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5-HT_{1A} and 5-HT_{2A} Receptor mRNAs and Binding Site Densities Are Differentially Altered in Schizophrenia

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We have investigated 5-HT_{1A} (serotonin$_{1A}$) and 5-HT_{2A} (serotonin$_{2A}$) receptor mRNA abundance and binding site densities in various neocortical and hippocampal regions of schizophrenics and control subjects. Age, agonal state (brain pH), and post mortem interval were included where necessary as covariates in our analyses. In schizophrenics, 5-HT_{1A} binding site densities, determined autoradiographically by [^{3}H]8-hydroxy-2,3-(dipropylamino)-tetralin ([^{3}H]8-OH-DPAT), were significantly increased (+23%) in the dorsolateral prefrontal cortex, with a similar trend in anterior cingulate gyrus. These increases were not accompanied by any change in 5-HT_{1A} receptor mRNA. No differences between the groups in [^{3}H]8-OH-DPAT binding or 5-HT_{1A} receptor mRNA were seen in superior temporal gyrus, striate cortex, or hippocampus. 5-HT_{2A} binding sites, determined by [^{3}H]ketanserin, were decreased in the dorsolateral prefrontal cortex (−27%) and parahippocampal gyrus (−38%) of schizophrenics, with a similar trend in cingulate gyrus, but not in superior temporal gyrus or striate cortex. 5-HT_{2A} receptor mRNA abundance was reduced in schizophrenics in the dorsolateral prefrontal (−49%), superior temporal (−48%), anterior cingulate (−63%) and striate (−63%) cortices, but not in parahippocampal gyrus. Parallel analyses of rat brain tissue showed no changes in 5-HT_{1A} or 5-HT_{2A} receptor mRNAs or binding site densities after chronic administration of haloperidol. These data show that schizophrenia is associated with alterations in the expression of central 5-HT_{1A} and 5-HT_{2A} receptors. They confirm reports of increased 5-HT_{1A} and decreased 5-HT_{2A} binding site densities in prefrontal cortex, and reveal more extensive decreases in 5-HT_{2A} receptor gene expression at the mRNA level. The resulting imbalance in the 5-HT_{1A} to 5-HT_{2A} receptor ratio, when considered in terms of the chemoarchitectural distribution of these receptors, may contribute to an impairment of corticocortical association pathways. The apparent dissociation of the normal relationships between the abundance of each 5-HT receptor and its mRNA in schizophrenia introduces a separate complexity to the data, which may give clues to the underlying molecular mechanisms. ***[Neuropsychopharmacology 15:442–455, 1996]***

KEY WORDS: *5-HT_{1A} receptor; 5-HT_{2A} receptor; Cortex; Serotonin receptor; In situ hybridization; Receptor autoradiography; Schizophrenia*

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There is considerable evidence for the involvement of 5-HT (serotonin) and its receptors in the pathophysiology and pharmacotherapy of schizophrenia. The evidence comes from transmitter and metabolite measurements, neuroendocrine investigations of 5-HT–mediated effects, platelet membrane studies, and from investigations of brain 5-HT receptors and uptake sites (for review, see Bleich et al. 1988; Breier 1995; Roth and Meltzer 1995).

Regarding 5-HT receptors, interest has centered on 5-HT_1 and 5-HT_2 classes, which have now been divided

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into genetic subtypes (Peroutka 1994), of which the 5-HT_{1A} and 5-HT_{2A} receptors have been investigated in most detail. Increased 5-HT_{1A} receptor binding site densities have been found in the prefrontal cortex in schizophrenia, using membrane binding (Hashimoto et al. 1991) and receptor autoradiography (Simpson et al. 1996; but see Joyce et al. 1993). 5-HT_{2A} receptor densities in the frontal cortex in schizophrenia appear to change in the opposite direction to the 5-HT_{1A} receptor, with four studies reporting decreases (Bennett et al. 1979; Mita et al. 1986; Arora and Meltzer 1991; Laruelle et al. 1993), although three found no change (Whitaker et al. 1981; Reynolds et al. 1983; Joyce et al. 1993). There are fewer data concerning these receptors in other areas of the cerebral cortex in schizophrenia. 5-HT_{1A} receptor binding sites are elevated in the posterior cingulate cortex and motor cortex but not in anterior cingulate gyrus (Joyce et al. 1993); Joyce and colleagues (1993) also reported an increase in hippocampal 5-HT_{1A} receptors, whereas another study did not (Hashimoto et al. 1991). 5-HT_{2A} receptor binding sites were found to be increased in the posterior cingulate cortex, lateral temporal cortex, and hippocampal subfields but not in anterior cingulate gyrus or motor cortex (Joyce et al. 1993), nor in occipital cortex (Laruelle et al. 1993).

The data as a whole suggest that 5-HT_{1A} receptors

Table 1. Demographic Details of Cases and Controls

No.	Age (year)	Sex	Cause of Death	pH	PMI (h)	Original Subtype	Onset (year)	Medication at Death
Schizophrenics								
1	71	F	Bronchopneumonia	6.62	48	Disorganized	38	Flupenthixol decanoate, amitryptiline
2	75	M	Left ventricular failure	6.02	56	Undifferentiated	32	Trifluoperazine
3	56	M	Myocardial infarction	6.09	48	Paranoid	29	Flupenthixol decanoate, trifluoperazine
4	30	M	Suicide—hanging	6.74	92	Paranoid	20	Flupenthixol decanoate
5	60	F	Bronchopneumonia	6.40	25	Disorganized	27	Fluphenazine decanoate, amitryptiline
6	43	F	Left ventricular failure	6.52	49	Paranoid	29	Zuclopenthixol decanoate, chlorpromazine
7	40	M	Left ventricular failure	6.68	76	Disorganized	32	Chlorpromazine
8	67	M	Fibrosing alveolitis	5.70	34	Paranoid	43	Flupenthixol decanoate
9	69	M	Bronchopneumonia	6.03	63	n/k	28	Zuclopenthixol decanoate, carbamazepine
10	28	F	Suicide—multiple injuries	6.67	32	Paranoid	20	Flupenthixol decanoate
11	44	M	Left ventricular failure	6.72	24	Paranoid	25	None
12	69	F	Bronchopneumonia	6.28	52	Disorganized	16	Thioridazine
13	64	M	Myocardial infarction	6.30	25	Paranoid	47	Chlorpromazine, amitryptiline
Mean	55	8M, 5F		6.37	48			
SD	16			0.33	21			
Controls								
1	70	M	Bronchitis	6.36	72			
2	67	F	Myocardial infarction	6.76	38			
3	52	M	Suicide—hanging	6.81	20			
4	75	F	Myocardial fibrosis	6.63	27			
5	68	M	Myocardial infarction	6.20	27			
6	79	F	Suicide—hemorrhage	6.27	51			
7	47	M	Myocardial infarction	6.72	21			
8	47	M	Myocardial infarction	6.94	38			
9	71	F	Myocardial infarction	6.25	27			
10	22	F	Multiple injuries	6.20	37			
11	49	M	Myocardial infarction	6.59	39			
12	61	M	Myocardial infarction	6.67	19			
13	45	M	Inhalation of vomit	6.77	20			
14	59	M	Myocardial infarction	6.69	72			
15	75	F	Cardiac rupture	6.75	27			
Mean	59	9M, 6F		6.58	36			
SD	15			0.25	17			

are increased and 5-HT_{2A} receptors are decreased in the prefrontal cortex in schizophrenia. Nevertheless, the data are not unequivocal, and the discrepancies need to be explored and explained. For example, alterations in 5-HT_{1A} and 5-HT_{2A} receptors may be anatomically localized, as exemplified by the different results found between anterior and posterior cingulate gyri in the study of Joyce and colleagues (1993). Another complication is that schizophrenics who commit suicide have higher 5-HT_{2A} receptor densities than those dying by natural causes (Laruelle et al. 1993; Meltzer 1994), and some studies have not analyzed data by mode of death. A third potential source of discrepancies in 5-HT_{2A} receptor data concerns the affinities of the ligands for other binding sites. For example, [^{3}H]ketanserin, as used by Mita et al. (1986) and Laruelle et al. (1993), is specific for 5-HT_{2A} relative to other 5-HT receptors but it can also detect α_1-adrenergic receptors (Hoyer et al. 1987). Similarly, radiolabeled LSD, used by Bennett et al. (1979), Whitaker et al. (1981), and Joyce et al. (1993), has a high affinity for multiple 5-HT receptors (see Roth and Meltzer 1995).

Interest in 5-HT receptors in schizophrenia, especially the 5-HT_{2A} receptor, has been heightened because of their role as a target of clozapine and other atypical antipsychotics (Meltzer 1992; Arranz et al. 1995; Burnet and Harrison 1995; Nordstrom et al. 1995; Schmidt et al. 1995). Therefore it seems important to investigate 5-HT receptors in schizophrenia further to resolve some of the current uncertainties. The present study was designed to contribute to this process in several ways. First, we examined 5-HT_{1A} and 5-HT_{2A} receptors at the mRNA level, because hybridization methods afford complete specificity as to the receptor subtype being investigated, overcoming the limitations of radioligands. Detection and quantitation of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in the human brain can be achieved by reverse transcription polymerase chain reaction (Burnet et al. 1994a) and by in situ hybridization histochemistry (ISHH; Burnet et al. 1995a). The latter technique has the advantage of high neuroanatomic resolution, which facilitates the integration of neurochemical data into models of psychosis-related circuitry (e.g., Benes et al. 1992; Lewis et al. 1992), and which is desirable given the increasing recognition of cytoarchitectural abnormalities in the disease (see Harrison 1995a). Hence, we used ISHH in the current study. Secondly, we performed receptor autoradiography with [^{3}H]8-hydroxy-2,3-(dipropylamino)-tetralin ([^{3}H]8-OH-DPAT) and [^{3}H]ketanserin, not only to corroborate existing data, but also to investigate in the same cases the two parameters of gene expression, viz. the encoded protein and the encoding mRNA. This combined approach allows the molecular mechanisms underlying altered receptor densities to begin to be investigated. Thirdly, we examined five cortical areas to inform about the distribution of any changes observed. Fourthly, we paid close attention to perimortem and demographic variables, which confound studies of this kind (Barton et al. 1993; Harrison et al. 1995). Finally, we treated rats with haloperidol to provide some indication as to the effect of long-term antipsychotic medication on these receptors and their transcripts.

MATERIALS AND METHODS

Tissue Collection and Processing

The demographic details of cases and controls used in the study are summarized in Table 1. The controls had no clinical history of neurologic or psychiatric disorders, and their brains were neuropathologically unremarkable, as were those from the schizophrenics. At autopsy, left hemisphere tissue was dissected from the following regions: striate cortex (Brodmann area [BA] 17), the middle part of the superior temporal gyrus (BA 22), anterior cingulate cortex (BA 24), dorsolateral prefrontal cortex (BA 46), and medial temporal lobe (hippocampus and parahippocampal gyrus). Tissue blocks were frozen in embedding compound on a dry ice/alcohol slurry and stored at −70°C. Next, 18-μm cryostat sections were cut and thaw mounted onto gelatin-subbed slides. Some sections were pretreated for ISHH and the remainder stored at −70°C before quantitative autoradiography. Brain pH, a measure of agonal state, was measured as described (Harrison et al. 1995). Because some brain areas were not available for study in each case, experiments were carried out on subgroups that remained matched for demographic variables between cases and controls.

Treatment of Rats with Haloperidol

Adult male Sprague-Dawley rats were administered haloperidol decanoate by intramuscular injection, 25mg/kg every 3 weeks (n = 7). Controls received the same volume of sesame oil (n = 7). After 16 weeks, rats were anesthetized, brains perfused with phosphate buffered saline (PBS) followed by 30% sucrose in PBS, removed and processed for ISHH and quantitative autoradiography as described for human tissues.

Receptor Autoradiography

In vitro receptor autoradiography was performed as previously described (Pazos et al. 1987a,b) using [^{3}H]8-OH-DPAT and [^{3}H]ketanserin (New England Nuclear) as the radioactive ligands for the detection of 5-HT_{1A} and 5-HT_{2A} receptor binding sites respectively. Nonspecific binding was determined by the addition of 50μmol/L 5-HT for [^{3}H]8-OH-DPAT and 50μmol/L methysergide for [^{3}H]ketanserin. Sections were apposed to ^{3}H-sensitive Hyperfilm (Amersham) at room temperature for up to 6 weeks.

In Situ Hybridization Histochemistry (ISHH)

ISHH was performed using oligodeoxyribonucleotide probes complementary to the rat and human 5-HT_{1A} and 5-HT_{2A} receptor genes (Burnet et al. 1995a). Briefly, probes were 3'-tail labeled with [α^{35}S]-dATP (1500Ci/mmol), in a 1:10 molar ratio, using terminal deoxynucleotidyl transferase and standard labeling buffer. The labeled probe was added to hybridization buffer at a final concentration of 2×10^4 cpm/μl. Sections were covered with glass coverslips (BDH, Poole, UK) and placed in a chamber humidified with 4 × SSC and 50% formamide and incubated overnight at 35°C. Post-hybridization washes were carried out in 1 × SSC for 5-HT_{2A} and 0.5 × SSC for 5-HT_{1A} receptor mRNAs at 55°C for 3 × 20 min followed by 2 × 60 minutes at room temperature. After a final rinse in water, slides were air dried and apposed to x-ray film (Hyperfilm betamax, Amersham, UK) at room temperature for 6 to 8 weeks. Experimental ISHH controls consisted of hybridization with probes in the sense orientation, ribonuclease pretreatment of sections, and incubation in the presence of 50-fold excess unlabeled antisense probe.

Image Analysis, Quantification, and Statistical Procedures

Autoradiograms were analyzed blind to diagnosis using a Kontron Vidas V2.1 image analyzer (Imaging Associates, Thame, UK). For 5-HT_{1A} receptor mRNA and binding sites, measurements were taken over dentate gyrus and CA1 of the hippocampus and over superficial and deep layers of the parahippocampal gyrus and neocortical regions. All cortical laminae were measured together for 5-HT_{2A} receptor mRNA and binding site densities. Optical density readings were calibrated to ^{35}S nCi/g and fmol/mg tissue equivalents for mRNA abundance and binding site densities, respectively, using appropriate microscales (Amersham, UK). The effects of post mortem interval, brain pH, age, and freezer storage time of samples on the levels of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs and binding sites were investigated using multiple regression. Overall effects of diagnosis and brain region were investigated using ANOVA with these pre- and postmortem factors as covariates where appropriate.

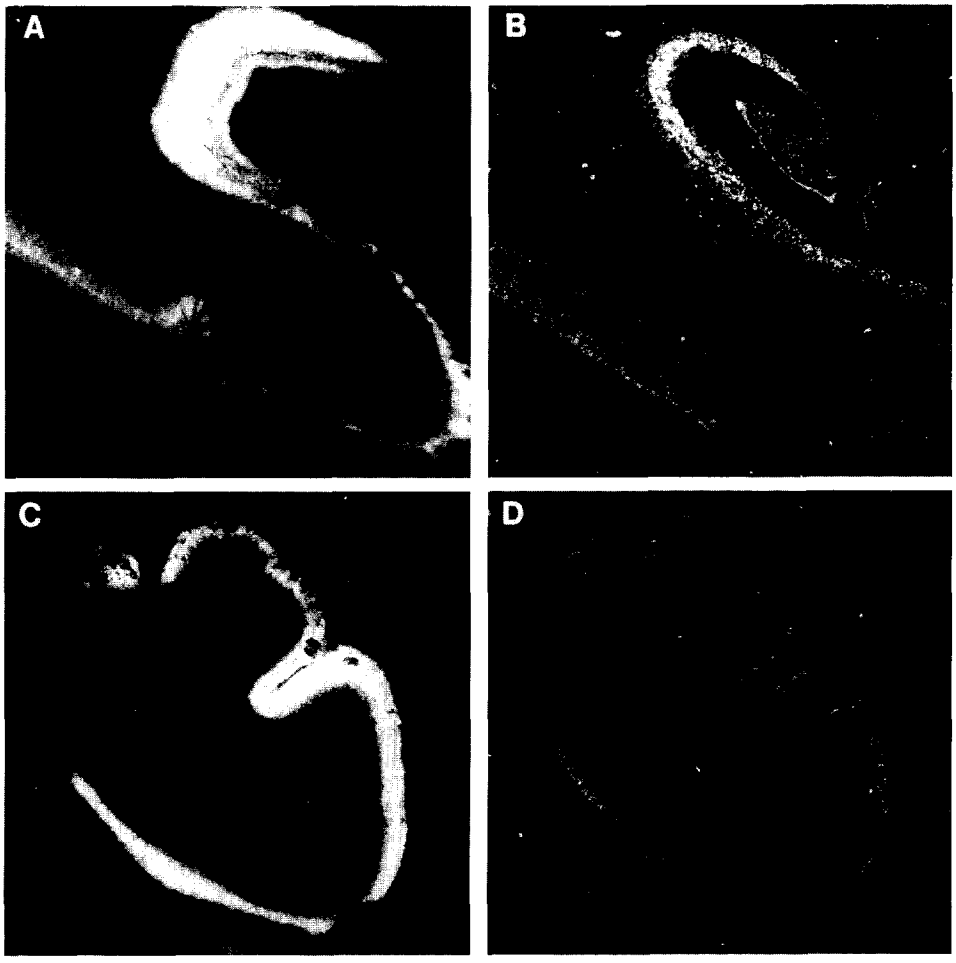

Figure 1. Autoradiograms showing the distribution of [^{3}H]8-OH-DPAT binding sites (**A** and **C**) and 5-HT_{1A} receptor mRNA (**B** and **D**) in the normal human hippocampus (**A** and **B**) and superior temporal gyrus (**C** and **D**).

RESULTS

The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs and binding site densities in neocortex and hippocampus (Figures 1 and 2, and data not shown) were as previously described (Pazos et al. 1987a,b; Burnet et al. 1995a). 5-HT_{1A} receptor mRNA could not reliably be detected in the deep layers of all neocortical samples, and its measurement was restricted to superficial cortical laminae unless otherwise specified. 5-HT_{2A} receptor mRNA was at very low levels in the hippocampus, and we therefore limited analysis of 5-HT_{2A} receptor expression in the medial temporal lobe to the parahippocampal gyrus. The experimental controls for ISHH produced minimal background signal (data not shown; Burnet et al. 1995a) and, in concert with the discrete distribution of each mRNA, confirm the specificity of the ISHH images. Nonspecific binding of [^{3}H]8-OH-DPAT and [^{3}H]ketanserin represented less than 10% of the total signal (data not shown).

There was a significant effect of age, brain pH, and post mortem interval on these mRNAs (Table 2). In all regions except BA22 5-HT_{2A} receptor mRNA declined with decreasing pH (increasing acidosis). 5-HT_{1A} receptor mRNA was only affected by pH in BA24. A negative correlation between age and 5-HT_{1A} receptor mRNA abundance was observed in parahippocampal gyrus and BA46. 5-HT_{2A} receptor mRNA abundance declined with age only in BA22. Post mortem interval affected 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in some regions (Table 2). Freezer storage time (range 1 to 30 months) did not affect the abundance of these mRNAs (data not shown).

Decreased [^{3}H]8-OH-DPAT binding site densities were associated with lower pH in dentate gyrus ($\beta = 0.589$, $p < .01$) and with increasing age in dentate gyrus ($\beta = -0.491$, $p < .05$) and BA22 ($\beta = -0.513$, $p < .05$). There were no other significant correlations of [^{3}H]8-OH-DPAT binding with pH or age, and none with post mortem interval or storage time. [^{3}H]ketanserin binding was not affected by these variables.

5-HT_{1A} Receptor mRNA Abundance and Binding Site Densities in Schizophrenia

There were no differences in 5-HT_{1A} receptor mRNA abundance between schizophrenics and controls in any of the brain regions examined (Figure 3A). In contrast, 5-HT_{1A} receptor binding densities (Figure 3B) were increased in schizophrenics in the superficial laminae of BA46, with a similar trend in BA24. 5-HT_{1A} receptor

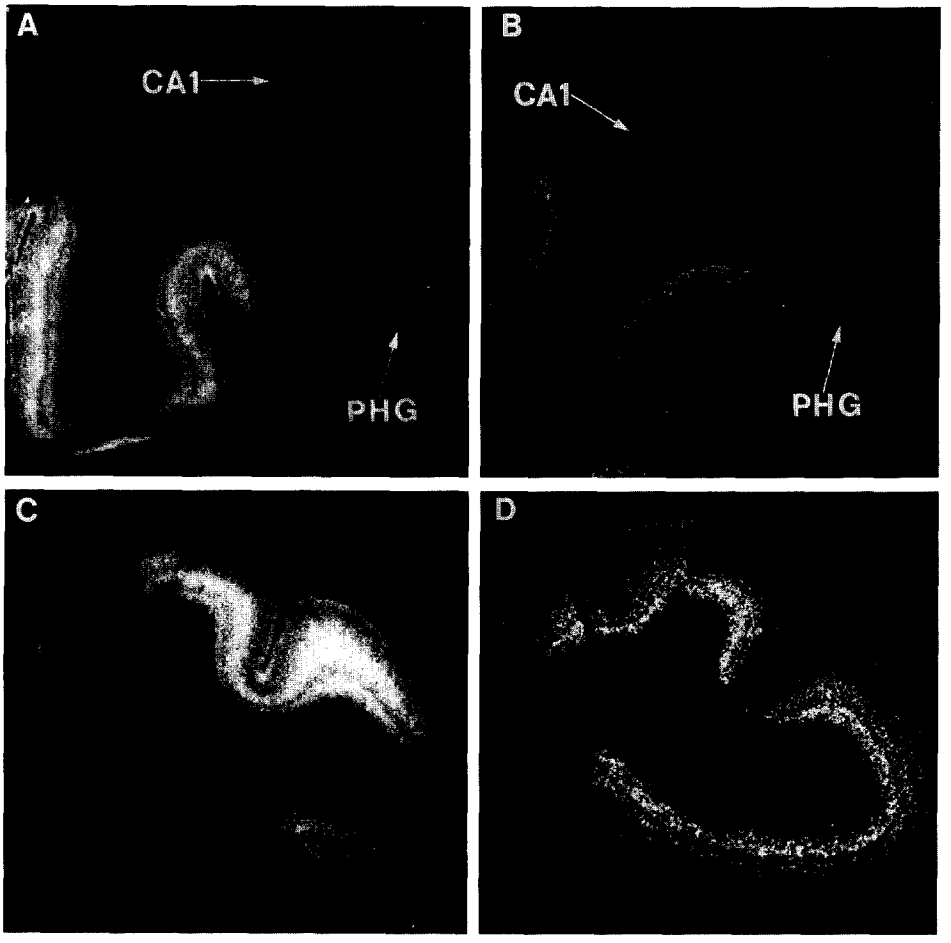

Figure 2. Autoradiograms showing the distribution of [^{3}H]ketanserin binding sites (**A** and **C**) and 5-HT_{2A} receptor mRNA (**B** and **D**) in the normal human hippocampus (**A** and **B**) and superior temporal gyrus (**C** and **D**). CA1: CA1 subfield; PHG: parahippocampal gyrus.

Table 2. Effect of Age, Brain pH, and Post Mortem Interval on 5-HT_{1A} and 5-HT_{2A} Receptor mRNAs Showing the β Values Arising from Linear Regression

Brain region	Age	pH	Post mortem interval
5-HT_{1A} receptor mRNA			
Dentate gyrus	−0.362	0.288	−0.592[b]
CA1	−0.430	0.172	−0.575[b]
PHG	−0.436[a]	0.334	−0.455[a]
BA22	−0.326	−0.039	−0.050
BA24	0.095	0.758[c]	0.146
BA46	−0.632[b]	0.043	0.073
5-HT_{2A} receptor mRNA			
Dentate gyrus	−0.034	0.667[b]	−0.246
BA17	−0.047	0.546[b]	−0.414[a]
BA22	−0.521[a]	0.345	−0.246
BA24	−0.287	0.591[c]	−0.382[a]
BA46	−0.162	0.671[c]	−0.369[a]

PHG: parahippocampal gyrus.

Increasing age, decreasing pH, and increasing post mortem interval are seen to be independently associated with reduced abundance of both mRNAs in some areas.

[a] $p < .05$.

[b] $p < .01$.

[c] $p < .001$.

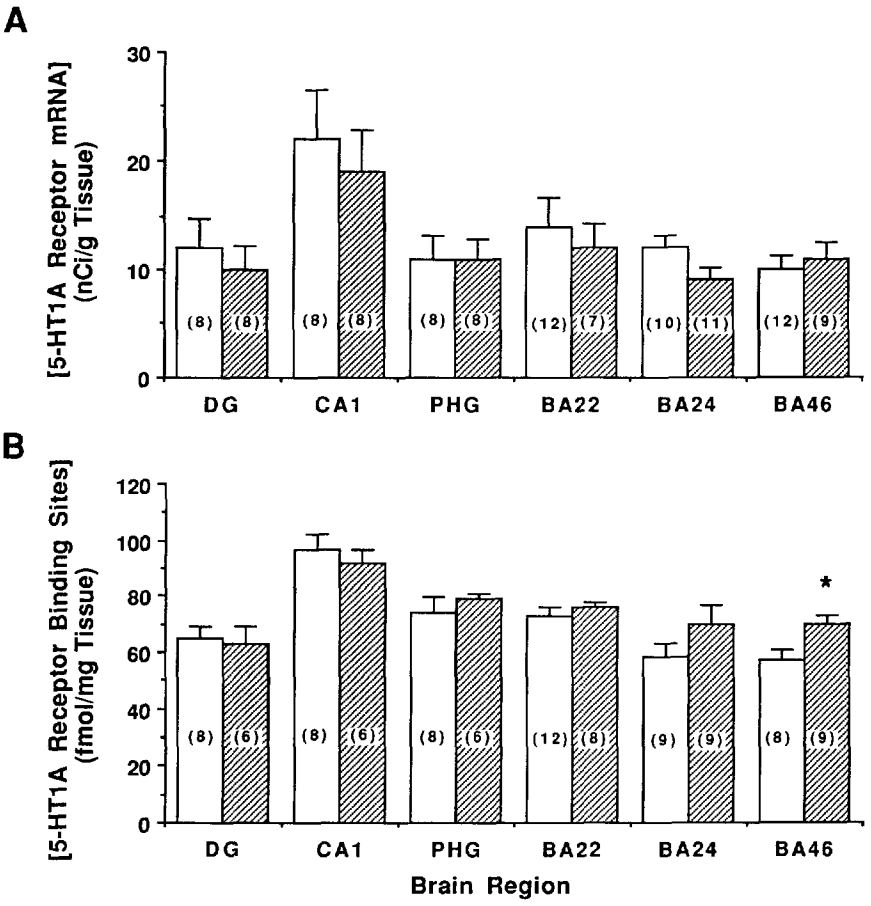

Figure 3. 5-HT_{1A} receptor mRNA abundance (**A**) and [^{3}H]8-OH-DPAT binding site densities (**B**) in controls (*open bars*) and schizophrenics (*shaded bars*) in dentate gyrus of the hippocampus (DG), CA1, parahippocampal gyrus (PHG), superior temporal gyrus (BA22), anterior cingulate gyrus (BA24) and dorsolateral prefrontal cortex (BA46). The number of cases studied in each area are indicated in parentheses. *$p < .01$.

Table 3. [^{3}H]8-OH-DPAT Binding Site Densities in the Deep Laminae of Cortical Regions of Controls and Schizophrenics

Brain Region (Deep Laminae)	[5-HT$_{1A}$ Receptor Binding Sites] (fmol/mg Tissue)	
	Control	Schizophrenic
PHG	46.1 ± 2.6	41.1 ± 3.2
BA22	28.5 ± 1.1	30.4 ± 0.8
BA24	23.3 ± 2.1	25.3 ± 2.2
BA46	22.1 ± 1.5	24.7 ± 1.7

No differences between the groups are seen, in contrast to the superficial laminae (Figure 3B).

binding site densities in the other regions, and in the deep laminae of all neocortical areas (Table 3), remained unaltered in schizophrenia.

5-HT$_{2A}$ Receptor mRNA Abundance and Binding Site Densities in Schizophrenia

5-HT$_{2A}$ receptor mRNA abundance was markedly decreased in all neocortical regions but not in parahippocampal gyrus (p = .079) of the schizophrenics, having controlled for the effect of pH, post mortem interval and age (Figure 4 and 5A). 5-HT$_{2A}$ receptor binding site densities were significantly reduced in BA46 and in the parahippocampal gyrus of the schizophrenics, with a similar trend in BA24 (p = .054) but not in BA17 or BA22 (Figure 5B). The decrease of 5-HT$_{2A}$ receptor mRNA and [^{3}H]ketanserin binding remained significant and of similar magnitude after omission of the two suicides in each group (data not shown).

5-HT$_{1A}$ and 5-HT$_{2A}$ Receptor Expression in Cortex and Hippocampus of Rats Treated with Chronic Haloperidol

Treatment of rats for 16 weeks with haloperidol decanoate did not affect 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor mRNAs, nor [^{3}H]8-OH-DPAT and [^{3}H]ketanserin binding site densities (Table 4).

DISCUSSION

The present study has identified anatomically and molecularly complex changes in 5-HT$_{1A}$ and 5-HT$_{2A}$ re-

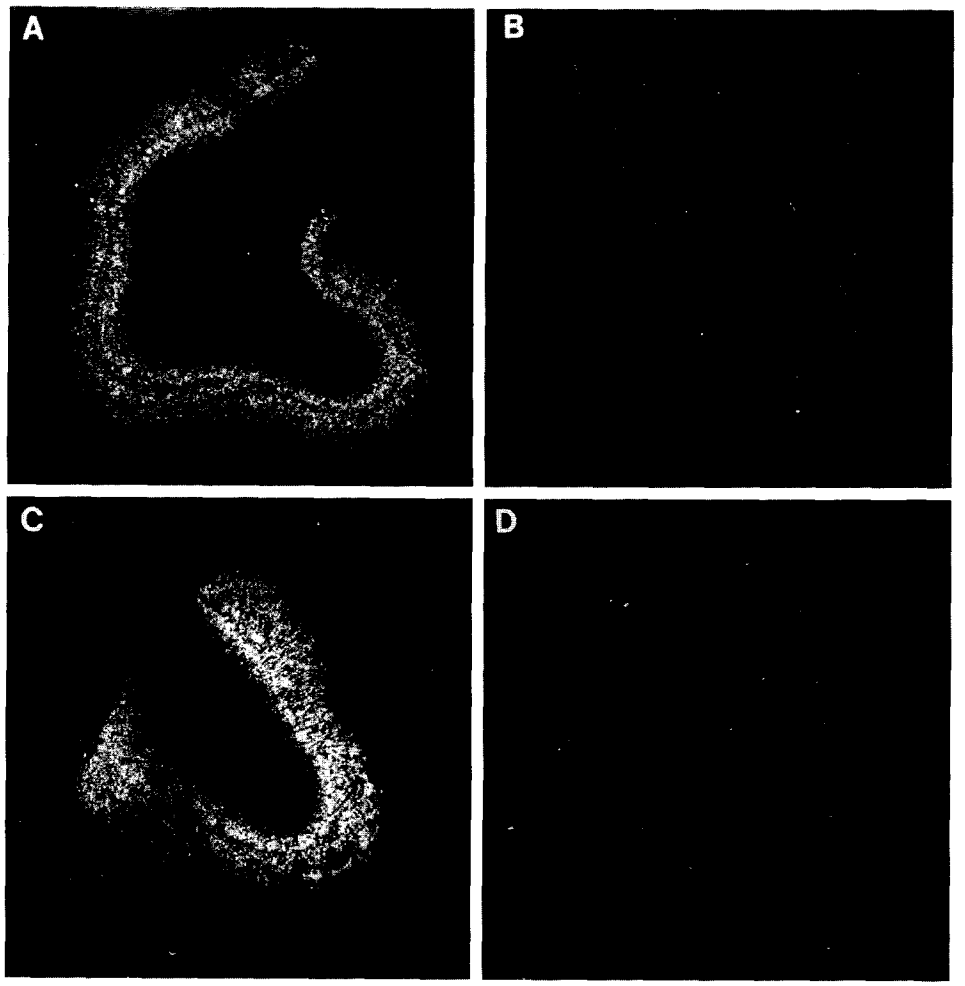

Figure 4. Representative autoradiograms of 5-HT$_{2A}$ receptor mRNA in superior temporal gyrus (**A** and **B**) and dorsolateral prefrontal cortex (**C** and **D**) illustrating the reduction of this mRNA in both areas in schizophrenics (**B** and **D**) compared with controls (**A** and **C**).

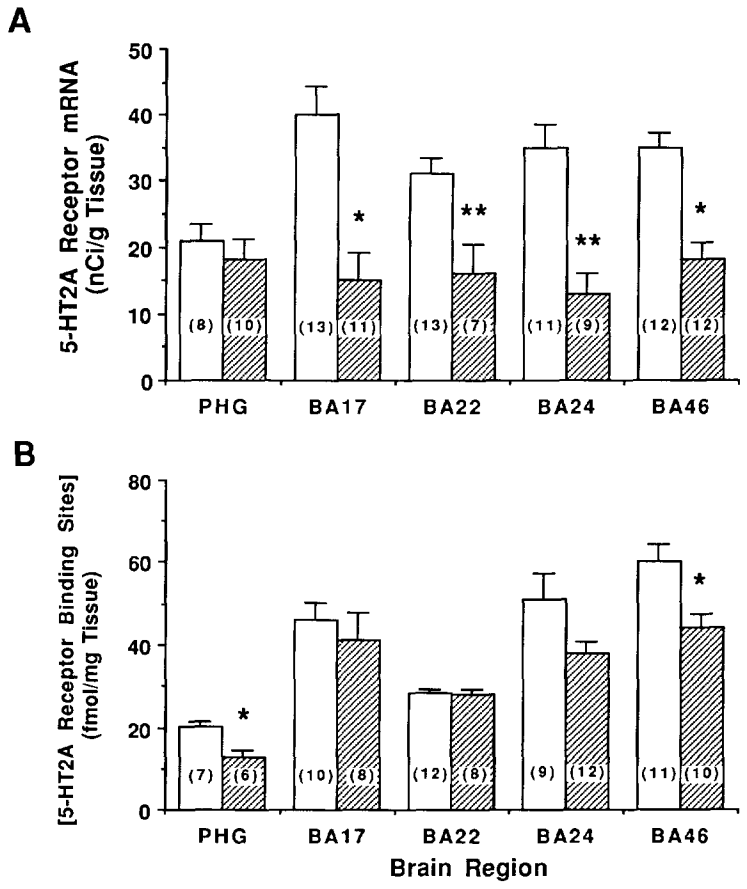

Figure 5. 5-HT_{2A} receptor mRNA abundance (**A**) and [^{3}H]ketanserin binding site densities (**B**) in controls (*open bars*) and schizophrenics (*shaded bars*). Abbreviations as in Figure 3. *$p < .01$, **$p < .001$.

ceptor gene expression in schizophrenia. Before discussing these data, some of the methodologic factors that affect their interpretation are outlined. There are also conceptual points about the interpretation of changes in mRNA abundance when they are not paralleled by that of the encoded receptor (as per the 5-HT_{2A} receptor data) and vice versa (as per the 5-HT_{1A} receptor data).

Factors Affecting 5-HT_{1A} and 5-HT_{2A} Receptors and Their mRNAs in Human Brain

Many demographic and perimortem factors influence 5-HT receptor expression in addition to any effect of schizophrenia. For example, 5-HT_{1A} and possibly 5-HT_{2A} receptor densities are reduced during aging (Dillon et al. 1991; Burnet et al. 1994a), and many mRNAs are highly susceptible to pre mortem acidosis (a marker of a poor agonal state) as well as to prolonged (>60 hour) post mortem interval (Barton et al. 1993; Harrison et al. 1995; Kingsbury et al. 1995). The present data illustrate the importance of these confounding factors (Table 2). As well as careful matching of our disease and control group for all the known variables of this kind (Table 1), we used multiple regression and covariate analysis to identify and control for them. We also limited our study to brains free of coincidental neurodegenerative pathologies, as these can affect 5-HT_{1A} and 5-HT_{2A} receptors (Blin et al. 1993; Francis et al. 1993). These methodologic features mean that the significant differences associated with a diagnosis of schizophrenia that we observed, though fewer in number than some previous studies, should be robust.

A separate potential confounder is the influence of antipsychotic medication on 5-HT_{1A} and 5-HT_{2A} receptors. In the absence of adequate brain tissue from drug-naive schizophrenics, it is difficult to overcome this problem. However, a number of considerations are relevant. We treated rats with chronic haloperidol to simulate the effect of long-term medication, which all of our cases had received, and found no changes in any parameter (Table 3). A similar result was observed after 2 weeks' haloperidol (Burnet et al. 1996). Other studies also show a lack of effect of haloperidol in rodents (Matsubara and Meltzer 1989) and, importantly, in primates (Lidow et al. 1995). Additionally, Mita et al. (1986) found that losses of 5-HT_{2A} receptors were similar in drug-free and drug-treated schizophrenic subjects. Coupled with the anatomically discrete nature of the 5-HT receptor abnormalities reported here, these findings provide some reassurance that the changes are not likely to be secondary to medication. However, it must be borne in mind that some typical antipsychotics,

Table 4. [^{3}H]8-OH-DPAT and [^{3}H]Ketanserin Binding Site Densities, and 5-HT$_{1A}$ and 5-HT$_{2A}$ Receptor mRNA Abundance, in Rats Treated with Haloperidol Decanoate for 16 weeks

		Binding sites (fmol/mg Tissue)		mRNA (nCi/g Tissue)	
Receptor	**Brain Region**	**Control**	**Haloperidol**	**Control**	**Haloperidol**
5-HT$_{1A}$	Hippocampus				
	Dentate Gyrus	161.9 ± 3.0	160.2 ± 3.1	196.4 ± 6.2	195.8 ± 5.8
	CA3c	189.3 ± 10.2	195.4 ± 9.4	180.1 ± 4.0	176.3 ± 4.0
	CA3a-b	102.3 ± 3.2	106.2 ± 3.6	98.9 ± 6.4	99.3 ± 5.0
	CA1	124.3 ± 3.8	126.4 ± 4.0	121.4 ± 4.2	117.7 ± 3.8
	Neocortex				
	Cingulate	51.2 ± 2.6	55.7 ± 3.6	50.9 ± 4.3	53.8 ± 3.8
	Frontoparietal				
	(deep layers)	57.4 ± 3.9	57.3 ± 4.1	54.6 ± 3.5	56.2 ± 3.1
	(superficial layers)	30.0 ± 2.0	29.1 ± 1.6	26.8 ± 2.0	27.4 ± 2.0
5-HT$_{2A}$	Neocortex				
	Cingulate	30.3 ± 4.1	31.4 ± 3.8	82.4 ± 7.9	80.3 ± 7.8
	Frontoparietal	24.8 ± 3.2	25.7 ± 3.4	61.5 ± 5.9	62.8 ± 6.0

Values are mean ± SEM. No differences are seen between the two groups.

especially those with a relatively high affinity for 5-HT$_{2A}$ receptors, may down-regulate 5-HT$_{2A}$ binding sites (Andree et al. 1986).

5-HT$_{1A}$ Receptor Expression in Schizophrenia

We found that 5-HT$_{1A}$ receptor densities, as determined by quantitative [^{3}H]8-OH-DPAT autoradiography, are increased in the prefrontal cortex (BA46) in schizophrenia (Figure 3B). This confirms and extends previous observations in other frontal regions (Hashimoto et al. 1991; Joyce et al. 1993; Simpson et al. 1996). The increase was limited to the superficial laminae (Table 3), as it was in the study of Simpson et al. (1996) in ventral prefrontal cortex. The laminar selectivity of the increase may be regionally specific, as it was not apparent in BA9 or in more posterior cortical areas (Joyce et al. 1993). We did not replicate the finding of Simpson et al. (1996) that the increase in prefrontal 5-HT$_{1A}$ receptors is greater in male than female schizophrenics (data not shown).

The interpretation of the laminar selectivity of the increase in 5-HT$_{1A}$ receptors requires consideration of the cell types that express them and their position in the local chemoarchitecture. In neocortex, including BA46, 5-HT$_{1A}$ receptor mRNA expression occurs in pyramidal neurons, more so in those of lamina III than of lamina V/VI (Burnet et al. 1995a). No expression in interneurons was seen. It is therefore likely that 5-HT$_{1A}$ receptors themselves are located primarily on the dendrites of lamina III pyramidal neurons (Gerard et al. 1994), which course upward toward the pial surface, giving rise to high densities of 5-HT$_{1A}$ receptors in the superficial laminae. Only dendrites of lamina V/VI pyramidal neurons will be present over the deeper laminae. From these considerations, it may be inferred that the increase of [^{3}H]8-OH-DPAT binding sites in superficial but not deep laminae arises selectively from lamina III pyramidal neurons. This neuron population is the main source of corticocortical association fibres (Jones 1984), and their activity is regulated at least partly by 5-HT$_{1A}$ receptors, which are inhibitory (see Francis et al. 1993). Increased 5-HT$_{1A}$ receptor densities might thereby inhibit transmission in these pathways and impair integration of frontal cortex activity with other cortical regions. Though clearly speculative, such a suggestion is in keeping with models that implicate dysfunction of these connections in schizophrenia (e.g., Hoffman and McGlashan 1994; Schlaepfer et al. 1994; Weinberger and Lipska 1995). A similar argument would suggest that the unaltered level of 5-HT$_{1A}$ receptor binding sites in deep laminae (Table 3) means that any postulated impairment of corticothalamic or corticostriatal circuits (which arise from lamina V/VI pyramidal neurons) in schizophrenia cannot be attributed to increased 5-HT$_{1A}$ receptors on those neurons.

Elevation of prefrontal 5-HT$_{1A}$ receptors was not accompanied by any increase of the encoding mRNA (Figure 3A). In normal human brain there is a correlation between these parameters (Burnet et al. 1994a), as there is in some experimental situations (e.g., Burnet et al. 1995b). Conversely, there are also precedents for mRNA-independent alterations of 5-HT$_{1A}$ receptor densities (Harrington and Peroutka 1991; Burnet et al. 1994b).

ISHH studies similar to those reported here have detected changes in mRNA levels which do correspond to changes in abundance of the encoded protein (e.g., Eastwood et al. 1995; Eastwood and Harrison 1995, and unpublished observations). A lack of methodologic sensitivity is therefore unlikely, and we conclude that the data indicate a genuine unaltered abundance of 5-HT_{1A} receptor mRNA. If so, the increase in 5-HT_{1A} receptors indicated by [^{3}H]8-OH-DPAT binding site densities must arise from translational or post-translational processes, such as a greater rate of translation per mRNA molecule, protein phosphorylation, or differences in the cellular distribution of the receptor. Furthermore, [^{3}H]8-OH-DPAT only binds to a 5-HT_{1A} receptor when it is coupled to its G protein; thus, an increase in the percentage of coupled receptors could produce the change observed in schizophrenia. This possibility could be assessed using recently available 5-HT_{1A} antagonists, such as [^{3}H]WAY100,635, which detect both forms of the receptor (Khawaga 1995). This ligand also has the advantage of high specificity for the 5-HT_{1A} receptor, overcoming the possibility that changes in [^{3}H]8-OH-DPAT binding might arise from another receptor subtype, such as the 5-HT_7 receptor (Lovenberg et al. 1993). Regardless of which mechanism explains the discrepancy between mRNA and binding site densities, its existence illustrates the value of measuring both parameters as a way to narrow down the points within the gene expression pathway at which the increase is likely to have occurred (Harrison 1995b).

Like Joyce et al. (1993), we found only a trend toward increased 5-HT_{1A} receptor binding sites in the superficial laminae in anterior cingulate gyrus (BA24) but, unlike their study, we did not find increased binding in the deep laminae (Table 3). Neither did we confirm the findings of increased 5-HT_{1A} receptors in hippocampus (Joyce et al. 1993) and temporal cortex (Hashimoto et al. 1991; Joyce et al. 1993.) Anatomic, including laminar, heterogeneity of 5-HT_{1A} receptor alterations in schizophrenics in the cingulate gyrus and temporal lobe may explain these differences.

5-HT_{2A} Receptor Expression in Schizophrenia

5-HT_{2A} receptor densities, as assessed by [^{3}H]ketanserin, were significantly reduced in the dorsolateral prefrontal cortex in schizophrenia (Figure 5B), confirming and extending earlier findings in several frontal regions (Bennett et al. 1979; Mita et al. 1986; Arora and Meltzer 1991; Laruelle et al. 1993). The magnitude of the reduction is similar (27%) to that observed using full saturation analyses of the orbitofrontal cortex (Laruelle et al. 1993). We also demonstrated reduced [^{3}H]ketanserin binding sites in the parahippocampal gyrus and a similar trend in the cingulate gyrus (BA24), indicating that the decrease is not limited to the frontal lobe. We observed no reduction in the striate cortex (BA17) in keeping with the finding of Laruelle et al. (1993). The increase in 5-HT_{2A} receptor binding site densities in the temporal cortex reported by Joyce et al. (1993) was not reproduced in this study. This difference may again be explained by anatomic heterogeneity, as Joyce and colleagues sampled middle temporal gyrus (BA21). Alternatively, the different profile of binding sites detected by [^{125}]LSD compared with [^{3}H]ketanserin may be relevant, as discussed previously. It raises the possibility that in schizophrenia there may be an increase in the density of non-5-HT_{2A} receptor binding sites that are detected by LSD, such as the 5-HT_{2C} receptor.

Decreased 5-HT_{2A} receptor mRNA in schizophrenia was present in all the neocortical regions we examined (Figure 5A). We emphasize that, although this mRNA proved to be particularly sensitive to pH and post mortem interval (Table 2), the reductions were present having controlled for these factors. We therefore view this conclusion as robust and an independent confirmation that 5-HT_{2A} receptor expression is reduced widely in the cortex in the disease. However, interpreting the origins and implications of this finding is complicated by the differential changes observed in 5-HT_{2A} receptor mRNA compared with binding site densities. In contrast to the 5-HT_{1A} receptor changes, we have to account for a change in the mRNA which is more widespread and of greater magnitude than the receptor it encodes. The reduction of 5-HT_{2A} receptor mRNA implies, by definition, impaired expression of the 5-HT_{2A} receptor gene. Preservation of the output of the gene expression pathway, i.e., receptor densities, suggests the occurrence of compensatory mechanisms. Thus, in schizophrenia, perhaps the decrease of 5-HT_{2A} receptor mRNA is counterbalanced by an increase in translational and/or post-translational processes that replenish binding sites. The compensatory mechanism could be said to be successful in BA17 and BA22, but incomplete in BA46, BA24, and parahippocampal gyrus. Of note, 5-HT_{2A} receptor mRNA and 5-HT_{2A} receptor binding sites often change differentially (Roth and Ciaranello 1991; Burnet et al. 1995b), and the expression of this gene has other unusual features, such as antagonist treatment leading to paradoxical receptor down-regulation (Blackshear et al. 1986), whereas agonists may produce up-regulation (Akiyoshi et al. 1993). The molecular basis for these phenomena is being elucidated (Ferry et al. 1993; Toth and Shenk 1994; Zhu et al. 1995) and may in time explain the pattern of 5-HT_{2A} gene expression alterations reported here.

The 5-HT_{2A} receptor changes can also be considered from the perspective of their effects on local circuitry. Most studies have concluded that 5-HT_{2A} receptors in the cortex are expressed primarily by interneurons, al-

though some electrophysiologic data suggest that pyramidal neurons express them as well (see Burnet et al. 1995a). Confirmation that pyramidal neurons do express 5-HT_{2A} receptors has come from recent ISHH data in both rat (Burnet et al. 1995a; Rahman et al. 1995) and human (Burnet et al. 1995a) brain. Indeed, in most cortical areas examined, 5-HT_{2A} receptor mRNA was detected at higher levels in pyramidal neurons than in interneurons. The fact that the receptor is expressed by neurons of both types makes discussion of the overall effects of a reduction in schizophrenia difficult to interpret at the cytoarchitectural level. A loss of pyramidal neuron 5-HT_{2A} receptors would be expected to decrease their excitation, compounding the effect discussed previously arising from an increase in 5-HT_{1A} receptors. However, a loss of 5-HT_{2A} receptors from interneurons, which are mostly inhibitory upon pyramidal neurons (Nieuwenhuys 1994), would counteract this process.

The unusual aspects of 5-HT_{2A} receptor gene regulation may also bear on a separate issue: decreased 5-HT_{2A} receptor expression in schizophrenia is intriguing given that clozapine also down-regulates the receptor (Lee and Tang 1984; Matsubara and Meltzer 1989; Wilmot and Szczepanik 1989; Burnet et al. 1996) and its mRNA (Burnet et al. 1996). Conventionally, therapeutic drugs are envisaged to act by ameliorating an underlying abnormality, such as D_2 receptor antagonism by antipsychotics counteracting the putative dopaminergic overactivity in schizophrenia. However, the present data suggest that, if the 5-HT_{2A} receptor is indeed an important target of atypical antipsychotics (Meltzer 1992; Schmidt et al. 1995), then a model of this kind is too simple. Perhaps the 5-HT_{2A} receptor changes in schizophrenia are part of a secondary response to the disease process, with further down-regulation being beneficial.

Finally, comparison of the 5-HT_{1A} and 5-HT_{2A} receptor data can help distinguish whether each alteration is a molecularly specific change or whether it reflects a more widespread functional or numeric change in the cell types expressing them. As 5-HT_{1A} and 5-HT_{2A} receptors changed differentially in most cortical areas, we can exclude differences in densities or overall gene expression rates of pyramidal neurons (the only cell type expressing both receptors) in schizophrenia as accounting for the data. On the other hand, decreased 5-HT_{2A} receptor mRNA could be due at least partially to a loss of the interneurons that express them (see Benes et al. 1991). Ideally these questions could be addressed by quantitative ISHH at a cellular resolution, but this may be problematic given the low level of interneuron 5-HT_{2A} receptor mRNA.

Further Considerations

The diverse changes in the 5-HT system in schizophrenia lack a unifying explanation. One parsimonious hypothesis is that the alterations might be secondary to pathology in 5-HT neurons. An involvement of the raphe in schizophrenia has been proposed on several grounds (Doty 1989; Shapiro 1993), and a recent report provides preliminary evidence for a loss of raphe 5-HT neurons (Chen et al. 1995). Equally, however, such presynaptic cellular abnormalities could be the result of chronic changes in post-synaptic cells, akin to the secondary cholinergic cell pathology in the basal forebrain in Alzheimer's disease (Harrison 1986). Distinguishing between these possibilities would be facilitated by studies of the long-term effects of developmental serotonergic lesions and of receptor up- and down-regulation (cf., Park et al. 1993; Pranzatelli et al. 1994). There is already evidence that neonatal dopaminergic lesions induce persistent changes in striatal 5-HT innervation (Mrini et al. 1995) and in cortical 5-HT_{2A} receptors (Radja et al. 1993). These data provide theoretical support for a developmental, presynaptic origin for 5-HT receptor changes in schizophrenia, as well as illustrating the close relationship between dopaminergic and serotonergic systems, whereby alterations in 5-HT parameters may be reconciled with the dopaminergic hypothesis of the disease (Kahn and Davidson 1993). Parenthetically, early 6-hydroxydopamine lesions affect the relationship between dopamine receptors and their mRNAs (Frohna et al. 1995), giving a precedent for a developmental origin of the 5-HT receptor mRNA/protein "uncoupling" seen here.

In summary, we have identified differential and localized alterations of 5-HT_{1A} and 5-HT_{2A} receptor gene expression in the cerebral cortex in schizophrenia. These findings broadly support and extend existing evidence for the involvement of these receptors in the disease. However, the underlying pathophysiologic mechanisms in schizophrenia are clearly complex with respect to the relationship between mRNA and receptor protein, and between one cell population and another. Future studies will therefore need to investigate how regulation of 5-HT receptor gene expression is coordinated as well as to integrate neurochemical with cytoarchitectural considerations.

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