 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-HT₃ receptor channel for Ca²⁺ 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-HT₃ receptor. In N1E-115 cells and in oocytes expressing cloned 5-HT₃ 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-HT₃ receptor induced by 5-HT (Yakel and Jackson 1988). Although the mechanism by which 5-HT induces 5-HT₃ receptor desensitization is unknown, it can be speculated that modulation of 5-HT₃ 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-HT₃ RECEPTOR ACTIVATION

Neurotransmitter release and metabolism in the CNS is modified by 5-HT₃ 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-HT₃ agonist 2-methyl-5-HT stimulated both basal and K⁺-induced dopamine (DA) release. The DA release induced by 5-HT₃ receptor stimulation was shown to be Ca²⁺-dependent and partially sensitive to tetrodotoxin (Blandina et al. 1989). The selective 5-HT₃ receptor antagonist ICS 205-930, but not the 5-HT₁/5-HT₂ 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-HT₃ receptors (Blandina et al. 1989).

The *in vitro* experiments performed with superfused striatal slices (Blandina et al. 1989) suggest that 5-HT₃ receptor antagonists either block postsynaptic receptors located on intrastriatal neurons involved in the regulation of DA release or block presynaptic 5-HT₃ 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-HT₃ recognition sites in the striatum, suggesting a lack of 5-HT₃ binding sites in the nerve terminals of the nigrostriatal dopaminergic system (Hamon, personal communication).

Acute *in vivo* administration of 5-HT₃ 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-HT₃ receptor antagonists, including ICS 205-930, GR38032F, GR65630, and MDL 72222.

The cellular site at which 5-HT₃ 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-HT₃ 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-HT₃ receptor agonist, in the nucleus accumbens (Chen et al. 1991), is effectively blocked by selective 5-HT₃ 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-HT₃ 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-HT₃ receptor antagonists within the VTA is exerted on a postsynaptic 5-HT₃ 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-HT₃ receptors that regulate DA release may be located in presynaptic dopaminergic nerve terminals.

The 5-HT₃ 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 [³H]noradrenaline release. This action of the 5-HT₃ receptor antagonists is only apparent, however, at concentrations that are two to three orders of magnitude higher than those required to block 5-HT₃ receptors (Feuerstein and Hertting 1986), and so the effect may not be specific.

The K⁺-induced increase in [³H]ACh release from the rat entorhinal cortex can be effectively reduced by activation of 5-HT₃ 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-HT₃ receptor antagonists is due to a direct blockade of the presynaptic inhibitory effect of 5-HT₃ receptor agonists on ACh release or to a blockade of 5-HT₃ 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-HT₃ receptors in nervous system tissue may participate in the modulation of both neuronal electrical activity and neurotransmitter release from nerve terminals. Apparently, 5-HT₃ 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-HT₃ receptor-gated Na⁺-K⁺ channel that is functionally linked to a selective Ca²⁺ 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-HT₃ receptor agonists is not clear. In frontocingulate and entorhinal cortices, 5-HT₃ receptor agonists activate PI metabolism (Edwards et al. 1991). Moreover, in medial prefrontal cortex, the 5-HT₃ 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-HT₃ receptor action, DA and ACh release may be differentially regulated by distinct subtypes of 5-HT₃ receptors or by different intracellular mechanisms linked to Ca²⁺ function.

5-HT₃ RECEPTORS AND CENTRAL NERVOUS SYSTEM DISORDERS

The development of drugs that are selective for the 5-HT₃ receptor, together with the availability of be-

havioral 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-HT₃ receptors in brain function. The 5-HT₃ 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-HT₃ 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 D₂ 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 D₂ receptor. In fact, the newly developed drugs with activity on emesis proved to be potent antagonists of the 5-HT₃ receptor (Fozard and Gittos 1983; Fake et al. 1987; Smith et al. 1988). The inability of 5-HT₃ receptor antagonists to block apomorphine-induced emesis showed that these compounds could not be exerting their effects through postsynaptic D₂ receptors. Because 5-HT increases the release of DA in various brain areas through a 5-HT₃ receptor-mediated mechanism, 5-HT₃ 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-HT₃ 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 D₂ 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-HT₃ 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-HT₃ 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-HT₃ receptors. In agreement with these results, intracerebroventricular administration of 5,7-dihydroxytryptamine, a selective neurotoxin for serotonergic neurons, fails to modify 5-HT₃ receptors in rat brain (Leysen, personal communication). The 5-HT₃ 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-HT₃ 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-HT₃ receptor antagonists exert a neuroleptic-like activity and support the view that 5-HT₃ receptors regulate central dopaminergic function (Tricklebank 1989).

The pharmacologic profile of 5-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ receptor antagonists, by blocking the action of 5-HT at the 5-HT₃ 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 Ca²⁺ channel activation by the 5-HT₃ 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-HT₃ receptor blockers have pharmacologic profiles similar to those of anxiolytic benzodiazepines, even though the 5-HT₃ receptor antagonists fail to bind to GABA_A receptor-associated benzodiazepine recognition sites (Jones et al. 1988). In contrast to benzodiazepines, however, 5-HT₃ receptor antagonists lack sleep-inducing, muscle-relaxant, and anticonvulsant effects. Moreover, 5-HT₃ receptor blockers fail to induce dependence after long-term treatment (Jones et al. 1988). Interestingly, the 5-HT₃ 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-HT₃ receptor antagonists exert their anxiolytic action in rodents is still unclear. In most tests that evaluate anxiety-related pharmacologic profiles, 5-HT₃ receptor antagonists show a dose-response relation within the range of those observed in other *in vivo* studies that evaluate 5-HT₃ receptor function (Butler et al. 1988). Moreover, the fact that structurally different 5-HT₃ receptor antagonists are endowed with anxiolytic activity strongly supports a role for 5-HT₃ 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-HT₃ receptor antagonists improve cognitive performance in behavioral tests that evaluate cognitive function (Costall et al. 1989). Consistent with the observation that 5-HT₃ 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-HT₃ 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-HT₃ receptor agonists and antagonists. Moreover, the availability of labeled receptor ligands with relatively high specific activity has allowed the identification of 5-HT₃ binding sites in mammalian brain. The 5-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ receptor as well as in primary cultures and cell lines of neuronal origin, 5-HT₃ receptor activation results in a fast depolarizing excitatory response that can be selectively blocked by 5-HT₃ receptor antagonists.

A growing body of evidence suggests the presence of distinct 5-HT₃ 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-HT₃ receptors (Derkach et al. 1989; Lamberts et al. 1989; Yang 1990). In addition, at odds with the failure of the 5-HT₃ 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-HT₃ receptor (Maricq et al. 1991). These data are consistent with the apparent absence of 5-HT₃ receptor mRNA in the mouse intestine (Maricq et al. 1991). Further evidence for the existence of multiple 5-HT₃ receptors has been provided by studies showing that the recovery of the 5-HT₃

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-HT₃ receptor function also support the concept of 5-HT₃ receptor heterogeneity. Thus, in the striatum and the nucleus accumbens, activation of 5-HT₃ receptors stimulates the release of DA (Blandina et al. 1988, 1989; Imperato and Angelucci 1989), whereas in cortical tissue the stimulation of 5-HT₃ receptors inhibits ACh release (Barnes et al. 1989b). The recent identification of a second transducing system linked to 5-HT₃ receptor function, namely PI hydrolysis (Edwards et al. 1991), further supports the existence of different 5-HT₃ receptor subpopulations in neural tissues. The possibility, however, that the increase of PI metabolism after 5-HT₃ 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-HT₃ receptor antagonists. The high concentration of 5-HT₃ binding sites in the area postrema, a brain area related to the control of emesis, suggests a potential role for 5-HT₃ receptor antagonists as potent antiemetics during tumor chemotherapy or radiotherapy. On the other hand, the ability of 5-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ 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-HT₃ receptor to understand the molecular mechanisms of receptor regulation. Moreover, further molecular biological studies should help elucidate whether there is only one 5-HT₃ receptor or whether receptor subtypes exist.

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