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5-HT$_6$ RECEPTORS AS EMERGING TARGETS FOR DRUG DISCOVERY

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■ **Abstract** 5-ht$_6$ receptors are the latest serotonin receptors to be identified by molecular cloning. Their high affinity for a wide range of drugs used in psychiatry, coupled with their intriguing distribution in the brain, has stimulated significant interest. Antisense oligonucleotides, antipeptide antibodies, selective radioligands, knockout mice, and selective antagonists of the 5-ht$_6$ receptor have recently become available. Surprisingly, 5-ht$_6$ receptors appear to regulate cholinergic neurotransmission in the brain, rather than the expected interaction as modulators of dopaminergic transmission. This interaction predicts a possible role for 5-ht$_6$ receptor antagonists in the treatment of learning and memory disorders. Furthermore, polymorphisms in the sequence of the 5-ht$_6$ receptor gene may provide a genetic tool to further our understanding of the differential responses of patients to antipsychotic medications.

INTRODUCTION

The multiplicity of actions of serotonin has been known for decades (1). Although the pharmacology of these responses suggested the likelihood of receptor subtypes (2, 3), the first identification of a discreet molecular species of known (deduced) amino acid composition came only a decade ago (4). This discovery presaged an explosion in the number of genetically identified receptors for serotonin (5). Attendant to the relatively fast pace of molecular discovery has been the slower pace of receptor characterization using nucleotide probes, radioligands, known chemical entities, and biochemical functional assays. More recently, the development of newly created probes such as selective antibodies, selective agonists and antagonists, knockout animals, and genetic linkages studies has been essential to further our understanding of the functions of individual serotonin receptor subtypes. Although our knowledge is far from complete, there is now substantial progress in the elucidation of functions of the most recently discovered serotonin receptor, 5-ht$_6$, and its possible therapeutic roles.

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MOLECULAR BIOLOGY OF 5-HT$_6$ RECEPTORS

The first cloning of the rat 5-ht$_6$ receptor reported a sequence predicted to encode a protein of 436 amino acids (6, 7). This sequence has been re-evaluated, and the receptor is now deduced to form a protein of 438 amino acids. The human homologue contains 440 amino acids (8). The human and rat amino acid sequences are 89% identical. Structural elements of this receptor sequence include a relatively short third intracellular loop (50 amino acids, rat; 57 amino acids, human) and a long carboxyl tail (120 amino acids), both of which are common to some other GPCRs that couple to adenylate cyclase stimulation (9). The 5-ht$_6$ receptor has a single glycosylation site in the amino terminus and multiple potential phosphorylation sites for protein kinase C in the cytoplasmic domains. The sequence also contains a leucine zipper motif in transmembrane (TM) III (7). The sequence has the highest amino acid identity (37%) to the *Drosophila melanogaster* cyclase stimulatory serotonin receptor (10) and the histamine H_2 receptor (11); lower identities are observed with 5-ht$_5$ and 5-HT$_7$ receptors. The rat and human sequences each contain two introns, one in the third intracellular loop and the other in the third extracellular loop (8). The intron in the third cytoplasmic loop appears at the same location as that described for the 5-ht$_{5a}$ and 5-ht$_{5b}$ receptors, as well as the dopamine D_2 and D_3 receptors (8). No additional subtypes have been cloned, and no functional splice variants have been identified. However, a truncated variant of the 5-ht$_6$ receptor, first noted by Monsma et al (6), was presumed to occur as a mis-splicing event and delete the seventh TM VII domain (12). Subsequently, Olsen and coworkers (13) showed that the human 5-ht$_6$ gene could give rise to an alternate splicing of the first intron, which produced a truncated variant of the receptor containing the amino terminus through TM IV. This variant is transcribed, and the transcripts are expressed in a limited subset of the brain regions (substantia nigra and caudate) occupied by mRNA for the full-length receptor.

The gene for the human 5-ht$_6$ receptor maps to chromosome region 1p35–p36, thus overlapping with the gene locus for the 5-HT$_{1D\alpha}$ receptor (8). The 5-ht$_6$ receptor sequence contains a Rsa1 restriction fragment–length polymorphism in the first extracellular loop (C267T).

MOLECULAR PHARMACOLOGY OF 5-HT$_6$ RECEPTORS

The 5-ht$_6$ receptor can be radiolabeled with [^{125}I]lysergic acid diethylamide (LSD) and couples to the stimulation of adenylate cyclase (6, 7). The distinctive properties of the pharmacology of the cloned rat 5-ht$_6$ receptor are its high affinity for a series of antipsychotic compounds, including clozapine and loxapine, as well as affinity for a number of tricyclic antidepressants such as amoxipine, clomipramine, and amitryptyline (6, 14, 15). A new potential antipsychotic compound, BIMG 80, also has moderate affinity for the 5-ht$_6$ receptor (16). Analysis of

binding studies using [^{125}I] LSD as a radioligand gives the rank order of affinities: methiothepin > 5-MeOT > 5-HT > tryptamine > 5-CT > sumatriptan >> 8-OH-DPAT. The affinity of 5-HT to the 5-ht$_6$ receptor is relatively low compared with other serotonin receptors. A similar receptor profile has been reported in N8TG2 cells, a neuroblastoma line. It displays a rank order of agonist potency in both radioligand binding and cAMP assays: 5-MeOT > 5-HT > tryptamine > 2-methyl tryptamine > 5-CT > α-methyl-5-HT (17). Responses to 5-HT in this cell line are antagonized by clozapine. Affinities of compounds for the human cloned 5-ht$_6$ receptor are equivalent to those determined for the rat, with the exception of four compounds: methiothepin (4-fold higher affinity for the human receptor), metergoline, and the atypical antipsychotics, tiopyrone and amperozide (>10-fold higher affinity for the rat receptor). Clozapine is a high-affinity antagonist at both human and rat 5-ht$_6$ receptors. The rank order of binding affinities for antagonists is methiothepin > clozapine = olanzapine > ritanserin >> risperidone. A nonconserved amino acid substitution of threonine (rat) for leucine (human) in TM III may contribute to the differences in binding affinities observed between the species homologues.

The primary signal transduction pathway of the 5-ht$_6$ receptor is the stimulation of adenylate cyclase (AC) activity. The rank order of both agonist and antagonist potencies, as well as their quantitative values, determined from AC stimulation matches closely with those determined in parallel using radioligand binding (15, 18). There are multiple isoforms of AC; for example, AC5 is a G_s-sensitive AC. It is highly localized in the striatum and nucleus accumbens, two major areas of 5-ht$_6$ localization. In contrast, AC1 and AC8 are calmodulin-stimulated ACs and are not activated by G_s proteins in vivo. AC1 and AC8 are neural-specific cyclases. AC1 is expressed in hippocampus, and AC8 is expressed in hippocampus and hypothalamus. The 5-ht$_6$ receptor, expressed in HEK 293 cells, interacts specifically with AC5 but not with AC1 or AC8 (19).

A limited number of mutations have been made experimentally to probe the binding pocket of the 5-ht$_6$ receptor. In TM V of many monoamine receptors, there are two ''conserved'' serine residues that are responsible for hydrogen bonding of hydroxyl groups of the cognate neurotransmitter (20, 21). In the many 5-HT receptors, the second serine is replaced by an alanine, and this replacement affects the binding of compounds such as N-1–substituted ergolines and tryptamines (22, 23). In the 5-ht$_6$ receptor, a threonine, rather than the ''expected'' alanine, occupies this position. Mutation of this residue to alanine (T196A) results in a decrease in the affinity of the mutant for LSD, 5-HT, and other N-1 unsubstituted ergolines (24). The magnitude of this change is consistent with a disruption of a hydrogen bond. In contrast, the N-1–methylated ergolines showed unchanged or enhanced affinity. Mutations were also made in TM III and TM VI (25, 26). In TM III, mutation of the conserved aspartic acid to asparagine (D106N) resulted in a loss of [^{3}H]LSD binding, although AC stimulation could still be elicited with both LSD and 5-HT. The potencies, however, were right shifted by 500-fold for LSD and 3600-fold for 5-HT (25). This change in affinity is consis-

tent with the loss of a charge-charge interaction. In contrast, mutation of the conserved tryptophan one helical turn upstream of this mutation (W102F) resulted in only a small (two- to sixfold) reduction of affinity for most test compounds. Finally, two adjacent residues near the distal end of TM VI were probed (A287L, N288S) as doubled mutants (25). This mutant displayed an elevated affinity for tryptamine derivatives with large substitutions on the 5′ position, as well as for ergopeptine ligands with large substituents on the 2′ position, possibly consistent with formation of a new hydrogen bond to Ser288. Studies such as these will aid molecular modeling approaches used in the design of selective ligands for this receptor.

REGULATION OF 5-HT$_6$ RECEPTORS

Since 5-ht$_6$ receptors may be important mediators of some of the beneficial actions of psychiatric drugs, it would be interesting to know if 5-ht$_6$ receptors act as autoreceptors. To investigate this possibility, Gerard et al (27) evaluated the impact of selective lesioning of the serotonergic system on the distribution of 5-ht$_6$ receptors by using 5,7-dihydroxytryptamine. Three weeks after administration of the toxin by microinfusion, only 10% of the serotonin transporter, a marker of serotoninergic neurons, remained in the anterior raphe region. In contrast, 5-ht$_6$ mRNA levels were unchanged. This observation indicates that 5-ht$_6$ receptors are not located on serotoninergic neurons, and therefore are not autoreceptors. In addition, the postsynaptic target cells of the serotoninergic projections do not up- or down-regulate their 5-ht$_6$ mRNA levels in response to the lesion, at the time point examined.

Glucocorticoids are known to affect serotoninergic systems and to be related to depression (28). Blockade of glucocorticoid synthesis, with metyrapone and aminogluthethimide, increases 5-ht$_6$ mRNA levels in the CA1 regions of the hippocampus (29). This effect can be partially reversed with corticosterone replacement. Metyrapone and aminogluthethimide have both been used in resistant depression, which has led to speculation that increases in receptor number with these treatments may enhance the effect of antidepressant ligand (29).

The developmental expression of mRNA for the 5-ht$_6$ receptor has been studied using RT-PCR. Expression of these transcripts first appeared on embryonic day 12 (E12), coincident with the appearance of the first serotonergic cell bodies of brain neurons, suggesting a possible role of the 5-ht$_6$ receptor in growth factor properties of 5-HT (30). Expression increased through postnatal day 15 and then stabilized through adult at the same level.

Selective Agonists and Antagonists for 5-ht$_6$ Receptors

At present, there are no fully selective agonists. However, a careful structure-affinity analysis of 5-HT derivatives has been presented (31). The most selective agonist is 2-methyl-5-HT. Modifications on the 5 position indicate that the

hydroxyl group is relatively unimportant for 5-HT binding. The conformation of the side chain that the 5-ht$_6$ receptor prefers for binding is that adopted by ergolines. An intact indole nucleus is favored for binding. Secondary and tertiary amines are preferred over the primary amine or the quaternary amine, whereas large dialkyl substitutions reduce affinity.

It was previously shown that many known antidepressants and antipsychotics are antagonists of the 5-ht$_6$ receptor (14). However, none was selective for this receptor. They typically have affinity for dopamine receptors, other 5-HT receptors, monoamine oxidase, and many other sites. Great strides have been made recently in the development of selective antagonists of the 5-ht$_6$ receptor.

The first reported 5-ht$_6$ antagonists were Ro-04–6790 [4-amino-N-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene sulphonamide] and Ro-63–0563 [4-amino-N-(2,6 bis-methylamino- pyridin-4-yl)-benzene sulphonamide] (32). They are both relatively high-affinity (pK_i = 7.3 and 7.9, respectively), selective competitive antagonists (pA_2 = 6.75 and 7.10), as evaluated in transfected cells. There are no significant differences in their affinities for rat compared with human 5-ht$_6$ receptors. Ro-04–6790 can be administered i.p. and is CNS penetrant. Ro-63–0563 can be administered i.v. and is also CNS penetrant. The preferred compound for in vivo use is Ro-04–6790, although neither compound achieves high brain levels.

These structures were evaluated for use as radioligands. [^{3}H]Ro 63–0563 was synthesized and had a specific activity of 29 Ci/mmol (33). It was used in membrane binding studies in rat striatal membranes. The measured dissociation constant was 11.7 nM and the B_{max} was 175 fmol/mg protein. However, poor levels of specific binding were observed (10–30%). In transfected cells, the ligand had a dissociation constant of 5 nM for human 5-ht$_6$ and 6.8 nM for rat 5-ht$_6$, and a better specific binding level was obtained (70%). The pharmacological profile of the 5-ht$_6$ receptor as determined using this radioligand was not significantly different from that measured with [^{3}H]LSD as a radioligand. The high nonspecific binding in native tissues limits its use as a radioligand for autoradiographic studies.

The next selective antagonist was SB-271046 (34). The initial hit from a high throughput screen was 4-bromo-N-[4-methoxy-3-(4-methylpiperazin-1-ylphenyl] benezenesulfonamide. It had a pK_i of 8.3 nM and was 50-fold selective over other binding sites. This compound was moderately brain penetrant but was rapidly cleared from the blood and therefore had low oral bioavailability. A series of bisaryl sulfonamides was prepared and evaluated. The 5-chloro-3-methylbenzothiophene derivative had a subnanomolar affinity but was metabolized by N-dealkylation in the rat to yield the corresponding NH-piperazine. Synthesis of this metabolite, which was detected at a high level in blood, resulted in a 5-ht$_6$ antagonist with high affinity (pK_i = 8.9 nM) and potency (pA_2 = 8.7 nM) and excellent selectivity. The compound was found to be moderately CNS penetrant (10%) and to have a low blood clearance, good half-life (4.8 h in rat), and 80% oral bioavailability. As such, 5-chloro-N-(4-methoxy-3-piperazin-1-yl-phenyl)-3-

methyl-2-benzothiophenesulfonamide (SB-271046) is a significant new tool for the study of 5-ht$_6$ receptor function.

In addition, another compound from the series {5-iodo-N-[4-methoxy-3-(4-methylpiperazin-1-yl-phenyl]benezenesulfonamide} has been radioiodinated to form [^{125}I]SB-258585 and evaluated for use as a radioligand (35). [^{125}I]SB-258585 had a specific activity of 2000 Ci/mmol. In binding assays on membranes derived from human 5-ht$_6$–transfected cells, a K_d = 0.8 nM was determined and 95% specific binding was obtained. Subsequent studies in native tissue homogenates (36) indicate that there is high specific binding (60–68%) in rat and pig striatal membranes and in human caudate-putamen membranes. The K_d in rat and porcine tissues was 2.8 nM with a B_{max} of 180 fmol/mg protein. In human caudate the K_d was 1.3 nM with a B_{max} of 215 fmol/mg protein. The rank order of affinities for a discriminating set of ligands was comparable to that previously determined for the cloned receptors and also by using alternate radioligands, thereby validating this as a new tool for the study of 5-ht$_6$ receptors. In addition, this ligand is useful for autoradiographic mapping studies (37) (see below).

IN VITRO STUDIES

Localization of mRNA for the 5-ht$_6$ Receptor

The distribution of mRNA encoding the 5-ht$_6$ receptor has been determined by Northern blot analysis in the rat brain and peripheral tissues (6). The highest expression was detected in the striatum with lower-density signals detected in the amygdala, cerebral cortex, and olfactory tubercle. mRNA was undetectable in cerebellum, hippocampus, hypothalamus, medulla, olfactory bulb, pituitary, retina, thalamus, and a number of peripheral tissues (heart, lung, kidney, liver, spleen, pancreas, skeletal muscle, smooth muscle, stomach, ovary, prostate, and testes). A second group observed 5-ht$_6$ mRNA signals in the hippocampus, hypothalamus, adrenal, and stomach (7). Initial in situ hybridization studies in the rat brain demonstrated high levels of mRNA in the striatum and olfactory tubercles (7). Other labeled structures included the nucleus accumbens, olfactory bulb, and hippocampus. Using in situ hybridization, Ward et al (38) have completed a detailed examination of the mRNA distribution for the 5-ht$_6$ receptor in the rat brain. Their study confirmed the high abundance of message in the olfactory tubercle, striatum, nucleus accumbens, dentate gyrus, and CA1, CA2, and CA3 fields of the hippocampus. Lower intensity labeling was found in the cerebellum, some diencephalic nuclei, amygdala, and several cortical layers (layers 2, 3, 4, and 6). In the striatum, the 5-ht$_6$ mRNA is extensively co-localized with enkephalin (68%), substance P (79%), and dynorphin (59%) output (39). Similar co-localization was detected in the substantia nigra. In the striatum, the 5-ht$_6$ transcripts are homogeneously distributed between the patch and matrix components as well as between cells projecting to the two major outflow pathways.

The distribution of the human 5-ht$_6$ mRNA has been evaluated by Northern blot analysis (8). It parallels the distribution shown in the rat brain, with the highest expression levels detected in the caudate. In the human brain, lower expression levels of 5-ht$_6$ mRNA were found in the hippocampus, amygdala, and thalamus.

Localization of the 5-ht$_6$ Receptor Protein

Immunohistochemistry The pattern of protein expression for the 5-ht$_6$ receptor has been determined using selective antibodies to a carboxyl-terminal domain of the receptor sequence (27). Receptor protein was abundant in the plexiform layer of the olfactory tubercle and in the frontal and entorhinal corticies, nucleus accumbens, striatum, hippocampus (striata oriens and radiatum of CA1 and molecular layer of the dentate gyrus), and molecular layer of the cerebellum. A moderate degree of immunoreactivity was found in the thalamus, the substantia nigra, the superficial layer of the superior colliculus, the motor trigeminal nucleus, and the facial nucleus (27). This pattern is consistent with that seen from determination of the mRNA distribution, indicating that the protein is close to the site of synthesis, as in dendrites or somata. Dendritic localization in the striatum and dentate gyrus has been visualized by immuno-electronmicroscopy. The strong distribution of the receptor protein in the extrapyramidal and limbic areas led to the suggestion that the 5-ht$_6$ receptor may control motor function and mood-dependent behaviors. Gerard et al (27) further suggested that the 5-ht$_6$ receptors may be on the target cells of dopaminergic neurons (but see below), which might explain part of the antipsychotic activity of clozapine.

Autoradiography Radioligand binding is an alternative to immunohistochemistry to map receptor protein in the rat brain. The first experiment used [^{3}H]clozapine as a label for 5-ht$_6$ receptors in membranes (40). Forty percent of the sites that Glatt et al (40) detected exhibited a 5-ht$_6$ profile. There were no differences in the density of these sites between cerebral cortex, striatum, and hippocampus. The [^{3}H]clozapine binding is consistent with data from in situ hybridization studies. Future studies using [^{3}H]clozapine for receptor autoradiography could provide a detailed map of 5-ht$_6$ receptors. Methiothepin is a ligand that has even higher affinity for the 5-ht$_6$ receptor. It has previously been radiolabeled with tritium, but it has not been found to be a suitable radioligand in the brain (41), as a result of its physico-chemical properties (e.g. lipophilicity) and poor receptor subtype selectivity.

Recently, a new radioligand, [^{125}I]SB-258582, has been introduced that is selective for the cloned 5-ht$_6$ receptor (35). Subsequent studies (37) indicate that there is high specific binding in native tissues and that this ligand is useful for autoradiographic mapping studies. In rat, high densities of sites were found in the cerebral cortex, nucleus accumbens, caudate-putamen, and CA1 and dentate gyrus of the hippocampus. A moderate density of labeling was detected in the thalamus

and substantia nigra. Furthermore, after lesioning with 6-hydroxydopamine (6-OHDA) to the median forebrain bundle, no changes in the levels of binding were found, although there was a complete loss of tyrosine hydroxylase immunoreactivity in the striatum and nigra. This indicates that the 5-ht_6 receptors may be on cholinergic or GABAergic interneurons in the caudate putamen or on striatal GABAergic neurons or on their terminal fields in the nigra.

c-fos Activation Neuronal activation had been monitored using antibodies to the immediate early gene c-fos after drug treatment. Typical and atypical antipsychotic compounds give characteristic distribution patterns of c-fos activation (42, 43). The high affinity of antipsychotic compounds at the 5-ht_6 receptor implies that part of their actions may be due to their action on this receptor. The selective 5-ht_6 antagonist SB-271046 was administered to rats for four days, and the brains were processed for c-fos immunoreactivity (44). Rats treated with clozapine or haloperidol were run in parallel for comparison. Clozapine enhanced c-fos levels in the median prefrontal cortex and nucleus accumbens, and haloperidol enhanced levels in the caudate putamen and nucleus accumbens. No enhancement was seen in the SB-271046–treatment group (although the caudate putamen was not examined). These data indicate that activity of clozapine as monitored in this assay is not primarily the result of its action at the 5-ht_6 receptor.

FUNCTIONS OF THE 5-HT_6 RECEPTOR

Cellular Responses

There are several early reports of ''atypical'' 5-HT receptors in cells lines, particularly NCB.20 cells. This cell line was created by fusing a mouse neuroblastoma line, N18TG2, and an embryonic hamster brain explant (45). In this cell line, 5-HT, 5-MeOT, and methysergide all stimulated cAMP production. Clozapine and spiperone were antagonists. This response was reinvestigated using the newer tools for characterization (46, 47). cAMP stimulation was inhibited by metergoline (K_b = 50 nM), but not by ICS 205–930 (47), consistent with a 5-ht_6 but not a 5-HT_4 or 5-HT_7 response profile. The parental mouse cell line (N18TG2) was also evaluated (17). 5-HT stimulates cAMP responses with a pharmacological profile similar to that of the cloned 5-ht_6 receptor. The rank order of agonist potency in both radioligand binding and second messenger assays was 5-MeOT > 5-HT > tryptamine > 2-Me-5-HT >> 5-CT > α-Me-5-HT. In binding assays methiothepin showed higher affinity than clozapine, while in second-messenger assays the antagonists methiothepin, clozapine, and mianserin exhibited similar potencies (pA_2 = 6.5). A molecular analysis of the N18TG2 cell line to evaluate the presence of mRNA for 5-HT receptor subtypes has not been reported.

In primary neurons, stimulatory AC responses mediated via a 5-ht$_6$–like receptor have also been described (48). In cultured mouse striatal neurons, the rank order of agonist potencies to stimulate cAMP production was 5-HT > LSD > 5-MeOT > 5-CT. The serotoninergic agonists 8-OH-DPAT, sumatriptan, and cisapride were inactive. This response was antagonized by methiothepin, nortriptyline, clozapine, and amitriptyline. In combination with high distribution of mRNA in the striatum, this pharmacological profile indicates that these were 5-ht$_6$ responses in native neurons.

Furthermore, mRNA for 5-ht$_6$ receptors has been detected in an immortalized serotoninergic cell line from rat raphe nuclei (49). This may serve as an interesting model system for future studies of 5-ht$_6$ receptor regulation in a neuronal context.

TISSUE RESPONSES

Potential functional correlates of 5-ht$_6$ receptors have also been observed in vitro (50). A study of glycogenesis in tissue slices from rat cortex may reflect a 5-ht$_6$–like profile. In this preparation, 5-HT, 5-MeOT, and tryptamine stimulated glycogen hydrolysis. Tricyclic antidepressants were among the most potent competitive antagonists of the response. Methiothepin was weaker than expected for a 5-ht$_6$ response in antagonizing the glycogen hydrolysis response; physicochemical properties of the compound may have limited its efficacy. N,N-dimethyltryptamine (N,N-DMT) also antagonized this response, but its efficacy was greater than methiothepin. At the human 5-ht$_6$ receptor, N,N-DMT was equipotent with 5-HT ($pK_i = 7.2$).

In pig caudate membranes (51), a rank order of agonist potencies similar to that determined for 5-ht$_6$ was observed: 5-HT = 5-MeOT > 5-CT. The agonists 8-OH-DPAT, sumatriptan, and renzapride were inactive. The antagonist rank order was methiothepin > clozapine >> ketanserin. Neither of these receptor profiles derived from striatal preparations exactly matches the rank order of potencies in the N18TG2 cell line or the rank order of binding affinities from the cloned rat receptor. However, cross species comparisons or methodological differences may obscure the true relationships.

Electrophysiology

At present, there are no available reports of electrophysiological studies on the 5-ht$_6$ receptor.

IN VIVO STUDIES OF 5-HT$_6$ RECEPTOR FUNCTION

Molecular Approaches to Function In Vivo

Antisense Oligonucleotides The first behavioral studies of possible 5-ht$_6$–mediated function have been attempted using antisense oligonucleotides (AOs) targeted to the 5-ht$_6$ receptor subtype (52). In these studies, the rats exhibited a

behavioral phenotype consisting of an increased number of yawns and stretches. This behavior was blocked by atropine, suggesting a role of the 5-ht$_6$ receptor in the control of cholinergic neurotransmission. If so, then a 5-ht$_6$ antagonist might be useful in the treatment of depression, anxiety, and/or memory disorders (52).

Using a similar approach, Yoshioka and colleagues (53) evaluated the effect of AOs in a conditioned fear stress paradigm (CFS). After seven days of AO administration to the lateral ventricle, the 5-ht$_6$ receptor number decreased by 30%. In these animals, but not the sense oligonucleotide controls, the CFS-induced 5-HT release was suppressed, although freezing behavior was unaffected. This result suggests a potential role for the 5-ht$_6$ receptor in some forms of anxiety.

Finally, a preliminary report has appeared linking the 5-ht$_6$ receptor to memory acquisition and feeding (54). Again using AOs applied i.c.v., rats were treated for six days and evaluated in the Morris water maze test. AO-treated rats had no differences in visual acuity or swim speed, but they had a shorter average latency and longer time spent on the learned platform than controls. In addition, they had a lower body weight. Confirmation of these fascinating results is awaited.

Knockout Mice Targeted gene disruption has served as a useful probe for receptor function (55). A constitutive knockout animal lacking functional 5-ht$_6$ receptors has been produced and evaluated in several tests. At present, the only detectable difference from the wild-type animals has been an increase in anxiety-like behavior in the elevated zero maze (56). Additional studies are required to fully probe the changes in behavior and physiology in this knockout mouse.

Selective Antagonist Studies of 5-ht$_6$ Receptor Function

The selective 5-ht$_6$ receptor antagonist Ro 04–6790 was administered to rats by systemic injection. The compound induced a behavioral syndrome that included a dose-dependent increase in yawning, stretching, and chewing and was similar to that seen with the antisense treatment (32). The maximal effect was obtained at a dose that gave a cerebrospinal fluid concentration sufficient to occupy more than 70% of the 5-ht$_6$ receptors. Further exploration of this syndrome revealed that the stretching component was dose dependent and statistically significant (57). Pretreatment with muscarinic antagonists inhibited the stretching induced by Ro 04–6790. A non-CNS penetrant muscarinic antagonist was unable to inhibit the behavior, indicating a central mechanism. In addition, haloperidol had no effect. As with the antisense treatment, the 5-ht$_6$ antagonists produced a stretching behavior that is likely to be mediated by an increase in cholinergic, but not dopaminergic, neurotransmission. In contrast, the yawning was neither dose dependent nor statistically significant. This was in contrast to the effect of AO treatment on yawning. A number of explanations are possible, but further studies are required to evaluate them.

The distribution and pharmacology of the 5-ht$_6$ receptor suggest a link with dopaminergic function. mRNA for the 5-ht$_6$ receptor is preferentially down-

regulated in rats in certain brain regions after a two-week treatment with clozapine or haloperidol (58). Bourson and colleagues (59) investigated the effects of Ro 04–6790 on dopaminergic function. Ro 04–6790 did not induce catalepsy and had no effect on haloperidol or SCH 23390–induced catalepsy. It did not elicit rotational behavior in rat with unilaterally lesion of the median forebrain bundle induced by 6-OHDA. Ro 04–6790 had no effect on L-Dopa or amphetamine-induced rotational behavior. In contrast, antagonism of the 5-ht$_6$ receptor inhibited rotational behavior in the lesioned rats in response to cholinergic antagonists such as scopolamine and atropine. Therefore, consistent with previous reports using oligonucleotides, 5-ht$_6$ receptors are involved in cholinergic but not dopaminergic neurotransmission.

Using a second and more highly brain penetrant 5-ht$_6$ antagonist, Routledge et al (60) demonstrated that SB-271046 significantly potentiated physostigmine-induced yawning. It was also tested in two models of cognition enhancement (61). SB-271046 improved retention in the water maze test of spatial learning and memory. The compound also produced a significant improvement in performance of aged rats in an operant-delayed alternation task. These results all suggest that the 5-ht$_6$ receptor is implicated in the control of central cholinergic function and may be an interesting avenue for the treatment of cholinergic defects in cognitive dysfunctions such as Alzheimer's disease. Taken in the context of the 6-OHDA lesioning studies along with autoradiography, functional 5-ht$_6$ receptors may be on cholinergic or GABAergic interneurons in the caudate putamen or on striatal GABAergic neurons or their terminal fields in the nigra. This distribution is consistent with the proposal that 5-ht$_6$ receptors may regulate motor function and control memory and mood (37).

POTENTIAL THERAPEUTIC INDICATIONS FOR 5-HT$_6$ RECEPTORS

The distribution of the 5-ht$_6$ receptor, as well as its affinity for antipsychotic compounds, has led to significant efforts to understand its possible role in psychiatry. Mapping and lesioning studies so far indicate that there is no direct involvement of 5-ht$_6$ receptors in dopaminergic neurotransmission. However, two genetic association studies have been recently reported with respect to the 5-ht$_6$ receptor gene. The first looked at association between the 5-ht$_6$ receptor gene and schizophrenia (62) in a Japanese population. Three hundred subjects were genotyped for the biallelic variation (267C/T); half were schizophrenic and half were healthy controls. No significant difference in allele frequencies was detected between the schizophrenic patients and the healthy controls. This suggested that the 5-ht$_6$ receptor gene may not contribute directly to schizophrenia. However, a second study evaluated the relationship between the C267T polymorphism and the clinical response of schizophrenic patients, who were refractory to typical

antipsychotics and to the atypical antipsychotic compound clozapine (63). Ninety-nine chlorpromazine-resistant patients of the same ethnic Chinese background were genotyped and their response to clozapine after a minimum of eight weeks was determined. The distribution of the three possible genotypes was in Hardy-Weinberg distribution. There were no differences in baseline scores. In 60.6% of patients, the Brief Psychiatric Rating Score (BPRS) decreased by over 20% from baseline after clozapine treatment. Patients with the 267T/T genotype had a significantly better response to clozapine than the other two groups. Although the group size was small, the results were significant. The changes in general symptoms were also close to significance. This parameter reflects somatic concern, anxiety, guilt, tension, and depressed mood, i.e. the emotional control systems. These results are particularly interesting since C267T is a silent mutation that does not change the amino acid sequence. It may, however, affect parameters such as RNA stability or translational efficiency. Although a larger study is needed to confirm these observations, they may suggest that the 5-ht_6 genotype may help predict patients' responses to clozapine.

A surprising outcome of the antisense studies, confirmed by the antagonist experiments, is the role of 5-ht_6 receptors in the control of central cholinergic function (59, 61). This is also supported by localization and lesion studies. Although the antagonist data have appeared in preliminary form only, it is exciting that the 5-ht_6 antagonists may have a role in the treatment of cognitive dysfunction. Other possible avenues presently under investigation are the link to depression and anxiety (53) and the effect on body weight (54). As the new pharmacological tools become more widely available, the larger picture of 5-ht_6 receptor function will be sketched.

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