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5-Hydroxytryptamine$_2$-Family Receptors (5-Hydroxytryptamine$_{2A}$, 5-Hydroxytryptamine$_{2B}$, 5-Hydroxytryptamine$_{2C}$): Where Structure Meets Function

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ABSTRACT. 5-Hydroxytryptamine$_2$ (serotonin$_2$, 5-HT$_2$)-family receptors are important for mediating many physiological functions, including vascular and nonvascular smooth muscle contraction, platelet aggregation, modulation of perception, mood, anxiety, and feeding behavior. A large number of psychopharmaceuticals, including atypical antipsychotic drugs, antidepressants, anxiolytics, and hallucinogens, mediate their actions, at least in part, via interactions with various 5-HT$_2$-family receptors. This review article summarizes information about structure-function aspects of 5-HT$_2$-family receptors. Evidence is presented that implies that conserved aromatic and charged residues are essential for ligand binding to 5-HT$_{2A}$ receptors. Additionally, findings are reviewed that are consistent with the hypothesis that residues located in intracellular loops 2 and 3 (i2 and i3) mediate coupling to specific G$_\alpha$-subunits such as G$_{\alpha q}$. Studies are reviewed that suggest that 5-HT$_2$-family receptors may be down-regulated by both agonists and antagonists, and usually this down-regulation is due to post-transcriptional mechanisms. Finally, a model for regulation of 5-HT$_2$-family receptors by receptor-mediated endocytosis is advanced, and the particular structural features responsible for the various endocytotic pathways are emphasized. Taken together, these results suggest that discrete domains of the receptor structure are important for ligand binding, G-protein coupling, and internalization. PHARMACOL. THER 79(3):231–257, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS. Serotonin, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, molecular model, signal transduction.

CONTENTS

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ABBREVIATIONS. CCK, cholecystokinin; DMT, *N*,*N*′-dimethyltryptamine; DOI, 4-iodo-2,5-dimethoxyphenylisopropylamine; DOM, 4-methoxy-2,5-dimethoxyphenylisopropylamine; GPCR, G-protein-coupled receptor; GRK, G-protein receptor kinase; 5-HT, 5-hydroxytryptamine, serotonin; LSD, lysergic acid diethylamide; NK-1, neurokinin-1; PI, phosphatidylinositol; PKC, protein kinase C; TM, transmembrane helix.

1. INTRODUCTION

Serotonin (5-hydroxytryptamine; 5-HT) is a major neurotransmitter involved in a large number of CNS processes, including the regulation of feeding behavior, aggression, mood, perception, pain, and anxiety (Bradley *et al.*, 1986; Roth, 1994). In the periphery, 5-HT is important for regulating vascular and nonvascular smooth muscle contraction, platelet aggregation, uterine smooth muscle growth, and gastrointestinal functioning (Hoyer *et al.*, 1994; Roth, 1994). To mediate these functions, a family of receptors, divided into 7 main types comprising at least 15 distinct receptors, has evolved (Hoyer *et al.*, 1994). Not surprisingly, with so many potential targets, a large number of psychopharmaceuticals that mediate their actions, at least in part, by modulating the number, activity, or both of one or more 5-HT receptors have been developed. It is likely that these changes in receptor number and activity are involved in the therapeutic actions of many drugs used in treating various CNS disorders such as schizophrenia (Mikuni and Meltzer, 1984), depression (Blier and de Montigny, 1980; Peroutka and Snyder, 1980a,b; Barbaccia *et al.*, 1983; Chaput *et al.*, 1986), mania (Mizuta and Segawa, 1988), and anxiety (Robinson *et al.*, 1989).

This review focuses on the 5-HT$_2$ family of receptors for the following reasons. First, drugs active at 5-HT$_2$ receptors are used in the treatment of many of the aforementioned disorders, including schizophrenia (Matsubara and Meltzer, 1989; Meltzer *et al.*, 1989; Canton *et al.*, 1990; Roth *et al.*, 1992; Schmidt *et al.*, 1995) and depression (Peroutka and Snyder, 1980a,b; Marek *et al.*, 1989; Fontaine, 1993; Palvimaki *et al.*, 1996). Second, allelic variations in 5-HT$_2$-family receptors have been associated with the responses to various serotonergic drugs, including clozapine, although not all investigators have noted these associations (Arranz *et al.*, 1995; Nothen *et al.*, 1995; Malhotra *et al.*, 1996; Arranz *et al.*, 1996; Erdmann *et al.*, 1996). Third, 5-HT$_2$ receptors represent the site of action of hallucinogens such as lysergic acid diethylamide (LSD), 4-bromo-2,5-dimethoxyphenylisopropylamine, and *N*,*N*′-dimethyltryptamine (DMT) (Aghjanian *et al.*, 1968; Glennon *et al.*, 1984; Titeler *et al.*, 1988). Fourth, the large body of information relating to the structure-function properties of 5-HT$_2$-family receptors makes this receptor family a convenient model system for probing these properties.

What follows, then, is a review of the structure-activity relations important for 5-HT$_2$ family receptors. After a general introduction to the 5-HT$_2$ family of receptors, there is a discussion of the molecular mechanisms responsible for binding of ligands to, and activation of, these receptors. Site-directed mutagenesis, molecular modeling, and structural studies are discussed in detail. Studies addressing the regulation of 5-HT$_2$-family receptors by various drugs, including agonists and antagonists, are then discussed. Finally, potential mechanisms of receptor regulation are discussed and integrated into an overview of the mechanisms responsible for regulation of G-protein-coupled receptors (GPCRs) in general.

2. HISTORY

2.1. *Serotonin, Lysergic Acid Diethylamide, and Schizophrenia*

5-HT was chemically identified by Rapport and Page at the Cleveland Clinic in 1948 (Rapport *et al.*, 1948) and identified as one of the major vasoconstricting substances found in defibrinated blood. In 1954, Wooley and Shaw observed striking structural similarities between LSD and 5-HT. Based on the observations that LSD may induce hallucina-

tions and that schizophrenia is characterized in part by hallucinosis, Wooley and Shaw (1954) proposed that 5-HT might be involved in the pathogenesis of schizophrenia. Others also suggested an involvement of 5-HT in the pathogenesis of schizophrenia on the basis of these effects of LSD (Luby *et al.*, 1959, 1962), although some differences between LSD-induced hallucinations and schizophrenia were soon evident (Hollister, 1962). Because of these criticisms and the observation that most typical antipsychotics blocked dopamine rather than 5-HT receptors, interest in 5-HT as an agent in schizophrenia waned for several years. With the discovery that clozapine was an effective antipsychotic drug lacking extrapyramidal side effects (Matz *et al.*, 1974), but having antiserotonergic activity (Fink *et al.*, 1984), and that clozapine down-regulated 5-HT$_2$ receptors (Reynolds *et al.*, 1983), interest in the involvement of 5-HT in schizophrenia became more widespread. At present, a large number of atypical antipsychotic drugs (e.g., risperidone, olanzapine, sertindole, seroquel, ziprasidone, and MDL100907) with relatively high affinities for 5-HT$_2$-family receptors have been developed (for a review, see Roth and Meltzer, 1994 and Roth *et al.*, 1995b).

2.2. 5-Hydroxytryptamine$_2$-Family Subtypes

As early as 1954, it was evident that subtypes of 5-HT receptors existed. Thus, based on organ-bath studies, Gaddum's group (Gaddum and Hameed, 1954; Gaddum *et al.*, 1955; Gaddum and Picarelli, 1957) suggested that 5-HT receptors could be divided into two types (M and D), one of which, the D-site, resembled the 5-HT$_2$ receptor. In 1978, using radioligand-binding techniques, Leysen and colleagues discovered a serotonergic component of neuroleptic receptors labeled with [^{3}H]spiperone. This site was later classified as the 5-HT$_2$ site (Peroutka and Snyder, 1979), and is now known as the 5-HT$_{2A}$ receptor. The rat 5-HT$_{2A}$ receptor was cloned in 1988 by Pritchett *et al.*

Around the same time, [^{3}H]mesulergine was found to bind selectively to a distinct 5-HT receptor in the choroid plexus (Pazos *et al.*, 1984a). This receptor, which bound 5-HT with high affinity, was named the 5-HT$_{1C}$ receptor. After the receptor was cloned (Julius *et al.*, 1988), it was clear that it was closely related to the 5-HT$_2$ receptor, and thus, it was named the 5-HT$_{2C}$ receptor.

Another 5-HT$_2$-like receptor, whose pharmacology is similar, but not identical to, the 5-HT$_{2A}$ receptor, is found in stomach fundus (McKenna *et al.*, 1990; Kursar *et al.*, 1994). This receptor recently was cloned and found to be structurally related, but not identical, to the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and thus, was named the 5-HT$_{2B}$ receptor (Kursar *et al.*, 1992; Schmuck *et al.*, 1994). The primary amino acid sequences of the various cloned members of the 5-HT$_2$ family are shown in Fig. 1. A dendrogram of 5HT$_2$-sequences is shown in Fig. 2.

2.3. 5-Hydroxytryptamine$_{2A}$ Receptors

5-HT$_{2A}$ receptors are expressed in large amounts in various cortical regions (Pazos *et al.*, 1985, 1987a; Roth *et al.*, 1987), with lower levels of expression in the basal ganglia and hippocampus. In the cortex, 5-HT$_{2A}$ receptors are found mainly in pyramidal cells (Willins *et al.*, 1997b), with some of the 5-HT$_{2A}$ receptor immunoreactivity associated with parvalbumin-immunoreactive interneurons.

5-HT$_{2A}$ receptors also occur in platelets (de Chaffoy de Courcelles *et al.*, 1985), vascular smooth muscle (Cohen *et al.*, 1981; Roth *et al.*, 1984, 1986), uterine smooth muscle (Wilcox *et al.*, 1992), and other tissues (Hoyer *et al.*, 1994). In most tissues (Table 1), 5-HT$_{2A}$ receptors activate phosphoinositide hydrolysis (Berridge *et al.*, 1982; Conn and Sanders-Bush, 1984; Roth *et al.*, 1984; de Chaffoy de Courcelles *et al.*, 1985). In the brain, 5-HT$_{2A}$ receptors may also regulate neurotrophin (Vaidya *et al.*, 1997) and c-fos mRNA expression (Moorman and Leslie, 1996), although the physiological significance of these findings is still unclear. In vascular smooth muscles, 5-HT$_{2A}$ receptors activate voltage-gated calcium channels (Nakaki *et al.*, 1985) by a protein kinase C (PKC)-mediated mechanism (Roth *et al.*, 1986; Roth and Chuang, 1987; Roth, 1990). Additionally, 5-HT$_{2A}$ receptors may regulate $Na^+/K^+/Cl^-$ co-transport (Mayer and Sanders-Bush, 1994).

2.4. 5-Hydroxytryptamine$_{2B}$ Receptors

5-HT$_{2B}$ receptors are found in many organs, including stomach fundus (Wainscott *et al.*, 1996), vascular smooth muscle (Ullmer *et al.*, 1995), spinal cord (Helton *et al.*, 1994), and brain (Choi and Maroteaux, 1996), and in some cell lines (Loric *et al.*, 1995). The cloned 5-HT$_{2B}$ receptor (Kursar *et al.*, 1994; Schmuck *et al.*, 1994) shares some sequence homology with the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Fig. 1).

In addition to being coupled to intracellular calcium release (Cox and Cohen, 1996; Ullmer *et al.*, 1996), activation of the ras proto-oncogene may be involved in mediating the effects of 5-HT$_{2B}$ receptor activation (Launay *et al.*, 1996) via an unknown cellular mechanism (Table 1). 5-HT$_{2B}$ receptors have also been suggested to be involved in regulating brain development (Choi *et al.*, 1997), based on *in vitro* studies.

2.5. 5-Hydroxytryptamine$_{2C}$ Receptors

5-HT$_{2C}$ receptors, formerly named 5-HT$_{1C}$, are found in the choroid plexus (Pazos *et al.*, 1984b), where they are linked to phosphatidylinositol (PI) hydrolysis (Conn *et al.*, 1986), as well as in many other brain regions (Pazos *et al.*, 1987b), including the cortex, basal ganglia, hippocampus, and hypothalamus (Molineaux *et al.*, 1989). Even though 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors share extensive sequence similarities, some differences in signal transduction characteristics have been noted (Berg *et al.*, 1994). Thus, Berg *et al.* (1994) found that 5-HT$_{2C}$, but not 5-HT$_{2A}$, receptors were able to modulate the activity of 5-HT$_{1B}$ receptors. 5-HT$_{2C}$ receptors, like 5-HT$_{2A}$ receptors, activate c-fos expression in the

```
CONSERVED      M-----------------------------------------------------------

5H2A-MUS     1 MEILCEDNISLSSIPNSLMQLGDDSRLYPNDFNSRDANTSEASNWTIDAENRTNLSCEGY   60
5H2A-RAT     1 MEILCEDNISLSSIPNSLMQLGDGPRLYHNDFNSRDANTSEASNWTIDAENRTNLSCEGY   60
5H2A-HAM     1 MEILCEDNTSLSSIPNSLMQVDGDSGLYRNDFNSRDANSSDASNWTIDGENRTNLSFEGY   60
5H2A-HUM     1 MDILCEENTSLSSTTNSLMQLNDDTRLYSNDFNSGEANTSDAFNWTVDSENRTNLSCEGC   60
5H2A-RH      1 MDILCEENTSLSSTTNSLMQLNEDTRLYSNDFNSGEANTSDAFNWTVESENRTNLSCEGC   60
5H2A-PIG     1 MDVLCEENTSLSSPTNSFMQLNDDTRLYHNDFNSGEANTSDAFNWTVDSENRTNLSCEGC   60
5H2C-MUS     1 MVNLGTAVRSL......LVHLIG...LLVWQFD.............ISISPVAAIVTDTF   38
5H2C-RAT     1 MVNLGNAVRSL......LMHLIG...LLVWQFD.............ISISPVAAIVTDTF   38
5H2C-HUM     1 MVNLRNAVHSF......LVHLIG...LLVWQCD.............ISVSPVAAIVTDIF   38
5H2B-MUS     1 MASSYKMSE.QSTTSEHILQKTCDHLILTNRSGLETDSVAEEMKQTVEGQGHTV......   53
5H2B-RAT     1 MASSYKMSE.QSTISEHILQKTCDHLILTDRSGLKAESAAEEMKQTAENQGNTV......   53
5H2B-HUM     1 MALSYRVSELQSTIPEHILQSTFVHVISSNWSGLQTESIPEEMKQIVEEQGNKL......   54

CONSERVED      ----------------W-AL-----II-TI-GN-LVI-AV--EK-L--ATNYFLMSLA-

5H2A-MUS    61 LPPTCLSILHLQE..KNWSALLTTVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2A-RAT    61 LPPTCLSILHLQE..KNWSALLTTVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2A-HAM    61 LPPTCLSILHLQE..KNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2A-HUM    61 LSPSCLSLLHLQE..KNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2A-RH     61 LSPSCLSLLHLQE..KNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2A-PIG    61 LSPPCFSLLHLQE..KNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAI  118
5H2C-MUS    39 NSSDGGRLFQFPDGVQNWPALSIVVIIIMTIGGNILVIMAVSMEKKLHNATNYFLMSLAI   98
5H2C-RAT    39 NSSDGGRLFQFPDGVQNWPALSIVVIIIMTIGGNILVIMAVSMEKKLHNATNYFLMSLAI   98
5H2C-HUM    39 NTSDGGR.FKFPDGVQNWPALSIVIIIIMTIGGNILVIMAVSMEKKLHNATNYFLMSLAI   97
5H2B-MUS    54 ................HWAALLILAVIIPTIGGNILVILAVALEKRLQYATNYFLMSLAI   97
5H2B-RAT    54 ................HWAALLIFAVIIPTIGGNILVILAVSLEKRLQYATNYFLMSLAV   97
5H2B-HUM    55 ................HWAALLILMVIIPTIGGNTLVILAVSLEKKLQYATNYFLMSLAV   98
                                          TM I

CONSERVED      AD-L-G--VMP---L-I-----WPLP--LC--W--LDVLFSTASIMHLCAIS-DRY-A--

5H2A-MUS   119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2A-RAT   119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAIWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2A-HAM   119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2A-HUM   119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2A-RH    119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2A-PIG   119 ADMLLGFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQ  178
5H2C-MUS    99 ADMLVGLLVMPLSLLAILYDYVWPLPRYLCPVWISLDVLFSTASIMHLCAISLDRYVAVR  158
5H2C-RAT    99 ADMLVGLLVMPLSLLAILYDYVWPLPRYLCPVWISLDVLFSTASIMHLCAISLDRYVAIR  158
5H2C-HUM    98 ADMLVGLLVMPLSLLAILYDYVWPLPRYLCPVWISLDVLFSTASIMHLCAISLDRYVAIR  157
5H2B-MUS    98 ADLLVGLFVMPIALLTIMFEAIWPLPLALCPAWLFLDVLFSTASIMHLCAISLDRYIAIK  157
5H2B-RAT    98 ADLLVGLFVMPIALLTIMFEATWPLPLALCPAWLFLDVLFSTASIMHLCAISLDRYIAIK  157
5H2B-HUM    99 ADLLVGLFVMPIALLTIMFEAMWPLPLVLCPAWLFLDVLFSTASIMHLCAISVDRYIAIK  158
              TM II                                  TM III
```

(Figure 1 continues)

brain (Moorman and Leslie, 1996), although the significance of this finding is unknown. Finally, 5-HT_{2C} receptors, like 5-HT_{2A} receptors, may regulate $Na^+/K^+/Cl^-$ cotransport in fibroblasts (Mayer and Sanders-Bush, 1994) (Table 1). The 5-HT_{2C} receptor, like the 5-HT_{2A} receptor, is heavily glycosylated (Abramowski and Staufenbiel, 1995) and migrates as a 60-kDa protein.

3. STRUCTURAL REQUIREMENTS FOR LIGAND RECOGNITION AT 5-HYDROXYTRYPTAMINE$_{2A}$ RECEPTORS: AGONIST BINDING

The 5-HT_2-family receptors comprise three members: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. Theoretically, each receptor has the prototypical heptahelical structure of GPCRs in which the seven helical domains are embedded in the plasma mem-

```
CONSERVED      -P------N-R--A--KI--VW-IS-G---P-P--G------------C-L-------F

5H2A-MUS  179 NPIHHSRFNSRTKAFLKIIAVWTISVGISMPIPVFGLQDDSKVF.KEGSCLLADD...NF  234
5H2A-RAT  179 NPIHHSRFNSRTKAFLKIIAVWTISVGISMPIPVFGLQDDSKVF.KEGSCLLADD...NF  234
5H2A-HAM  179 NPIHHSRFNSRTKAFLKIIAVWTISVGVSMPIPVFGLQDDSKVF.KQGSCLLADD...NF  234
5H2A-HUM  179 NPIHHSRFNSRTKAFLKIIAVWTISVGISMPIPVFGLQDDSKVF.KEGSCLLADD...NF  234
5H2A-RH   179 NPIHHSRFNSRTKAFLKIIAVWTISVGISMPIPVFGLQDDSKVF.KEGSCLLADD...NF  234
5H2A-PIG  179 NPIHHRRFNSRTKAFLKIIAVWTISVGISMPIPVFGLQDDSKVF.KEGSCLLADD...NF  234
5H2C-MUS  159 SPVEHSRFNSRTKAIMKIAIVWAISIGVSVPIPVIGLRDESKVFVNNTTCVLNDP...NF  215
5H2C-RAT  159 NPIEHSRFNSRTKAIMKIAIVWAISIGVSVPIPVIGLRDESKVFVNNTTCVLNDP...NF  215
5H2C-HUM  158 NPIEHSRFNSRTKAIMKIAIVWAISIGVSVPIPVIGLRDEEKVFVNNTTCVLNDP...NF  214
5H2B-MUS  158 KPIQANQCNTRATAFIKITVVWLISIGIAIPVPIKGIETDV.INPHNVTCELTKDRFGSF  216
5H2B-RAT  158 KPIQANQCNSRTTAFVKITVVWLISIGIAIPVPIKGIEADV.VNAHNITCELTKDRFGSF  216
5H2B-HUM  159 KPIQANQYNSRATAFIKITVVWLISIGIAIPVPIKGIETDV.DNPNNITCVLTKERFGDF  217
                                TM IV

CONSERVED      ---GS---FF-PL-IM--TY-LTI--L--------------------------------

5H2A-MUS  235 VLIGSFVAFFIPLTIMVITYFLTIKSLQKEATLCVSDLSTRA.KLSSFSFL....PQSSL  289
5H2A-RAT  235 VLIGSFVAFFIPLTIMVITYFLTIKSLQKEATLCVSDLSTRA.KLASFSFL....PQSSL  289
5H2A-HAM  235 VLIGSFVAFFIPLTIMVITYFLTIKSLQKEATLCVSDLSTRA.KLASFSFL....PQSSL  289
5H2A-HUM  235 VLIGSFVSFFIPLTIMVITYFLTIKSLQKEATLCVSDLGTRA.KLASFSFL....PQSSL  289
5H2A-RH   235 VLIGSFVSFFIPLTIMVITYFLTIKSLQKEATLCVSDLGTRA.KLASFSFL....PQSSL  289
5H2A-PIG  235 VLIGSFVSFFIPLTIMVITYFLTIKSLQKEATLCVSDLGTRA.KLASFSFL....PQSSL  289
5H2C-MUS  216 VLIGSFVAFFIPLTIMVITYFLTIYVLRRQTLMLLRGHTEEELRNISLNFL....KCCCK  271
5H2C-RAT  216 VLIGSFVAFFIPLTIMVITYFLTIYVLRRQTLMLLRGHTEEELANMSLNFL....NCCCK  271
5H2C-HUM  215 VLIGSFVAFFIPLTIMVITYCLTIYVLRRQALMLLHGHTEEP.PGLSLDFL....KCC.K  270
5H2B-MUS  217 MVFGSLAAFFVPLTIMVVTYFLTIHTLQKKAYLVKNKPPQRLTRWTVPTVFLREDSSFSS  276
5H2B-RAT  217 MLFGSLAAFFAPLTIMIVTYFLTIHALRKKAYLVRNRPPQRLTRWTVSTVLQREDSSFSS  276
5H2B-HUM  218 MLFGSLAAFFTPLAIMIVTYFLTIHALQKKAYLVKNKPPQRLTWLTVSTVFQRDETPCSS  277
                     TM V

CONSERVED      ------------------------------------Q-I-NE--A-K-LG-VF--F--MW

5H2A-MUS  290 SSEKLFQRSIHREP....GSYAGRR.........TMQSISNEQKACKVLGIVFFLFVVMW  336
5H2A-RAT  290 SSEKLFQRSIHREP....GSYAGRR.........TMQSISNEQKACKVLGIVFFLFVVMW  336
5H2A-HAM  290 SSEKLFQRSIHREP....GSYTGRR.........TMQSISNEQKACKVLGIVFFLFVVMW  336
5H2A-HUM  290 SSEKLFQRSIHREP....GSYTGRR.........TMQSISNEQKACKVLGIVFFLFVVMW  336
5H2A-RH   290 SSEKLFQRSIHRDP....GSYTGRR.........TMQSISNEQKACKVLGIVFFLFVVMW  336
5H2A-PIG  290 SSEKLFQRSIHREP....GSY.GRR.........TMQSISNEQKACKVLGIVFFLFVVMW  335
5H2C-MUS  272 K.GDEEENAPNPNP....DQKPRR.KKKEKRPRGTMQAINNEKKASKVLGIVFFVFLIMW  325
5H2C-RAT  272 KNGGEEENAPNPNP....DQKPRR.KKKEKRPRGTMQAINNEKKASKVLGIVFFVFLIMW  326
5H2C-HUM  269 RNTAEEENSANPNQ....DQNARRRKKKERRPRGTMQAINNERKASKVLGIVFFVFLIMW  324
5H2B-MUS  277 PEKVAMLDGSHRDKILPNSSDETLMRRMSSVGKRSAQTISNEQRASKALGVVFFLFLLMW  336
5H2B-RAT  277 PEKMVMLDGSHKDKILPNSTDETLMRRMSSAGKKPAQTISNEQRASKVLGIVFLFFLLMW  336
5H2B-HUM  278 PEKVAMLDGSRKDKALPNSGDETLMRRTSTIGKKSVQTISNEQRASKVLGIVFFLFLLMW  337
                                                              TM VI
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(Figure 1 continues)

brane. For the following discussion, the numbering scheme for the rat 5-HT$_{2A}$ receptor is followed, because it is this receptor for which the most detailed analyses have been published.

3.1. *Anchoring of Polar Residues*

3.1.1. Transmembrane helix III aspartic acid (D155). Investigators have proposed many detailed molecular models of 5-HT$_2$-family receptors in which at least one of the amine moieties of 5-HT and other agonists is anchored, in part, by a highly conserved aspartic acid residue (155 or cognate residue) found in transmembrane helix III (TMIII) for the 5-HT$_{2A}$ (Hibert *et al.*, 1991; Westkaemper and Glennon, 1991; Edvardsen *et al.*, 1992; Trumpp-Kallmeyer *et al.*, 1992; Gallaher *et al.*, 1993; Kristiansen *et al.*, 1993; Wang *et al.*, 1993; Westkaemper and Glennon, 1993; Choudhary *et al.*, 1995; Weinstein and Zhang, 1995; Almaula *et al.*, 1996) and 5-HT$_{2C}$ receptors (Kristiansen and Dahl, 1996). With one exception, the various models propose that D155 anchors the terminal nitrogen of 5-HT (Fig. 3). One study

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CONSERVED      CPFFITN-----C---CN------LL--FVW-GY--S--NPL-YTLFNK--R-AF--Y--

5H2A-MUS   337 CPFFITNIMAVICKESCNENVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  396
5H2A-RAT   337 CPFFITNIMAVICKESCNENVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  396
5H2A-HAM   337 CPFFITNIMAVICKESCNEHVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  396
5H2A-HUM   337 CPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  396
5H2A-RH    337 CPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  396
5H2A-PIG   336 CPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLSSAVNPLVYTLFNKTYRSAFSRYIQ  395
5H2C-MUS   326 CPFFITNILSVLCGKACNQKLMEKLLNVFVWIGYVCSGINPLVYTLFNKIYRRAFSKYLR  385
5H2C-RAT   327 CPFFITNILSVLCGKACNQKLMEKLLNVFVWIGYVCSGINPLVYTLFNKIYRRAFSKYLR  386
5H2C-HUM   325 CPFFITNILSVLCEKSCNQKLMEKLLNVFVWIGYVCSGINPLVYTLFNKIYRRAFSNYLR  384
5H2B-MUS   337 CPFFITNLTLALC.DSCNQTTLKTLLEIFVWIGYVSSGVNPLIYTLFNKTFREAFGRYIT  395
5H2B-RAT   337 CPFFITNVTLALC.DSCNQTTLKTLLQIFVWVGYVSSGVNPLIYTLFNKTFREAFGRYIT  395
5H2B-HUM   338 CPFFITNITLVLC.DSCNQTTLQMLLEIFVWIGYVSSGVNPLVYTLFNKTFRDAFGRYIT  396
                                                 TM VII

CONSERVED      C-Y-----------------------------------------------------

5H2A-MUS   397 CQYKENRKPLQLILVNTIPTLA.........YKSSQLQVGQKKNSQEDAEP.TANDCSMV  446
5H2A-RAT   397 CQYKENRKPLQLILVNTIPALA.........YKSSQLQVGQKKNSQEDAEQ.TVDDCSMV  446
5H2A-HAM   397 CQYKENRKPLQLILVNTIPALA.........YKSSQLQAGQNKDSKEDAEP.TDNDCSMV  446
5H2A-HUM   397 CQYKENKKPLQLILVNTIPALA.........YKSSQLQMGQKKNSKQDAKT.TDNDCSMV  446
5H2A-RH    397 CQYKENKKPLQLILVNTIPALA.........YKSSQLQMGQKKNSKQDAKT.TDNDCSMV  446
5H2A-PIG   396 CQYKENKKPLQLILVNTIPALA.........YKSSQLQTGQKENSKQDDKA.TENDCTMV  445
5H2C-MUS   386 CDYKPDKKP.PVRQIPRVAATA.........LSGRELNVNIYRHTNERVVR.KANDTEPG  434
5H2C-RAT   387 CDYKPDKKP.PVRQIPRVAATA.........LSGRELNVNIYRHTNERVAR.KANDPEPG  435
5H2C-HUM   385 CNYKVEKKP.PVRQIPRVAATA.........LSGRELNVNIYRHTNEPVIE.KASDNEPG  433
5H2B-MUS   396 CNYRATKSVKALRKFSSTLCFGNSMVENSKFFTKHGIRNGINPAMYQSPMRLRCSTIQSS  455
5H2B-RAT   396 CNYQATKSVKVLRKCSSTLYFGNSMVENSKFFTKHGIRNGINPAMYQSPVRLRSSTIQSS  455
5H2B-HUM   397 CNYRATKSVKTLRKRSSKIYFRNPMAENSKFFKKHGIRNGINPAMYQSPMRLRSSTIQSS  456

CONSERVED      ----------------------S--------------------------

5H2A-MUS   447 TLGNQHSEEMCTDNIETVNEKVSCV.........................  471
5H2A-RAT   447 TLGKQQSEENCTDNIETVNEKVSCV.........................  471
5H2A-HAM   447 TLGKQQSEETCTDNINTVNEKVSCV.........................  471
5H2A-HUM   447 ALGKQHSEEASKDNSDGVNEKVSCV.........................  471
5H2A-RH    447 ALGKQHSEDASKDNSDGVNEKVSCV.........................  471
5H2A-PIG   446 ALGKQHSEDAPADNSNTVNEKVSCV.........................  470
5H2C-MUS   435 IEMQVENLELPVNPSNVVSERISSV.........................  459
5H2C-RAT   436 IEMQVENLELPVNPSNVVSERISSV.........................  460
5H2C-HUM   434 IEMQVENLELPVNPSSVVSERISSV.........................  458
5H2B-MUS   456 SIILLDTLL.TENDGDKAEEQVSYILQERAGLILREGDEQDARAPWQVQE  504
5H2B-RAT   456 SIILLNTFL.TENDGDKVEDQVSYI.........................  479
5H2B-HUM   457 SIILLDTLLLTENEGDKTEEQVSYV.........................  481
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FIGURE 1. Alignment of 5-HT$_2$-family receptor. Completely conserved residues are shown above the aligned sequences. Putative transmembrane regions are shaded. Sequences are from mouse (mus), rat, hamster (ham), human (hum), rhesus monkey (rh), and pig.

(Edvardsen *et al.*, 1992) implied that D120, in TMII, anchored the protonated terminal amine, although interactions with D155 were also noted.

Site-directed mutagenesis studies of D155 (Wang *et al.*, 1993) demonstrated that D155 was essential for optimal agonist and antagonist binding. In this study, a D155N mutant receptor was found to have lower affinity for 5-HT, 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI), ketanserin, mianserin, and spiperone, but not for LSD. The magnitude of the decrease in binding, measured in $\Delta\Delta G$ (0.8–1.5 kcal/mol) (Wang *et al.*, 1993), was much less than that predicted by molecular dynamics simulations (3–20 kcal/mol) (Edvardsen *et al.*, 1992). These mutagenesis results suggest that although D155 is essential for anchoring 5-HT and related ligands to the 5-HT$_{2A}$ receptor, its contribution is overestimated by molecular dynamics simulations.

In this regard, it is of interest to note that although Weinstein's group (Weinstein and Zhang, 1995) has predicted that LSD is at least partially anchored by D155, simulations by Westkaemper and Glennon (1993) suggest that

TABLE 1. Comparison of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ Receptors

Receptor	Signal transduction	Brain distribution	Splice variants	Other tissues
5-HT$_{2A}$	PI, c-fos, ion flux, PKC activation	Cortex > striatum > hippocampus	None identified yet	Vascular and nonvascular smooth muscle, platelets, uterus
5-HT$_{2B}$	Ras activation, Ca^{2+} flux	Possibly small amounts in multiple brain regions	None identified yet	Stomach fundus, other smooth muscles, some blood vessels
5-HT$_{2C}$	PI, ion fluxes, c-fos	Choroid plexus > hippocampus > striatum	Yes	Spinal cord

LSD and 5-HT are anchored slightly differently with respect to D155. The Westkaemper model, therefore, may explain why LSD is unaffected by the D155N mutation, whereas 5-HT shows diminished affinity for the D155N mutant receptor. It will be important for future studies to probe more carefully the effect of various types of substituents at the D155 locus (e.g., D155A, D155Q, D155E, D155N) coordinately with substitutions on the amine moiety of 5-HT. In particular, D155N mutations in conjunction with amino-carboxyl substitutions on 5-HT would be most informative. This suggestion is based on the following reasoning: If D155 anchors the charged terminal amine of 5-HT, a D155N mutation would be predicted to have high affinity for carboxylate derivatives of 5-HT and low affinity for amine derivatives. It is also worth noting that the magnitude of change (0.8–1.5 kcal/mol) is much smaller than that of other GPCRs (see, for example, Strader *et al.*, 1988), particularly in view of the fact that a change in charge (Asp→Asn) that should induce a propulsion of 5-HT *out of* the binding pocket was constructed. Taken together, these considerations suggest that residues in addition to D155 may serve to stabilize the positively charged moieties of 5-HT$_2$-family ligands.

3.1.2. Transmembrane helix III Ser (S159). Weinstein's group (Weinstein and Zhang, 1995) first proposed that at least one additional residue in TMIII (S159) helps to anchor the charged terminal amine moiety of 5-HT and related ligands. This group (Almaula *et al.*, 1996) recently has shown that mutation of this residue (S159A) caused a 17.6-fold decrease (1.2 kcal/mol) in the affinity of 5-HT and smaller changes in the affinities of *N,N′*-dimethyl-5-HT (bufotenine, 4-fold, 0.6 kcal/mol) and LSD (1.4-fold, 0.15 kcal/mol). The results were interpreted to suggest that the terminal nitrogen of 5-HT is within 1.8 Å of S159, whereas for bufotenine, this distance was proposed to be 4.7 Å and for LSD, 5 nm. These authors also suggested that the altered positioning of these three ligands provides a clue to their differential efficacies as agonists. Thus, at the native receptor, bufotenine is a partial agonist, whereas at the S159A receptor, bufotenine functions as a full agonist relative to 5-HT (Almaula *et al.*, 1996).

3.1.3. Transmembrane helix V Ala/Ser (A242S). Studies performed some years ago with [^{3}H]mesulergine (Pazos *et al.*, 1984a) indicated that there were species differences in 5-HT$_{2A}$ receptors. In these studies, Pazos *et al.* (1984a) found that [^{3}H]mesulergine bound to 5-HT$_{2A}$ receptors in rat cortex, but did not label human receptors. These results suggested that differences in the primary amino acid sequences of rat and human receptors might be important for different pharmacological properties of these two sites. After the cloning of the rat and human 5-HT$_{2A}$ receptors (Pritchett *et al.*, 1988; Saltzman *et al.*, 1991), it was noted that there were only three amino acid differences in the putative transmembrane domains (Kao *et al.*, 1992), of which only one (Ala242) is near the putative binding domain for ligands in the rat 5-HT$_{2A}$ receptor. Changing this amino acid from the human to the rat sequence (S242A mutation) markedly increased the affinity of mesulergine for the rat receptor (Kao *et al.*, 1992).

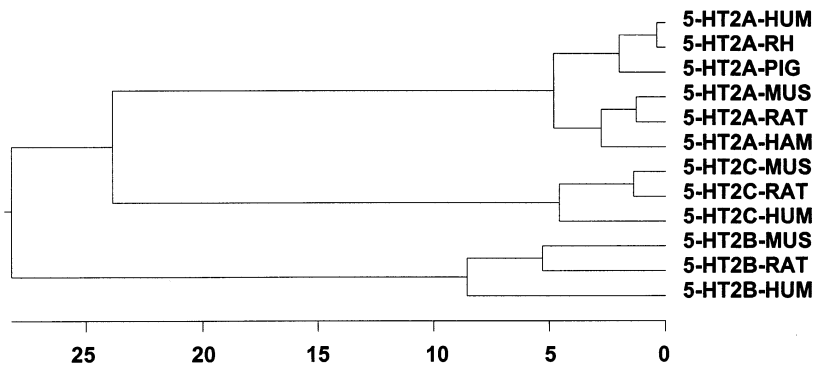

FIGURE 2. Dendrogram of 5-HT$_2$ receptors. Horizontal axis represents the percentage difference. Clustering was done using the Clustal method with PAM 250 residue weight table.

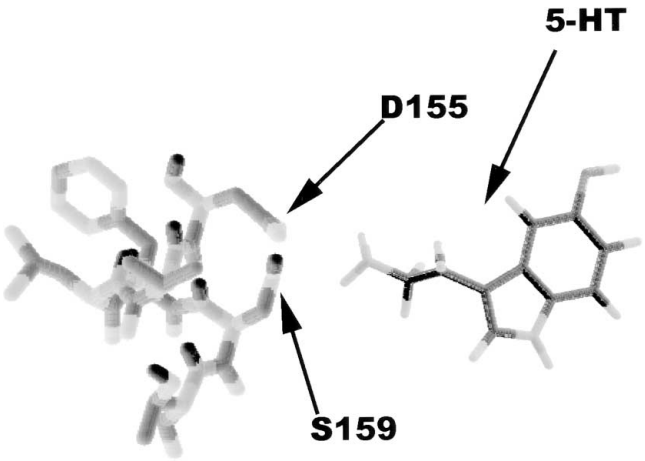

FIGURE 3. Potential roles of aspartic acid 155 (D155) and serine 159 (S159) in 5-HT binding. Shown is a model of 5-HT binding to TMIII in which the terminal amine moiety of 5-HT is anchored, in part, by D155 and S159, as suggested by mutagesis results reported in Almaula *et al.* (1996) and Wang *et al.* (1993) and by models reported by Weinstein and Zhang (1995) and Almaula *et al.* (1996).

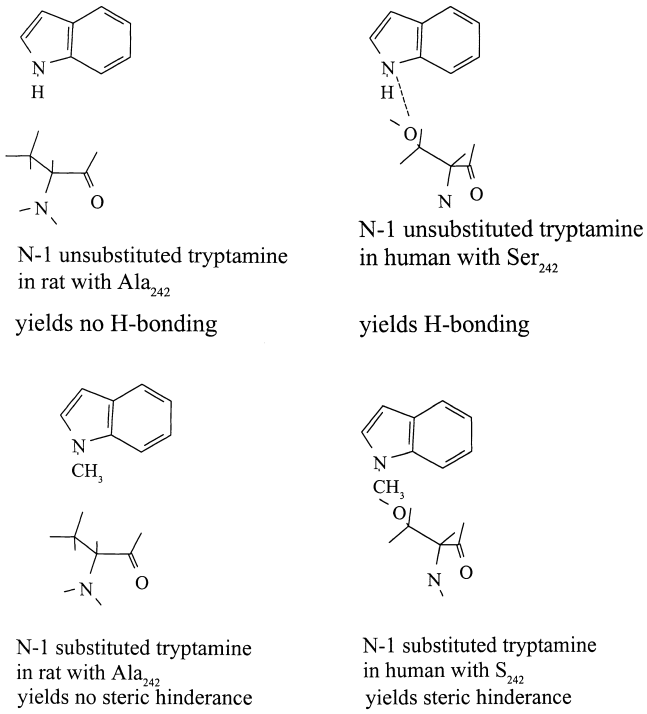

FIGURE 4. Structures of N-1-substituted and N-1-unsubstituted tryptamines and potential binding modes to rat versus human 5-HT$_{2A}$ receptors. Shown are structures of representative N-1-substituted and -unsubstituted tryptamines. According to the results of Johnson *et al.* (1994, 1995), the N-1-substituted tryptamines and ergolines have higher affinities for the rodent 5-HT$_{2A}$ receptors and lower affinities for the human 5-HT$_{2A}$ receptors because of an Ala→Ser switch in the rat versus human receptors, respectively.

Structure-activity studies (Johnson *et al.*, 1994) showed that A242 was important for the relatively higher affinity that N-1-substituted ergolines and tryptamines had for rat versus human 5-HT$_{2A}$ receptors. Based on these results, Johnson *et al.* (1994) proposed that S242 on the human receptor serves as a hydrogen-bonding member for N-1-unsubstituted tryptamines and ergolines, which have higher affinities for human as compared with rat receptors. On the other hand, S242 likely provides steric hindrance of N-1-substituted ergolines and tryptamines and makes their affinities lower for the human versus the rat receptor. Thus, N-1-substituted ergolines and tryptamines are more readily accommodated in the rat receptor, which has an alanine at position 242 (see Fig. 4 for structures of N-1-substituted ergolines and tryptamines).

3.1.4. Other residues involved in anchoring charged groups. Other residues have been identified by molecular modeling studies that could be involved in anchoring charged groups of 5-HT and other agonists, including Tyr370 (Y370) (Westkaemper and Glennon, 1993), which may interact with D155, and Trp151 (W151), which could act to stabilize the positively charged amine moiety of 5-HT and other ligands (Roth *et al.*, 1997b). For the 5-HT$_{2C}$ receptor, Kristiansen and Dahl (1996) have proposed an interaction of the indole nitrogen with S133 (Helix II).

3.2. *Anchoring of Aromatic Moieties*

A fundamental feature of all 5-HT$_{2A}$ agonists is the presence of an aromatic moiety. Thus, all classes of agonists (see Fig. 5), including indoles, phenylisopropylamines, ergolines, and phenylpiperazines, are characterized by aromatic residues that are nearly equidistant from a charged amine. Many molecular models have proposed that at least one aromatic residue is involved in stabilizing the binding of aromatic moieties of 5-HT and related agonists to 5-HT$_{2A}$ (Hibert *et al.*, 1991; Trumpp-Kallmeyer *et al.*, 1992; Edvardsen *et al.*, 1992; Choudhary *et al.*, 1993; Kristiansen *et al.*, 1993; Moereels and Janssen, 1993; Westkaemper and Glennon, 1993; Zhang and Weinstein, 1993; Holtje and Jendretzki, 1995; Weinstein and Zhang, 1995; Roth *et al.*, 1997b) and 5-HT$_{2C}$ (Kristiansen and Dahl, 1996) receptors.

3.2.1. Conserved phenylalanines involved in agonist binding. Hibert *et al.* (1991) initially proposed that several highly conserved phenylalanines were involved in stabilizing the binding of the aromatic ring of 5-HT and related compounds to 5-HT$_{2A}$ receptors. The proposed phenylalanines included Phe243 (F243) in TMV and Phe340 (F340) in TMVI. Others have proposed that Phe240 (F240), F243, and F244 (all in TMV) alone, or in combination, may anchor the aromatic portion of 5-HT$_{2A}$ (Edvardsen *et al.*, 1992; Moereels and Janssen, 1993; Westkaemper and Glennon, 1993; Zhang and Weinstein, 1993; Weinstein and Zhang, 1995) and 5-HT$_{2C}$ agonists (Kristiansen and Dahl, 1996). At present, no direct experimental evidence either refutes or supports the involvement of TMV phenylalanines in agonist binding.

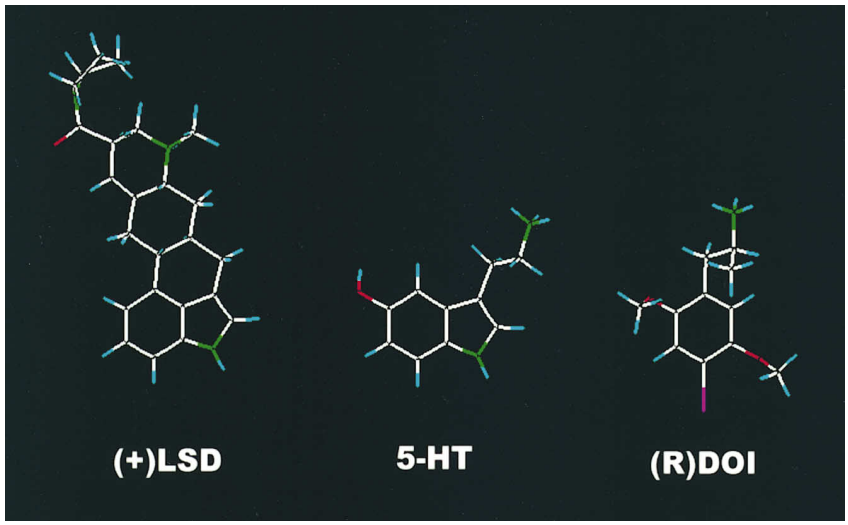

FIGURE 5. Structures of representative 5-HT$_2$-family agonists. Shown are representative energy-minimized structures for LSD, 5-HT, and DOI.

In addition to the TMV phenylalanines, Phe339 (F339) and Phe340 (F340) in TMVI have also been implicated in agonist binding by molecular modeling studies of 5-HT$_{2A}$ (Hibert *et al.*, 1991; Edvardsen *et al.*, 1992; Trumpp-Kallmeyer *et al.*, 1992; Moereels and Janssen, 1993; Westkaemper and Glennon, 1993; Zhang and Weinstein, 1993; Weinstein and Zhang, 1995) and 5-HT$_{2C}$ (Kristiansen and Dahl, 1996) receptors. Considerable mutagenesis evidence supports the hypothesis that F340 is involved in agonist binding (Choudhary *et al.*, 1993; Roth *et al.*, 1995a, 1997a,b).

In initial studies, Choudhary *et al.* (1993) found that mutation of F340 (F340L), but not F339 (F339L), dramatically decreased the affinities and efficacies of several agonists, including DOI and 5-HT, for the 5-HT$_{2A}$ receptor. Later studies examined the effect of this mutation on agonist binding to high- and low-affinity states of the 5-HT$_{2A}$ receptor (Roth *et al.*, 1997a). The F340L mutation caused decreases in affinity for several tested agonists at both the high- (1.8- to 6600-fold, 0.25–3.8 kcal/mol) and low- (18- to 255-fold, 1.3–2.4 kcal/mol) affinity states of the 5-HT$_{2A}$ receptor.

The F340L mutation also induced large decreases in efficacies (E_{max} values) and potencies (K_{act} values) for activating PI hydrolysis when compared with the native receptor. Several agonists (quipazine, α-methyl-5-HT, 5-O-methyl-DMT, and DMT) had negligible efficacies at the F340L mutant receptor, but were full or partial agonists at the native receptor. Potencies for activating PI hydrolysis were diminished by 116- to 542-fold (Roth *et al.*, 1997a). These results suggested that F340 is essential for agonist binding and efficacy. Mutations of the adjacent phenylalanine (F339L) or unrelated phenylalanines (F125L) did not uniformly affect agonist binding or efficacy (Choudhary *et al.*, 1993; Roth *et al.*, 1997a). Taken together, these results suggest that F340 is involved in agonist binding, whereas nearby phenylalanines (F339, F125) are not.

Baldwin (1993, 1994) suggested that another phenylalanine found in TMVII (F365) lies near the binding pocket of biogenic amine receptors. Mutation of F365 (F365L) diminished agonist efficacy by 44–70%, but had no effect on agonist binding affinity (Roth *et al.*, 1997b). These results suggest that F365 is not directly involved in agonist binding, but may be involved in signal transduction.

3.2.2. Conserved tryptophan residues essential for agonist binding and efficacy. In addition to phenylalanines, a number of tryptophans have also been predicted to be involved in the binding of agonists to 5-HT$_{2A}$ (Hibert *et al.*, 1991; Edvardsen *et al.*, 1992; Trumpp-Kallmeyer *et al.*, 1992; Kristiansen *et al.*, 1993; Zhang and Weinstein, 1993; Holtje and Jendretzki, 1995; Weinstein and Zhang, 1995; Roth *et al.*, 1997b) and 5-HT$_{2C}$ (Kristiansen and Dahl, 1996) receptors. Most explicit models suggesting involvement of specific tryptophans have implicated Trp336 (TMVI) (Hibert *et al.*, 1991), Trp151 (TMIII), Trp200 (TMIV), and Trp367 (TMVII) (Edvardsen *et al.*, 1992; Weinstein and Zhang, 1995) for 5-HT$_{2A}$ receptors. For 5-HT$_{2C}$ receptors, Kristiansen and Dahl (1996) did not specifically predict that any of these tryptamines were involved in agonist recognition. None of these models has explicitly identified the contribution of the various tryptophan residues for agonist recognition, although it appears that these residues are generally situated in regions that stabilize aromatic or aliphatic portions of 5-HT and related ligands.

Roth and colleagues recently have tested these models by constructing a number of mutations of various tryptophans, including Trp76 (W76A; TMI), Trp200 (W200A, TMIV), Trp336 (W336A; TMVI), and Trp367 (W367A; TMVII). With the exception of the W76A mutation, mutation of these conserved tryptophan residues drastically diminished agonist affinities and efficacies at 5-HT$_{2A}$ receptors (Roth *et al.*, 1997b). One previous study (Weinstein and Zhang, 1995) explicitly predicted that W151 (TMIII) and W200 would be involved in 4-methoxy-2,5-dimethoxyphenylisopropylamine (DOM), but not 5-HT, binding and that W367 would be involved in 5-HT, but not DOM, binding.

Some support for this model comes from the Roth study (Roth *et al.*, 1997b), which found that the W200A mutation diminished DOM binding. All the tested mutations (W200A, W336A, W367A) however, greatly diminished agonist potency and efficacy for PI hydrolysis for both DOM and 5-HT (Roth *et al.*, 1997b), a result suggesting that the effect of the tryptophan mutations may be generalized rather than agonist specific. Taken together, these results demonstrate that highly conserved tryptophan residues are essential for agonist binding and efficacy at 5-HT_{2A} receptors, although the exact mechanism by which this occurs remains unknown.

3.2.3. Role of conserved tyrosines for agonist binding and efficacy. Two independently derived molecular models have predicted that a highly conserved tyrosine (Y370; TMVII) has important, but mechanistically distinct, roles in agonist binding (Westkaemper and Glennon, 1993; Weinstein and Zhang, 1995). Thus, Weinstein's group (Weinstein and Zhang, 1995) has proposed a direct role for Y370 in DOM binding, whereas Westkaemper has suggested that Y370 actually stabilizes the negative charge from Asp155 (D155) (Westkaemper and Glennon, 1993). On the basis of theoretical proximity, Roth *et al.* (1997b) have suggested that Y370 interacts with Asn92 (TMII). A mutation of Y370 (Y370A) yielded a receptor with greatly diminished affinity for 5-HT and DOM, but not for α-methyl-5-HT or bufotenine (Roth *et al.*, 1997b). In all cases though, the Y370A mutant had greatly decreased efficacies and potencies for activating PI hydrolysis when compared with the native receptor (Roth *et al.*, 1997b). It is not now possible to determine whether Y370 is involved in helping to anchor the charged amine moiety, as suggested by Westkaemper and Glennon (1993) or if Y370 is involved in an aromatic-aromatic type interaction, as was seen for F340. Future studies in which Y370F mutations are constructed will help to clarify whether a strictly aromatic moiety is needed or whether the para-hydroxyl group of tyrosine assists in ligand binding.

3.3. *Nonconserved Residues Involved in Subtype-Selective Agonist Binding*

In addition to conserved residues involved in anchoring agonists to 5-HT_2-family receptors, there must also be residues that are involved in subtype-specific differences. Thus, for instance, the 5-HT_{2A} receptor has relatively low affinity for MK-212 and 5-HT, whereas the 5-HT_{2C} receptor has higher affinity for these agonists (Roth *et al.*, 1992). Very few studies have been concerned with subtype selectivity of agonist binding at 5-HT_2-family receptors. Dahl's group has performed dynamics simulations with 5-HT at both 5-HT_{2A} (Edvardsen *et al.*, 1992) and 5-HT_{2C} (Kristiansen and Dahl, 1996) receptors, although no predictions were made about the higher affinity of 5-HT for the 5-HT_{2C} receptor than for the 5-HT_{2A} receptor.

Chimeric receptor studies (Choudhary *et al.*, 1992) have demonstrated that residues located in several helices are essential for the preferential agonist affinity seen for 5-HT_{2C} receptors. In these studies, Choudhary *et al.* (1992) found that helices VI and VII were most important for defining agonist selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors. Limited 5-HT_{2A}→5-HT_{2C} mutagenesis studies have been carried out (Roth *et al.*, 1993). In these studies (Roth *et al.*, 1993), the following 5-HT_{2A}→5-HT_{2C} mutations were constructed: T81I and T82I in TMII and F125L, M132L, and T134A in TMIII. None of these mutations significantly affected agonist or antagonist affinities.

4. STRUCTURAL REQUIREMENTS FOR LIGAND RECOGNITION AT 5-HYDROXYTRYPTAMINE$_{2A}$ RECEPTORS: RECEPTOR-G-PROTEIN COUPLING

4.1. *Direct G-Protein Coupling*

Several studies have attempted to identify the region(s) of the 5-HT_{2A} and 5-HT_{2C} receptors essential for coupling to $G_{\alpha q}$-family G-proteins. By constructing chimeric 5-$HT_{1B/2A}$ receptors, Oksenberg *et al.* (1995) found that the third intracellular loop (i3) was important for $G_{\alpha q}$ coupling. These authors were able to change the G-protein specificity of the 5-HT_{2A} receptor from activation of PI hydrolysis to inhibition of adenylate cyclase activity by swapping i3 of the 5-HT_{2A} receptor for i3 of the 5-HT_{1B} receptor.

Studies with other GPCRs have implied that amphipathic α-helices are involved in receptor-G-protein interactions (Hamm *et al.*, 1988; Duerson and Clapham, 1993). Experiments with the guinea pig 5-HT_{2A} receptor (Watts *et al.*, 1994) imply that a putative α-helix may not be involved in 5-HT_{2A}-$G_{\alpha q}$ coupling. In these studies, Watts *et al.* (1994) noted that the guinea pig 5-HT_{2A} receptor has tandem substitutions that could disrupt a potential α-helix. Despite this potential disruption of a putative α-helical region, the guinea pig receptor was coupled to PI hydrolysis. Direct structural studies are necessary to determine whether this region of the guinea pig receptor actually is devoid of helical structure, and is involved in G-protein coupling. In this regard, recent studies by Hyde *et al.* (1997) suggest that the 5-HT_{2A} receptor interacts with $G_{\alpha q}$ via the carboxyl terminus of i3. In these studies, Hyde *et al.* (1997) reported that peptides corresponding to the carboxyl terminus of i3 may also directly activate purified $G_{\alpha q}$. These results suggest that the 5-HT_{2A} receptor and $G_{\alpha q}$ may interact via the carboxyl terminus of the i3 loop.

4.2. *Constitutively Active 5-Hydroxytryptamine$_2$-Family Receptors*

Sanders-Bush's group was the first to demonstrate that 5-HT_{2C} receptors expressed in the choroid plexus may have intrinsic constitutive activity (Barker *et al.*, 1994; Westphal and Sanders-Bush, 1994; Westphal *et al.*, 1995). Others (Hartman and Northup, 1996) found that if $G_{\alpha q}$ was co-

expressed with 5-HT$_{2C}$ receptors, the 5-HT$_{2C}$ receptor displayed constitutive activation in COS-7 cells. In these studies, 5-HT$_{2C}$ receptors expressed without $G_{\alpha q}$ did not show constitutive activity (Hartman and Northup, 1996). On the other hand, Labrecque *et al.* (1995) found that 5-HT$_{2C}$ receptors, when overexpressed in Sf-9 cells without co-expression of $G_{\alpha q}$, also display constitutive activation. These results suggest that the cellular milieu in which 5-HT$_{2C}$ receptors are expressed modifies the extent to which they display constitutive activity.

An explanation for constitutive activity of GPCRs comes from recently proposed thermodynamic models of receptor-G-protein interactions (Lefkowitz *et al.*, 1993). According to this modified ternary complex model of receptor-G-protein interaction (Fig. 6), GPCRs may spontaneously interact with G-proteins and induce GTP hydrolysis and activation of second messenger systems. In heterologous expression systems in which receptor levels may be artificially increased, the statistical probability of this reaction occurring is increased (see Fig. 6). This modified model also proposes that some antagonists may behave as inverse agonists (e.g., antagonists with negative intrinsic activities), whereas others act as neutral antagonists.

In this regard, Barker *et al.* (1994) showed that several antagonists, such as clozapine, mianserin, and ritanserin, had negative intrinsic activity and decreased basal PI hydrolysis in the absence of agonists. Other antagonists (e.g., mesulergine, Br-LSD) had neutral antagonist activity and were able to inhibit agonist-induced stimulation of PI hydrolysis, but had no effect on basal activity. Interestingly, constitutively active 5-HT$_{2C}$ receptors showed a higher basal level of phosphorylation, which was diminished by incubation with antagonists with negative intrinsic activity (Westphal *et al.*, 1995). The ability of an antagonist to induce down-regulation of 5-HT$_{2C}$ receptors and its negative intrinsic activity in Sf-9 cells were not correlated (Labrecque *et al.*, 1995). These results suggest that the ability of an antagonist to down-regulate 5-HT$_{2C}$ receptors is not related to its intrinsic activity, but merely to the presence of antagonist activity.

More recent studies by Teitler's group (Casey *et al.*, 1996; Herrick-Davis *et al.*, 1997) have examined regions of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors responsible for constitutive activity targeting residues previously implicated at α_1-adrenergic receptors (Kjelsberg *et al.*, 1992). These researchers found that various mutations of cognate residues in both the 5-HT$_{2A}$ (Cys322) and 5-HT$_{2C}$ (S312) receptors at the carboxyl terminus of i3 caused both receptors to have greater amounts of constitutive activity when compared with native receptors. These results correlate well with Hyde's results (Hyde *et al.*, 1997) that the i3 loop of the 5-HT$_{2A}$ receptor interacts with $G_{\alpha q}$ via the carboxyl terminus of i3 . In agreement with the findings of Barker *et al.* (1994) and Westphal and Sanders-Bush (1994), several antagonists can be classified as having negative intrinsic activity and others as neutral antagonists at the mutant constitutively active receptors.

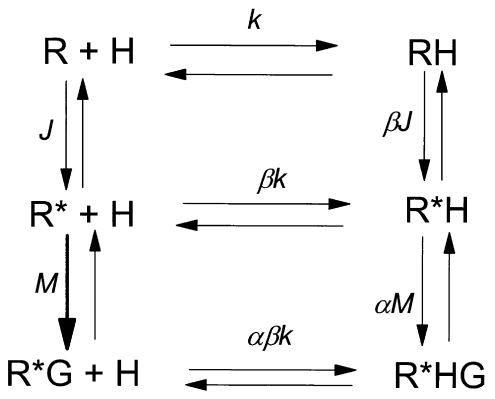

FIGURE 6. Shown is a modified ternary complex model in which H = ligand, R = receptor, and G = G-protein, as modified from Roth *et al.* (1997a), Lefkowitz *et al.* (1993), and Samama *et al.* (1994). According to this model, inverse agonists (antagonists with negative intrinsic activity) promote the inactive state of the receptor (HR), whereas constitutively active mutants promote the spontaneous association of R* with G.

4.3. *Mutations of 5-Hydroxytryptamine$_2$-Family Receptors That Alter G-Protein Coupling: Evidence for Allosteric Activation of $G_{\alpha q}$*

In addition to sites important for agonist binding that may be expected to directly modify the ability of agonists to activate PI hydrolysis, there are other sites that presumably are some distance from the ligand-binding domain and that allosterically modify agonist efficacy.

Shih's group was the first to identify such a site for 5-HT receptors: Asp120 (D120), which is located in TMII (Wang *et al.*, 1993). Previous work by Limbird's group (Horstman *et al.*, 1990) had implicated this site as one for allosteric regulation of GPCRs in general. In the Wang *et al.* (1993) studies, a D120N mutation yielded a receptor devoid of agonist efficacy. Agonist-binding affinity was decreased by 7- to 13-fold; this alteration in binding affinity is less than one would predict if Asp120 were directly involved in ligand binding

Studies by Sealfon *et al.* (1995) suggested that the role of D120 was to stabilize a helix-helix interaction between TMII and TMVII. In these studies, Sealfon *et al.* (1995) demonstrated that mutation of an asparagine (Asn) in TM-VII to aspartic acid (Asn→Asp) was able to rescue the deficient phenotype of the D120N mutation. Thus, the inability of the D120N mutation to activate PI hydrolysis was reversed by the double mutation. These results suggest that TMII and TMVII interact and that this interaction is essential for signal transduction of the 5-HT$_{2A}$ receptors, perhaps by facilitating helical movements (Sealfon *et al.*, 1995), as the same group had suggested earlier for the gonadotropin-releasing hormone receptor (Zhou *et al.*, 1994).

Investigators have also identified other sites that may have allosteric effects on receptor function. Thus, Burns *et*

al. (1997) recently reported that RNA editing occurs in regions of intracellular loop 2 (i2) to yield several isoforms of the 5-HT_{2C} receptor. In the most common isoform (47% of total receptor in whole brain), the tripeptide sequence valine-asparagine-valine (VNV) exists. Other isoforms are present at lesser amounts: VNI (18% in whole brain), VSV (11% in whole brain), VSI (10% in whole brain), and INV, ISV, and INI (1–5% each in whole brain) (Burns *et al.*, 1997). Interestingly, the VSV isoform was somewhat defective in inducing second messenger production compared with the INI isoform (Burns *et al.*, 1997). These results imply that in addition to i3, the i2 loop is important for $G_{\alpha q}$ coupling and that mutations of this region may affect receptor-mediated signal transduction. In addition to RNA editing, isoforms of the 5-HT_{2C} receptor splicing variants that alter signal transduction have also been found (Canton *et al.*, 1996).

The location of the various sites that modify agonist binding and efficacy are shown in Fig. 7. Taken together, these results indicate that a central core of residues is important for direct agonist binding and that peripheral residues presumably are involved in receptor-effector coupling.

5. MECHANISMS OF ANTAGONIST BINDING TO 5-HYDROXYTRYPTAMINE$_2$-FAMILY RECEPTORS

5.1. *Molecular Modeling and Mutagenesis of Ketanserin-like Antagonists to the 5-Hydroxytryptamine$_{2A}$ and 5-Hydroxytryptamine$_{2C}$ Receptors*

Dahl's group has performed the most extensive modeling studies of 5-HT_{2A} and 5-HT_{2C} receptors, by using both bacteriorhodopsin (Edvardsen *et al.*, 1992; Kristiansen *et al.*, 1993) and rhodopsin-based (Kristiansen and Dahl, 1996) models. In initial studies (Edvardsen *et al.*, 1992), they predicted that ritanserin, a 5-$HT_{2A/2C}$ antagonist, interacted with a large number of conserved (D120, D155, W151, W200, F240, F243, W336, F339, F340) and nonconserved (Leu123, Ser131, Ile135) residues located in various helices. Later studies (Kristiansen *et al.*, 1993) performed with ketanserin identified many of the same residues, but, interestingly, suggested that F339, and not F340, interacted with ketanserin. As well, much attention was paid to potential interactions with highly conserved tryptophans (W151, W200, W336). These initial studies were performed with

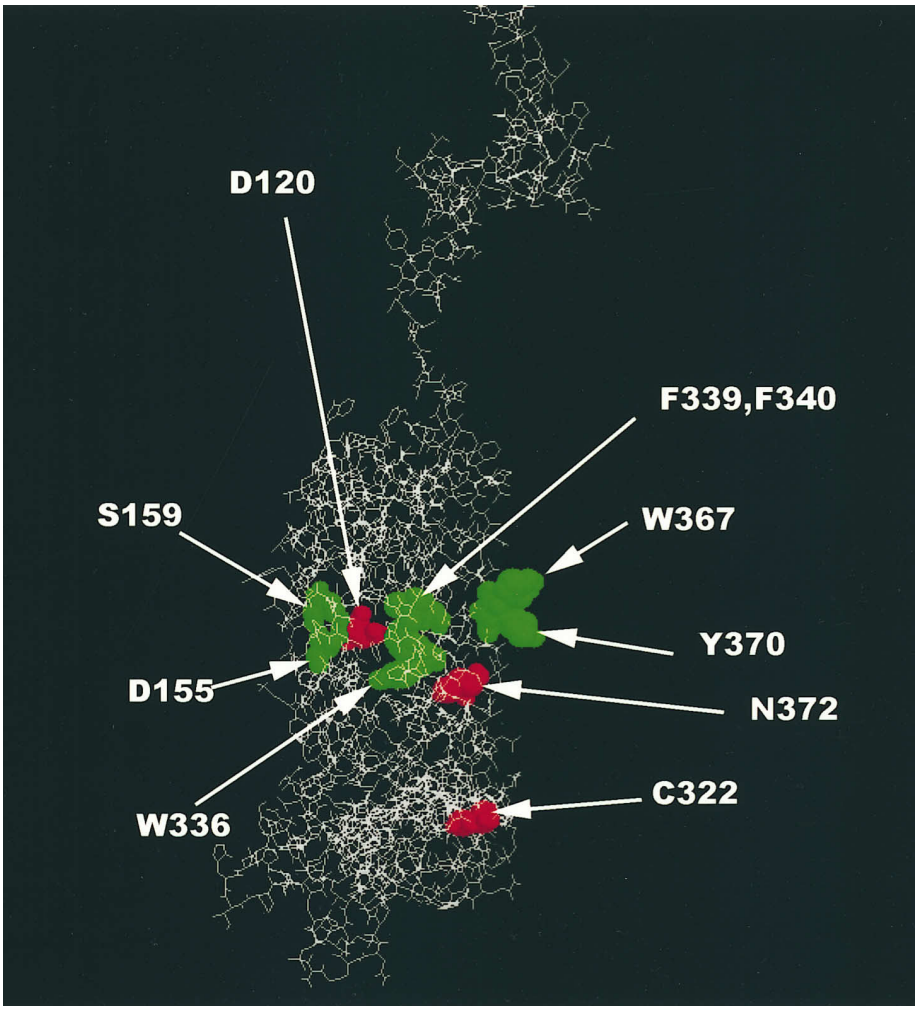

FIGURE 7. Residues essential for agonist binding and efficacy at 5-HT_{2A} receptors. Shown in green are residues identified by site-directed mutagenesis studies that appear to be directly involved in agonist binding (Choudhary *et al.*, 1993; Wang *et al.*, 1993; Almaula *et al.*, 1996; Roth *et al.*, 1997b) and those in red, studies that have been suggested to modulate agonist binding and efficacy by allosteric effects (Wang *et al.*, 1993; Sealfon *et al.*, 1995).

bacteriorhodopsin templates, which are likely to yield models with somewhat different helical topologies and orientations when compared with rhodopsin-based templates (for a review, see Baldwin, 1994) because recent high-resolution studies of rhodopsin (Schertler and Hargrave, 1995) indicate that its helical topology and orientation is different from that of bacteriorhodopsin.

More recent studies of the 5-HT$_{2C}$ receptor (Kristiansen and Dahl, 1996) used a rhodopsin-based template. Again, the residues identified for antagonist binding included cognates to D155, W151, W336, F339, F340, and F365. Because this group now had carried out simulations of ketanserin with the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, and because ketanserin has 30-fold differences in affinities for each receptor, they were able to predict residues essential for binding selectivity.

Earlier studies by Pierce *et al.* (1992) suggested that the p-fluorobenzoyl moiety of ketanserin and related compounds docks near the extracellular ends of the transmembrane helices. Their hypothesis suggests that the benzoyl carbonyl in ketanserin-like compounds may interact with Ser336 or Lys350 of the 5-HT$_{2C}$ receptor. The corresponding residues of the 5-HT$_{2A}$ receptor are Ala365 (for Ser336) and Ala (for Lys360). Alternatively, Kristiansen and Dahl (1996) suggested that Cys362 of the rat 5-HT$_{2C}$ receptor, which corresponds to Ser372 of the 5-HT$_{2A}$ receptor, is important for subtype selectivity. Additionally, Ser139 of TMIII, which is conserved among the 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_{2B}$ receptors, was proposed by Kristiansen and Dahl to be important for anchoring the p-fluorobenzoyl moiety of ketanserin. Alternative models have suggested that the benzoyl moiety forms a hydrogen bond with the 5-HT$_{2A}$ receptor (for example, see Andersen *et al.*, 1994).

Extensive site-directed mutagenesis studies of the 5-HT$_{2A}$ receptor have evaluated the effects of these mutations on binding of ketanserin (Choudhary *et al.*, 1993; Wang *et al.*, 1993; Choudhary *et al.*, 1995; Roth *et al.*, 1997b). Several amino acids have been identified as essential for ketanserin binding, including Trp76 (TMI) (Roth *et al.*, 1997b), Asp155 (Wang *et al.*, 1993), Trp336 (Roth *et al.*, 1997b), Phe339 (Choudhary *et al.*, 1993, 1995), Trp367 (Roth *et al.*, 1997b), and Tyr370 (Roth *et al.*, 1997b). Interestingly, although the F340A and F340L mutations did not affect the affinity of ketanserin for the 5-HT$_{2A}$ receptor, the F340Y mutation substantially diminished ketanserin's affinity (Choudhary *et al.*, 1995). These results suggest that although F340 is not in direct contact with ketanserin, it is located close to ketanserin's binding site because the F340Y mutation introduces a potentially bulky hydroxyl group that may sterically force ketanserin out of the binding pocket.

In general, there is fairly good agreement when comparisons are made between molecular modeling studies of ketanserin binding and site-directed mutagenesis studies (see Table 2). Thus, Kristiansen and colleagues (Kristiansen *et al.*, 1993; Kristiansen and Dahl, 1996) correctly identified many of the residues essential for ketanserin binding. Unfortunately, no studies have been performed to test their predictions for subtype-selective binding of ketanserin and related compounds (Kristiansen and Dahl, 1996). Further studies on structure-activity relations between ketanserin analogues and 5-HT$_{2A}$ receptors are likely to yield interesting information.

5.2. *Molecular Modeling of Ergoline and Ergopeptines to 5-Hydroxytryptamine$_{2A}$ Receptors*

A large number of investigators have also attempted to model interactions of ergolines and ergopeptines to 5-HT$_2$-family receptors. Westkaemper and Glennon (1993) modeled the binding of LSD to the 5-HT$_{2A}$ receptor and suggested that the diethyl amide side chain is surrounded by

TABLE 2. Comparison of Binding Models of Ketanserin with Site-Directed Mutagenesis Results

Residue	Kristiansen *et al.*, 1993	Holtje and Jendretzki, 1995	Mutagenesis data (Roth *et al.*, 1993, 1997b; Wang *et al.*, 1993; Choudhary *et al.*, 1993, 1995; Almaula *et al.*, 1996)
Thr88	+		−
Ile89	+		−
Gly91	+		
Asn92	+		
Val95	+		
Asp120	+		−
Leu123	+		
Gly124	+		
Val127	+		
Met128	+		
Ser131	+		
Trp151	+	+	
Asp155	+	+	+
Ser159	+	+	−
Trp200	+		+
Ser203	+		
Ile206	+	+	
Ser207	+		
Ile210	+		
Ser239	+	+	
Phe240	+		
Phe243	+		
Phe244	+		
Leu247	+		
Met250	+		
Met335	+		
Trp336	+	+	+
Phe339	+		+
Phe340	−	+	−
Ala343	−	+	
Asn343	+		
Leu362	+		
Phe365	+	−	
Trp367	−		+
Tyr370	−		+

Shown are results from molecular modeling studies that have specifically predicted residues essential for ketanserin binding to 5-HT$_{2A}$ receptor, as well as mutagenesis studies that have determined residues essential for ketanserin binding.

several amino acids, including Cys148 (TMIII), Ile215 (TMIV), Ile345 (TMVI), and Ile358 (TMVII). They also proposed that the Ser207 (TMIV) OH is directed toward the indole nitrogen so that LSD is slightly shifted in the binding pocket when compared with 5-HT.

Weinstein and Zhang (1995) also modeled the binding of LSD to the 5-HT_{2A} receptor and identified the following residues as being involved in anchoring LSD to the receptor: Asp155, Phe158, Ser159, Ile163 (TMIII), Ile197, Trp200, Thr201, Val204, Met208 (TMIV), Ser239, Phe240, Phe243, Phe244 (TMV), Ile368, Leu371, Ser372, Ser373, and Asn376 (TMVII). Thus, the two extant molecular models describing LSD's binding to the 5-HT_{2A} receptor differ considerably with respect to the individual amino acids predicted to be involved in binding. Studies using mutants only have shown that Ser159 is not involved in anchoring LSD to the 5-HT_{2A} receptor (Almaula *et al.*, 1996).

Choudhary *et al.* (1993, 1995) examined the effects of point mutations of conserved and nonconserved phenylalanine residues on ergoline and ergopeptine binding. Three amino acids were targeted: Phe125 (TMII), Phe339 (TMVI), and Phe340 (TMVI), and both conservative (Phe→Tyr) and nonconservative (Phe→Ala, Phe→Leu) mutations were constructed. These authors found that the F340L and F340A mutations drastically diminished the affinities of several ergolines for the 5-HT_{2A} receptor, including mesulergine, LY53857, lisuride, amersergide, ergonovine, lergotrile, methysergide, and metergoline. By contrast, the F340L and F340A mutations had minimal effects on ergopeptine binding. In this study, the binding of several ergopeptines (bromocryptine, ergotamine, ergocryptine, and ergocornine) was evaluated at receptors bearing the F340L and F340A mutations, and Choudhary *et al.* (1995) found that their affinities were minimally affected by these mutations.

Additionally, Choudhary *et al.* (1995) examined the effect of the F340Y mutation on the binding of ergolines and ergopeptines. These researchers showed that the F340Y mutation had a minimal effect on ergoline and ergopeptine binding. The data were then analyzed with the aid of a molecular model of the 5-HT_{2A} receptor by using a bacteriorhodopsin template. Choudhary *et al.* (1995) suggested that the bulky peptide portion of the ergopeptines prevents them from binding far enough into the binding pocket to interact with F340. These results suggest that the nature of the substituent at the 8-position of the ergoline nucleus gives rise to differing modes of binding for two classes of structurally similar molecules.

6. UNANSWERED ISSUES REGARDING MECHANISMS OF LIGAND BINDING TO 5-HYDROXYTRYPTAMINE$_2$-FAMILY RECEPTORS

At present, all the available structure-function studies of 5-HT_2-family receptors have used molecular modeling, site-directed mutagenesis, analogue binding, or some combination of these approaches. Currently, no direct studies have determined how ligands bind to 5-HT_2 receptors. In this regard, with the exception of rhodopsin, there is no structural information about the helical packing of 5-HT_2-family receptors or other members of the GPCR superfamily. In the future, as technological advances make the routine production of large quantities of pure receptor protein available, direct structural studies will be possible. Until that time, the available studies must be viewed with caution, because it is possible that mutations can exert their effects on receptor function by indirect effects (for discussion, see Fersht *et al.*, 1987; Fersht, 1988; Serrano *et al.*, 1991).

7. REGULATION OF 5-HYDROXYTRYPTAMINE$_{2A}$ RECEPTORS

7.1. *5-Hydroxytryptamine$_{2A}$ Receptors Are Down-Regulated and Desensitized by Agonists* in Vitro *and* in Vivo

As is the case for the majority of GPCRs, 5-HT_{2A} receptors can be down-regulated after exposure to an agonist. Thus, for instance, daily LSD administration down-regulates 5-HT_{2A} receptors (Buckholtz *et al.*, 1985, 1988) *in vivo*, as does DOI administration (Buckholtz *et al.*, 1988; McKenna *et al.*, 1989; Pranzatelli, 1991).

Similar results have been found for some, but not all, *in vitro* model systems. Thus, for instance, Leysen and co-workers found that DOM caused a rapid down-regulation and desensitization of 5-HT_{2A} receptors in smooth muscle cells *in vitro* (Leysen *et al.*, 1989; Pauwels *et al.*, 1990; Leysen and Pauwels, 1990). Similar results were found by Ivins and co-workers in P11 cells (Ivins and Molinoff, 1991; Ferry *et al.*, 1993). The down-regulation of 5-HT_{2A} receptors in P11 cells is due to a PKC-mediated alteration in mRNA stability (Ferry *et al.*, 1994; Ferry and Molinoff, 1996). On the other hand, an up-regulation of 5-HT_{2A} receptors by agonists has been described in cultured cerebellar granule cells (Akiyoshi *et al.*, 1993; Chen *et al.*, 1995a,b). This up-regulation appeared to require activation of a calcium/calmodulin-sensitive kinase (Chen *et al.*, 1995a). In NIH 3T3 cells, 5-HT_{2A} receptors may be desensitized, but not down-regulated, by acute (Roth *et al.*, 1995a) and chronic (Grotewiel and Sanders-Bush, 1994) agonist administration. These results suggest that the precise effects of agonists on 5-HT_{2A} receptors depend on the cellular milieu in which they are expressed. Thus, for instance, in immature cerebellar neurons, agonist exposure increases levels of 5-HT_{2A} receptors. In most other cells, agonists induce a characteristic down-regulation and desensitization of 5-HT_{2A} receptors and receptor activity.

The cellular mechanisms responsible for 5-HT_{2A} receptor down-regulation and desensitization have been incompletely described. For some time, it has been clear that PKC activation may induce desensitization of 5-HT_{2A} receptors (Roth *et al.*, 1986; Kagaya *et al.*, 1990, 1993). More recent studies have attempted to examine the mechanism(s) responsible for agonist-induced desensitization.

Studies with chimeric 5-HT$_{2A}$/thrombin receptors have implied that 5-HT$_{2A}$ receptors may be desensitized by PKC activation, but not via direct phosphorylation of the 5-HT$_{2A}$ receptor (Vouret-Craviari *et al.*, 1995). As well, selective PKC antagonists did not attenuate rapid agonist-induced desensitization in HEK-293 cells (Vouret-Craviari *et al.*, 1995). By contrast, studies with platelets demonstrated that H-7, which inhibits PKC, was able to attenuate agonist- and PKC-dependent desensitization (Kagaya *et al.*, 1990). Time-course studies showed that the rapid (10 min–2 hr) and delayed (>6 hr) phases of agonist-induced desensitization were not affected by down-regulating various PKC isoforms, whereas the intermediate phase (2–4 hr) was attenuated by down-regulating PKC (Roth *et al.*, 1995a). The PKC subtype involved in desensitization has also been examined by using subtype-selective antibodies. These studies (Roth *et al.*, 1995a) revealed that the α and ϵ, but not ζ, isoforms were most likely involved in the PKC-mediated desensitization of 5-HT$_{2A}$ receptors in NIH 3T3 cells. Taken together, these results suggest that although PKC may be involved in some phases of 5-HT$_{2A}$ desensitization, other cellular processes are also involved.

To examine other molecular targets for agonist-mediated desensitization, Roth *et al.* (1995a) studied the effect of PKC activation and agonist exposure on levels of $G_{\alpha q}$—the G-protein responsible for coupling 5-HT$_{2A}$ receptors to phospholipase C. Chronic agonist or PKC exposure did not alter the levels of $G_{\alpha q}$ (Roth *et al.*, 1995a). Using mutant receptors, Roth *et al.* (1995a) also found that mere agonist occupancy was not sufficient for agonist-induced desensitization, but that second-messenger production was essential for agonist-mediated desensitization. These results imply that kinases other than PKC might be involved in agonist-mediated desensitization.

Others have examined the effects of agonists and antagonists on the subcellular distribution of 5-HT$_{2A}$ receptors *in vitro*. Two studies (Vouret-Craviari *et al.*, 1995; Berry *et al.*, 1996) found that agonists induced a rapid internalization of 5-HT$_{2A}$ receptors *in vitro*. Dual-label fluorescence confocal microscopic studies disclosed that 5-HT$_{2A}$ receptors are internalized via endosomes (Berry *et al.*, 1996). Whether agonist-mediated internalization also occurs *in vivo* is not known.

That desensitization, internalization, and down-regulation are distinct biochemical processes for 5-HT$_{2A}$ receptors was shown by the Roth *et al.* (1995a) studies. In these experiments, NIH 3T3 cells stably transfected with a 5-HT$_{2A}$ cDNA were chronically exposed to quipazine or DOI. A time-dependent desensitization of 5-HT$_{2A}$ receptor-mediated PI hydrolysis was measured without a change in receptor number. Berry *et al.* (1996) found that chronic agonist administration also did not change the subcellular distribution of 5-HT$_{2A}$ receptors. These results imply that 5-HT$_{2A}$ receptors may be desensitized by mechanisms distinct from those involved in down-regulation and internalization.

7.2. *General Mechanisms of Receptor Internalization*

Over the past several years, investigators have developed a general model of GPCR internalization (Fig. 8). Because receptor internalization has been thoroughly reviewed elsewhere (Chuang *et al.*, 1996; Ferguson *et al.*, 1996a,b; Bohm *et al.*, 1997a,b), only the salient points are discussed here. Gaps in our understanding of 5-HT receptor regulation are also emphasized.

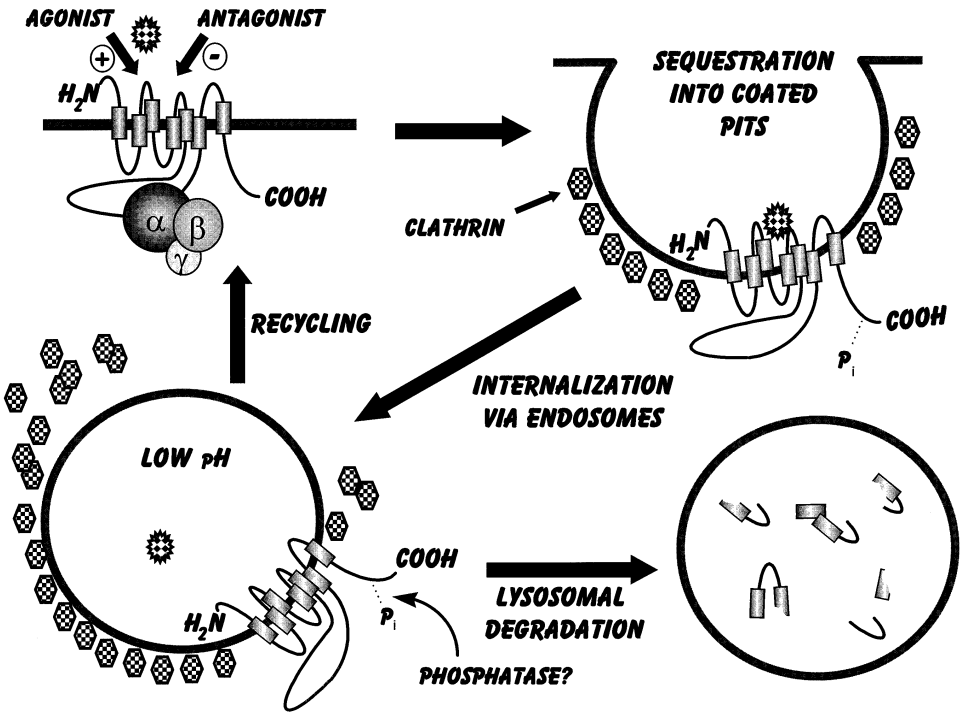

FIGURE 8. Model of 5-HT$_{2A}$ receptor internalization and recycling. After binding ligand (agonist or antagonist), 5-HT$_{2A}$ receptors are recruited to clathrin-coated vesicles where they undergo endocytosis via endosomes (see Berry *et al.*, 1996; Willins *et al.*, 1997a). 5-HT$_{2A}$ receptors may then be recycled to the cell surface or, possibly, undergo degradation via lysosomes. By analogy with β-adrenergic receptors, it is possible that 5-HT$_{2A}$ receptors may undergo phosphorylation and then dephosphorylation via a specific phosphatase found in endosomes.

After agonist binding, receptors such as the β-adrenergic receptor may be phosphorylated by G-protein receptor kinases (GRKs). GRKs are a family of at least six members (GRK1, GRK2, GRK3, GRK4, GRK5, GRK6) (for a review, see Sterne-Marr and Benovic, 1995), each of which has a distinct tissue and regional brain distribution and substrate specificity. GRK2, also known as βARK1, phosphorylates the β_2-adrenergic receptor. Following phosphorylation of the β_2-adrenergic receptor by GRK2, the receptor becomes uncoupled from heterotrimeric G-proteins. GRK2 and GRK3 are thought to be targeted to the β_2-adrenergic receptor via interactions with the βγ-subunits of the G-protein heterotrimeric complex. Other GRKs (GRKs4–6) are localized in the membrane in the absence of receptor activation by agonists (Ferguson *et al.*, 1996a,b).

Another family of proteins known as arrestins (for a review, see Sterne-Marr and Benovic, 1995) also bind to GPCRs, with β-arrestin being the prototypic member of the family (Lohse *et al.*, 1990). Desensitization of β-adrenergic receptors appears to require not only GRK-mediated phosphorylation, but also the binding of β-arrestin (Lohse *et al.*, 1992). β-Arrestin_1 and β-arrestin_2 appear to prefer the β-adrenergic receptor, but can also bind to m_2-muscarinic acetylcholine receptors (Sterne-Marr and Benovic, 1995; Ferguson *et al.*, 1996a,b).

Following β-arrestin binding, the β_2-adrenergic receptor migrates to coated vesicles via a specific interaction between clathrin and β-arrestin (Fig. 8) (Goodman *et al.*, 1996). These results suggest that β-arrestin functions as a scaffolding or sorting protein, which allows for the specific localization of desensitized and phosphorylated receptors to coated pits (Fig. 8).

The finding that β-arrestin might help to target GPCRs to coated vesicles is important in light of earlier studies that identified GPCRs in coated vesicles isolated from the brain (see Table 3). As can be seen, a large number of GPCRs have been localized to coated vesicles, including opiate (Bennett *et al.*, 1985), β-adrenergic (Chuang *et al.*, 1986), muscarinic (Silva *et al.*, 1986), adenosine (Gonzalez-Calero *et al.*, 1990), and D_1- and D_2-dopamine (Ozaki *et al.*, 1994). Opiate and β-adrenergic receptors are uncoupled from G-proteins in purified coated vesicles (Bennett *et al.*, 1985; Chuang *et al.*, 1986), whereas some other GPCRs retain this coupling in purified coated-vesicle preparations (Silva *et al.*, 1986; Gonzalez-Calero *et al.*, 1990).

More recent studies have attempted to localize GPCRs to coated vesicles via histochemical techniques during agonist-mediated internalization. Thus, β_2-adrenergic (von Zastrow and Kobilka, 1994), cholecystokinin (CCK) (Roettger *et al.*, 1995), substance P (Grady *et al.*, 1995), m_1-muscarinic (Tolbert and Lameh, 1996), and 5-HT_{2A} (Berry *et al.*, 1996) have all been localized to clathrin-coated vesicles after short periods of agonist administration.

Other evidence in favor of the hypothesis that GPCRs use coated pits for the initial phases of endocytosis comes from the observation that dynamin is essential for agonist-induced endocytosis (Zhang *et al.*, 1996). Dynamin is a 110-kDa protein, (for a recent review, see Damke, 1996), with intrinsic GTPase activity, and is required for coated-vesicle formation. α-Adaptin, another protein involved in coated-pit formation (Damke, 1996), may assist in targeting dynamin to coated vesicles. In the studies by Caron's group (Zhang *et al.*, 1996), GTPase-deficient mutants of dynamin were shown to block agonist-mediated internalization of β_2-adrenergic receptors. Taken together, all these studies are consistent with a model proposing that clathrin-coated vesicles play a central role in agonist-mediated internalization of GPCRs in general, and 5-HT_{2A} receptors in particular.

Other studies have suggested that smooth-surfaced vesicles called caveolae are also involved in GPCR internalization. Thus, Lisanti's group (Chun *et al.*, 1994) first noted that endothelin receptors were internalized via caveolin-coated vesicles in transiently transfected COS cells. Other cell surface receptors, such as tyrosine kinase receptors (Wu *et al.*, 1997) and G-protein α-subunits (Li *et al.*, 1995), also occur in caveolae, a finding suggesting that this pathway is important for GPCR trafficking. Direct tests of this hypothesis, however, have yielded mixed results. Thus, Roettger *et al.* (1995) demonstrated that CCK receptors were internalized via caveolae and clathrin-coated pits, whereas muscarinic (Tolbert and Lameh, 1996) and 5-HT_{2A} (Berry *et al.*, 1996) receptors are internalized only via clathrin-coated pits and not via caveolae.

The receptor domains essential for internalization have been studied extensively for a wide variety of GPCRs. Studies with the β_2-adrenergic receptor have identified a highly conserved motif (NPXXY) that may be essential for agonist-mediated internalization of some GPCRs (Barak *et al.*, 1994, 1995). These authors found that mutation of the terminal tyrosine of this motif yielded a receptor that was deficient in internalization, among other functions. This motif resembles those for internalization of other membrane proteins, such as the insulin receptor and the low-density lipoprotein receptor. Analogous mutations of the neurokinin-1 (NK-1) receptor had a small, but significant, effect on internalization. Studies of gastrin receptors (Slice *et al.*, 1994) and angiotensin II receptors (Thomas *et al.*, 1996; Laporte *et al.*, 1996) showed no effect of a mutation of the NPXXY motif on agonist-mediated internalization.

A large number of studies have examined various domains of GPCRs essential for agonist-induced internalization. The first studies that examined a specific GPCR domain essential for internalization were those of Valiquette *et al.* (1990). These workers found that mutation of Y350 and Y354 of the β_2-adrenergic receptor increased agonist-induced down-regulation, but did not affect sequestration. Subsequently, Lameh *et al.* (1992) found that the i3 (cytoplasmic loop III) was essential for internalization of the m_1-muscarinic receptor. There is a similar requirement of i3 or i2 residues for gastrin receptors (Benya *et al.*, 1994), the STE2 yeast GPCR (Stefan and Blumer, 1994), the m_4-muscarinic receptor (Van Koppen *et al.*, 1994), the gonadotropin-releasing hormone receptor (Arora *et al.*, 1995), the

TABLE 3. Neurotransmitter Receptors Localized to Coated Vesicles or Caveolae

Date	Receptor	G-Protein coupled	Coated vesicle	Caveolae	Reference
1985	Opiate	No	Yes	N/A	Bennett *et al.*, 1985
1985	GnRH	Not assessed	Yes	N/A	Hazum *et al.*, 1985
1986	β-Adrenergic	No	Yes	N/A	Chuang *et al.*, 1986
1986	Muscarinic acetylcholine	Yes	Yes	N/A	Silva *et al.*, 1986
1990	α_1-Adenosine	Yes	Yes	N/A	Gonzalez-Calero *et al.*, 1990
1991	Glutamate	N/A	Yes	N/A	Martin *et al.*, 1991
1993	Metabotropic glutamate	Yes	Yes	N/A	Martin *et al.*, 1993
1993	GABA	N/A	Yes	N/A	Tehrani and Barnes, 1993
1994	Endothelin	Not assessed	No	Yes	Chun *et al.*, 1994
1994	β_2-Adrenergic	Not assessed	Yes	N/A	von Zastrow and Kobilka, 1994
1994	D_1- and D_2-Dopamine	Yes	Yes	N/A	Ozaki *et al.*, 1994
1995	CCK	N/A	Yes	Yes	Roettger *et al.*, 1995
1995	Substance P and NK-1	Not assessed	Yes	N/A	Grady *et al.*, 1995
1996	m_1-Muscarinic	Not assessed	Yes	No	Tolbert and Lameh, 1996
1996	5-HT$_{2A}$	Not assessed	Yes	No	Berry *et al.*, 1996

GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; N/A, not available.

AT-1 angiotensin receptor (Hunyady *et al.*, 1996), and the B_2-bradykinin receptor (Prado *et al.*, 1997).

A large number of studies have found that the carboxyl terminus of the receptor is important for internalization of the thyrotropin-releasing hormone receptor (Nussenzveig *et al.*, 1993; Haraguchi *et al.*, 1994), the angiotensin 1A receptor (Chaki *et al.*, 1994; Hunyady *et al.*, 1994; Thomas *et al.*, 1995), the calcitonin receptor (Findlay *et al.*, 1994), the parathyroid hormone receptor (Huang *et al.*, 1995), the V_2-vasopressin receptor via a palmitoylation-sensitive group (Schulein *et al.*, 1996), the thrombin receptor (Shapiro *et al.*, 1996), the NK-1 receptor (Bohm *et al.*, 1997a,b), and the B_2-bradykinin receptor (Prado *et al.*, 1997). Taken together, these results suggest that *multiple* domains may be involved in GPCR internalization in general, and 5-HT receptor internalization in particular.

Only one study has investigated mutations of the 5-HT$_{2A}$ receptor and the effects on internalization (Vouret-Craviari *et al.*, 1995). This study found that deletion of a portion of the carboxyl terminus of the 5-HT$_{2A}$ receptor had no effect on internalization. Interestingly, a 5-HT$_{2A}$/thrombin chimeric receptor, in which the C-terminus of the thrombin receptor replaced the C-terminus of the 5-HT$_{2A}$ receptor, allowed the receptor to become sensitive to PKC activation. In Vouret-Craviari *et al.*'s (1995) study, only the distal portion of the C-terminus of the 5-HT$_{2A}$ receptor was deleted, and the NPXXY motif (AA 376-380) was left intact.

7.3. *Paradoxical Regulation of 5-Hydroxytryptamine$_{2A}$ Receptors by Antagonists*

Typically, GPCRs are up-regulated or sensitized after chronic agonist administration. 5-HT$_2$-family receptors (5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$) differ by being down-regulated by antagonists. Thus, Peroutka and Snyder (1980a,b) were the first to demonstrate that several antidepressants with antagonist activity at 5-HT$_{2A}$ receptors induced down-regulation. Since then, many 5-HT$_{2A}$ antagonists have been shown to cause "down-regulation" of radiolabeled 5-HT$_{2A}$ receptors, including mianserin (Blackshear and Sanders-Bush, 1982; Helmeste and Tang, 1983), ritanserin and setoperone (Leysen *et al.*, 1986; Twist *et al.*, 1991), and ketanserin (Eison *et al.*, 1989; Pranzatelli, 1991).

In general, antagonists cause 5-HT$_{2A}$ receptor down-regulation, although there is a novel 5-HT$_{2A}$ antagonist (SR 46349B) that causes an up-regulation of 5-HT$_{2A}$ receptors *in vivo* (Rinaldi-Carmona *et al.*, 1993) and *in vitro* (Rinaldi-Carmona *et al.*, 1994). Why SR 36349B causes up-regulation of 5-HT$_{2A}$ receptors *in vitro* and *in vivo* whereas other 5-HT$_{2A}$ antagonists induce down-regulation is unknown. Interestingly, in cells stably expressing the F340L mutation, quipazine functions as an antagonist rather than an agonist (Roth *et al.*, 1997a). In cells expressing the F340L mutation, quipazine behaves like an SR 36349B-like antagonist and induces an up-regulation of the F340L mutant receptor. These results suggest that under appropriate circumstances, antagonists may up-regulate 5-HT$_{2A}$ receptors, as well as down-regulate them.

The mechanism of the antagonist-mediated down-regulation of 5-HT$_{2A}$ receptors has been investigated *in vivo* and *in vitro*. *In vivo* studies with mianserin (Roth and Ciaranello, 1991) showed no effect of chronic mianserin treatment on 5-HT$_{2A}$ mRNA levels. A similar lack of effect on 5-HT$_{2A}$ mRNA levels was seen for imipramine (Burnet *et al.*, 1994). By contrast, Butler *et al.* (1993) found that mianserin caused a small (10–15%) increase in 5-HT$_{2A}$ mRNA levels. Because mianserin can decrease 5-HT$_{2A}$ receptor number while perhaps causing a slight increase in 5-HT$_{2A}$ mRNA, it is unlikely that the mechanism by which mianserin decreases 5-HT$_{2A}$ receptors *in vivo* is transcriptional.

Studies by Toth and Shenk (1994) have implied that mianserin may cause 5-HT$_{2A}$ down-regulation via transcriptional mechanisms. In C6-glioma cells, mianserin causes a

down-regulation of 5-HT_{2A} receptors accompanied by a decrease in 5-HT_{2A} mRNA and mediated by specific sequences in the 5′-untranslated region of the gene. Clozapine can also decrease 5-HT_{2A} mRNA levels *in vivo* in the cingulate cortex, but not in other cortical regions (e.g., frontal cortex, piriform cortex) (Burnett *et al.*, 1996). Buspirone, on the other hand, has been shown to induce increases in 5-HT_{2A} mRNA levels in the hippocampus (Chen *et al.*, 1995b). Other treatments that can increase 5-HT_{2A} mRNA levels include *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline administration (Raghupathi *et al.*, 1996), electroconvulsive shock (Burnet *et al.*, 1995), estrogen (Summer and Fink, 1995), dopamine agonists (Laprade *et al.*, 1996), and serotonergic agonists (in myometrial and cerebellar granule cells) (Akiyoshi *et al.*, 1993; Rydelek-Fitzgerald *et al.*, 1993).

7.4. *Typical and Atypical Antipsychotic Drugs Down-Regulate 5-Hydroxytryptamine$_{2A}$ Receptors*

It has been know for many years that in addition to binding to D_2-dopamine receptors, a large number of antipsychotic drugs also bind to 5-HT_2 receptors (Leysen *et al.*, 1978; Peroutka *et al.*, 1980). In addition, more recent studies, which have examined the binding characteristics of antipsychotic drugs to cloned 5-HT receptors, have revealed high affinities for typical and atypical antipsychotic drugs at a variety of 5-HT receptors, including 5-HT_{2A} (Roth *et al.*, 1995b), 5-HT_{2C} (Canton *et al.*, 1990; Roth *et al.*, 1992; Kuoppamaki *et al.*, 1995), 5-HT_6 (Roth *et al.*, 1994; Kohen *et al.*, 1996), and 5-HT_7 (Roth *et al.*, 1994) receptors. In general, typical antipsychotic drugs may be distinguished from atypical antipsychotic drugs by the former's higher affinities for various 5-HT receptors (e.g., 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, and/or 5-HT_7) than for D_2- or D_4-dopamine receptors (Meltzer *et al.*, 1989; Roth *et al.*, 1995b). Antipsychotic drugs, both typical and atypical, function as antagonists at 5-HT_{2A} (Roth *et al.*, 1986), 5-HT_{2C} (Canton *et al.*, 1994), and 5-HT_6 (Kohen *et al.*, 1996) receptors.

In the case of atypical antipsychotic drugs, it has been clear for many years that chronic treatment with clozapine causes a down-regulation of 5-HT_{2A} receptors (Reynolds *et al.*, 1983; Mikuni and Meltzer, 1984; Andree *et al.*, 1986; Hall *et al.*, 1995; Kuoppamaki *et al.*, 1995). Other atypical antipsychotic drugs, such as fluperlapine and setoperone (Matsubara and Meltzer, 1989), iloperidone (Strupczewski *et al.*, 1995), ORG 5222 and amperozide (Svartengren, 1993; Kuoppamaki *et al.*, 1995), when administered chronically, also down-regulate 5-HT_{2A} receptors. Apparently, risperidone does not induce 5-HT_{2A} receptor down-regulation *in vivo* (Kuoppamaki *et al.*, 1995). Some typical antipsychotic drugs, such as amoxapine (Helmeste and Tang, 1983), loxapine (Matsubara and Meltzer, 1989), chlorpromazine, cis-fluphenthixol, and thioridazine (Andree *et al.*, 1986), also down-regulate 5-HT_{2A} receptors. In general, the ability of an antipsychotic drug to down-regulate 5-HT_{2A} receptors is proportional to its 5-HT_{2A} antagonist activity. Thus, the typical antipsychotic drugs that induce 5-HT_{2A} down-regulation (amoxapine, loxapine, chlorpromazine, cis-fluphenthixol, and thioridazine) all have relatively high 5-HT_{2A} receptor affinities, although these affinities are still less than those for D_2-dopamine receptors (for a discussion, see Meltzer *et al.*, 1989).

The mechanism responsible for the paradoxical down-regulation of 5-HT_{2A} receptors by antipsychotic drugs has only recently come under study. As previously mentioned, Burnett *et al.* (1996) found that clozapine was without significant effects on 5-HT_{2A} receptor mRNA levels in the frontal and piriform cortices, but caused a significant decrease in 5-HT_{2A} mRNA levels in the cingulate cortex. The decreased levels of 5-HT_{2A} receptor mRNA in the cingulate cortex correlated well with clozapine's ability to decrease 5-HT_{2A} receptor levels, a finding suggesting that in this region, clozapine down-regulates 5-HT_{2A} receptor via transcriptional mechanisms. By contrast, the lack of effect in the frontal cortex, where the bulk of the 5-HT_{2A} receptors are located, suggests that clozapine's effects are non-transcriptional in this brain region.

In this regard, Willins *et al.* (1997a,b) recently have shown that clozapine causes 5-HT_{2A} receptor internalization *in vitro* and alters the subcellular distribution of 5-HT_{2A} receptors *in vivo*. Willins *et al.* (1997a,b) showed that chronic clozapine administration caused an apparent decrease in 5-HT_{2A}-like immunoreactivity in the apical dendrites of pyramidal neurons and an increase in intracellular immunoreactivity. These results are reminiscent of recent studies (Roettger *et al.*, 1997) reporting that CCK antagonists caused internalization of the CCK receptor *in vitro*. These authors found that various CCK antagonists induced a dose-dependent internalization of CCK receptors without causing CCK receptor phosphorylation. In this regard, Berry *et al.* (1996) showed that ketanserin caused internalization of the 5-HT_{2A} receptor, albeit with lower efficacy than did quipazine. Taken together, these results suggest that antagonists may also cause the internalization of GPCRs, and that this internalization may be important for mediating the effects of chronic antagonist exposure on receptor levels and activity.

As discussed in Section 7.2, a number of studies of GPCRs have implied that receptor phosphorylation, followed, perhaps, by binding of arrestin-like proteins, is a key event in inducing receptor internalization. It is unlikely that receptor phosphorylation is a universal signal for receptor internalization, however, because antagonists can induce internalization apparently with causing receptor phosphorylation (Roettger *et al.*, 1997). It is likely, therefore, that other cellular mechanisms are involved in GPCR internalization in general and 5-HT_{2A} receptor internalization in particular. In this regard, Worley's group (Brakeman *et al.*, 1997) recently identified a protein called Homer, which they suggest is involved in trafficking of metabotropic glutamate receptors in neurons. Whether Homer is involved in trafficking of other GPCRs or is specific for metabotropic glutamate receptors is unknown.

7.5. *Regulation of 5-Hydroxytryptamine$_{2A}$ Receptors by Antidepressants*

Peroutka and Snyder (1980a,b) were the first to demonstrate that 5-HT$_{2A}$ receptors may be down-regulated by antidepressants. They discovered that chronic treatment with several different antidepressants of distinct chemical classes induced a down-regulation of 5-HT$_2$ receptors. Shortly thereafter, Kellar *et al.* (1981) found that although chronic treatment with a variety of antidepressants induced 5-HT$_2$ receptor down-regulation, electroconvulsive treatment induced an up-regulation of 5-HT$_2$ receptors. A number of other studies have also shown that various antidepressants, including amitriptyline (Peroutka and Snyder, 1980a,b), desipramine (Hall *et al.*, 1984; Goodwin *et al.*, 1984; Metz and Heal, 1986), imipramine (Barbaccia *et al.*, 1983), iprindole (Kendall *et al.*, 1982; Eison *et al.*, 1991), mianserin (Blackshear and Sanders-Bush, 1982; Roth and Ciaranello, 1991), clomipramine (De Ceballos *et al.*, 1985; Schoups and De Potter, 1988), and monoamine oxidase inhibitors (Goodnough and Baker, 1994a,b) can down-regulate 5-HT$_{2A}$ receptors.

By contrast, treatment with the 5-HT-selective reuptake inhibitor fluoxetine has been reported to cause no change (Cadogan *et al.*, 1993; Goodnough and Baker, 1994a,b) or an increase (Klimek *et al.*, 1994) in 5-HT$_{2A}$ receptors, as measured by radioligand binding. Others who have evaluated the responsiveness of 5-HT$_{2A}$ receptors after chronic fluoxetine administration have detected either increases (Cadogan *et al.*, 1993) or no changes in 5-HT$_{2A}$-mediated PI hydrolysis (Romero *et al.*, 1994). Tilakaratne *et al.* (1995) measured c-fos responses induced by 5-HT$_{2A}$ agonists after chronic fluoxetine administration and found increased c-fos responses.

Treatment with other 5-HT-selective reuptake inhibitors have also yielded mixed results. For paroxetine, increases (Cadogan *et al.*, 1993), decreases (Koshikawa *et al.*, 1989), or no effects (Bakish *et al.*, 1997) on 5-HT$_{2A}$ receptors, as measured by radioligand binding, have been reported. Citalopram has been reported to down-regulate 5-HT$_2$ receptors (Kubota *et al.*, 1989; Klimek *et al.*, 1994).

7.6. *Regulation of 5-Hydroxytryptamine$_{2C}$ Receptors*

The regulation of 5-HT$_{2C}$ receptors has not been studied as extensively as that of 5-HT$_{2A}$ receptors. Like 5-HT$_{2A}$ receptors, 5-HT$_{2C}$ receptors are subject to striking developmental regulation (Roth *et al.*, 1991). Additionally, down-regulation by antagonists has been demonstrated for 5-HT$_{2C}$ receptors (Pranzatelli *et al.*, 1993; Pranzatelli and Tailor, 1994; Kuoppamaki *et al.*, 1994, 1995). Similar to the 5-HT$_{2A}$ receptor, chronic citalopram and fluoxetine administration induces an up-regulation of 5-HT$_{2C}$ receptor activity (Laakso *et al.*, 1996). In this regard, two studies have demonstrated that many antidepressants are antagonists at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Akiyoshi *et al.*, 1996; Palvimaki *et al.*, 1996). It is conceivable, then, that the up-regulation of 5-HT$_{2C}$ receptors is secondary to blockade of these receptors by typical and atypical antidepressants. Unfortunately, the mechanisms responsible for drug-induced changes in 5-HT$_{2C}$ receptor number have not been studied *in vivo*. *In vitro* studies, on the other hand, have demonstrated a rapid desensitization of 5-HT$_{2C}$ receptors after agonist exposure (Akiyoshi *et al.*, 1995).

8. UNANSWERED ISSUES FOR 5-HYDROXYTRYPTAMINE$_2$-RECEPTOR REGULATION

At present, there is almost no information about the structural features of 5-HT$_2$-family receptors essential for regulation by either agonists or antagonists. 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are unique in comparison with other GPCRs in that they are down-regulated by both agonists and antagonists. The molecular mechanisms responsible for this apparent down-regulation are unknown. Because a large number of psychiatrically important drugs, including atypical antipsychotics, antidepressants, and anxiolytics, can all induce down-regulation of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, a knowledge of the mechanisms responsible for these alterations could have clinical significance. Additionally, because it is unlikely that antagonist-induced internalization of 5-HT$_{2A/2C}$ receptors is mediated by phosphorylation, it is likely that novel mechanisms of receptor internalization will need to be invoked to determine how these receptors are internalized. Thus, a detailed study of the structure-activity relations of 5-HT$_2$-family receptors, particularly with regard to regulatory properties, will no doubt lead to novel insights about GPCR regulation.

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