Article

Ionotropic Glutamate Receptors and Expression of *N*-Methyl-D-Aspartate Receptor Subunits in Subregions of Human Hippocampus: Effects of Schizophrenia

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Objective: Multiple quantifiable biologic abnormalities have been localized to the hippocampus in schizophrenia. Alterations in glutamate-mediated transmission at *N*-methyl-D-aspartic acid (NMDA)-sensitive receptors in hippocampus have been implicated in the pathophysiology of the illness. The authors tested the hypothesis that glutamatergic transmission within and efferent from hippocampus is altered in schizophrenia.

Method: The authors analyzed postmortem hippocampal tissue from individuals with schizophrenia and from healthy individuals. The tissue samples had been collected by two brain tissue banks, one in Maryland and the other in Melbourne, Australia. Ionotropic receptor binding for the NMDA, kainate, and ^{3}H-amino-3-hydroxy-5-methylisoxazol-4-propionate (AMPA) receptors was quantified by using usual radioligand techniques. In situ hybridization autoradiography was used to quantify mRNA for the NMDA receptor subunits NR1, NR2A, and NR2B.

Results: Ligand binding to the ionotropic glutamate receptors (NMDA, kainate, and AMPA) did not differ significantly overall or in any subregion between the schizophrenia tissue and the healthy comparison tissue. The only exception was AMPA receptor binding in hippocampal subregion CA2, which was slightly but significantly less in schizophrenia. However, the level of mRNA for the NMDA receptor subunits NR1 and NR2B was significantly different between groups; in several hippocampal subregions, the level of NR1 mRNA was lower and the level of NR2B mRNA higher in schizophrenia.

Conclusions: Because the NR1 subunit of the NMDA receptor is critical to full receptor activity, a reduction of NR1 in hippocampus in schizophrenia suggests a functional impairment in glutamatergic transmission at the NMDA receptor, resulting in reduced glutamatergic transmission within and possibly efferent from the hippocampus in schizophrenia. This defect could underlie a hypoglutamatergic state in regions of limbic cortex, consistent with published results from other lines of research in schizophrenia.

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Several lines of evidence, including in vivo human imaging results and postmortem tissue findings, suggest abnormalities of human hippocampal structure and function in schizophrenia. In vivo, hippocampal size in persons with schizophrenia is reduced bilaterally, albeit mildly, especially in anterior regions (1–5). Structural and histologic abnormalities in postmortem hippocampal tissue from affected persons have been repeatedly reported (6–11), although some of these findings have not been consistently replicated (12). Moreover, in vivo functional studies of persons with schizophrenia have directly demonstrated an alteration in neuronal activity in the limbic system and/or parahippocampal gyrus, as measured by positron emission tomography and [^{18}F]fluorodeoxyglucose or regional cerebral blood flow techniques (13–17). In addition, considerable evidence of compromised cognitive function, especially in short-term memory and attention, exists in schizophrenia (18–20); these dysfunctions may represent the behavioral correlates of hippocampal pathology.

Studies from our laboratory using an in vivo animal preparation of psychosis with phencyclidine (PCP) support this focus. Because PCP and its congener ketamine induce psychotic-like phenomena in normal persons (21–23) and psychosis exacerbation in schizophrenia (21, 24), PCP's actions in animals have been studied to shed light on the mechanisms of psychosis, and even of schizophrenia, in humans (25). Changes in neuronal activity and immediate early gene activation/repression in rat brain after PCP occur predominantly in limbic regions, including hippocampus (26, 27). Moreover, an increase in *N*-methyl-D-aspartic acid (NMDA)-sensitive glutamate receptor binding occurs exclusively in rat hippocampus 12–24 hours after PCP administration (28, 29). The selective PCP agonist MK801 also demonstrates these actions. One parsimonious explanation for these PCP-induced neurochemical

TABLE 1. Characteristics of Postmortem Brain Tissue From Subjects With Schizophrenia and Normal Comparison Subjects

Brain Tissue Collection and Study Group	Subjects' Sex[a]		Subjects' Age at Death (years)		Postmortem Interval (hours)[b,c]	
	Male	Female	Mean	SD	Mean	SD
Maryland Brain Collection						
Schizophrenia (N=12)	4	8	50.8	15.7	11.4	4.3
Comparison (N=16)	6	10	49.1	17.5	16.7	5.7
Melbourne Brain Collection 1						
Schizophrenia (N=7)	7	0	37.0	13.9	43.9	16.5
Comparison (N=7)	7	0	35.9	13.4	34.8	9.7
Melbourne Brain Collection 2						
Schizophrenia (N=12)	10	2	41.8	16.3	38.8	14.4
Comparison (N=12)	10	2	42.5	16.7	42.7	15.0

[a] Significant difference between brain tissue collections (F=15.83, df=2, 63, p=0.0001).
[b] Time between death and autopsy for tissue from subjects whose death was witnessed; for other tissue, time halfway between time the subject was found dead and time the subject was last seen alive.
[c] Significant difference between brain tissue collections (F=41.32, df=2, 63, p=0.0001).

changes in rat hippocampus is that PCP administration causes inhibition at the NMDA-sensitive glutamate receptor in the trisynaptic perforant pathway, and the effect is additive within the hippocampal projection fields and becomes greatest in the Schaffer collateral terminal field of CA1. This inhibition could result in a functionally significant reduction in glutamatergic output from the hippocampal cortex to other limbic regions, including but not limited to an effect on the anterior cingulate cortex. We have speculated that this reduction in excitatory output from hippocampus and reduced afferent cingulate stimulation is associated with psychosis across psychotic diagnoses, whether the psychotic symptoms are induced by PCP or associated with schizophrenic psychosis (25).

These findings, along with additional data (25, 30), encouraged a hypothesis of reduced glutamatergic output from hippocampus and insufficient afferent activation of the anterior cingulate cortex as a mechanism for human psychosis and for aspects of schizophrenia, especially positive psychotic symptoms and possibly cognitive dysfunction. To test this hypothesis further, we collected postmortem brain tissue from persons with schizophrenia and a healthy comparison group and measured ionotropic glutamate receptor binding and NMDA receptor subunit composition in hippocampus, looking for evidence of a regional endogenous alteration in glutamate-mediated neurotransmission in schizophrenia.

Method

Materials

^{3}H-Glutamic acid (54.1 Ci/mmol), ^{3}H-amino-3-hydroxy-5-methylisoxazol-4-propionate (AMPA) (56.6 Ci/mmol), and ^{3}H kainic acid (58 Ci/mmol) were obtained from New England Nuclear (Boston). NMDA, AMPA, kainate, quisqualate, and potassium thiocyanate were purchased from Sigma (St. Louis). Deoxyadenosine 5′-a-(^{35}S) thiotriphosphate trimethylammonium salt (^{35}S-dATP) (1000 Ci/mmol) was obtained from Amersham (Piscataway, N.J.).

Postmortem Human Tissue

Postmortem human brain tissue was analyzed in three different tissue sets, one from the Maryland Brain Collection of the Maryland Psychiatric Research Center, Baltimore, and two from the Melbourne Brain Collection of the Mental Health Research Institute, Parkville, Victoria, Australia. The tissue from the Maryland Brain Collection was collected from 12 normal subjects and 16 subjects with schizophrenia. The tissue from the Melbourne Brain Collection was collected from 19 normal subjects and 19 subjects with schizophrenia. The normal and schizophrenia groups within each sample were matched as closely as possible on age, sex, and postmortem interval. (For donors whose deaths were witnessed, the postmortem interval was the time between death and autopsy. For others, the postmortem interval was halfway between the time the donor was found dead and the time the donor was last seen alive.) Tissue was rapidly frozen to –70°C and stored until required. The tissue storage time at –70°C before assay did not differ between the schizophrenia group and the normal comparison group. In addition, in the tissue sets from the Melbourne Brain Collection, the pH of the brain tissue was measured, as described previously (31), and did not differ between the two groups. Coronal tissue slices were cut approximately 2 cm thick at a mid-anterior level of the hippocampus, and the hippocampus was identified and blocked. Blocks posterior to the fimbria but anterior to the anterior-posterior midpoint of the hippocampus were used, obtained similarly in the schizophrenia tissue and the comparison tissue in each tissue set. However, neither landmarks nor histologic characteristics were used to standardize the anterior-posterior position. Regions in the hippocampal tissue that were measured are identified in Figure 1. Blocks were covered with mounting media (Lipshaw, Detroit) to reduce desiccation and stored at –70°C until further processing. Sections were cut at 20 μm using a cryostat, thaw-mounted onto Probe-On microscope slides (Fisher, Pittsburgh), and stored at –70°C until further processing. For tissue from Melbourne Brain Collection, frozen 20 μm sections were cut in Australia from hippocampal tissue blocks and placed on Probe-On slides. The slides were placed in a slide box with desiccant and air freighted to the United States in excess dry ice.

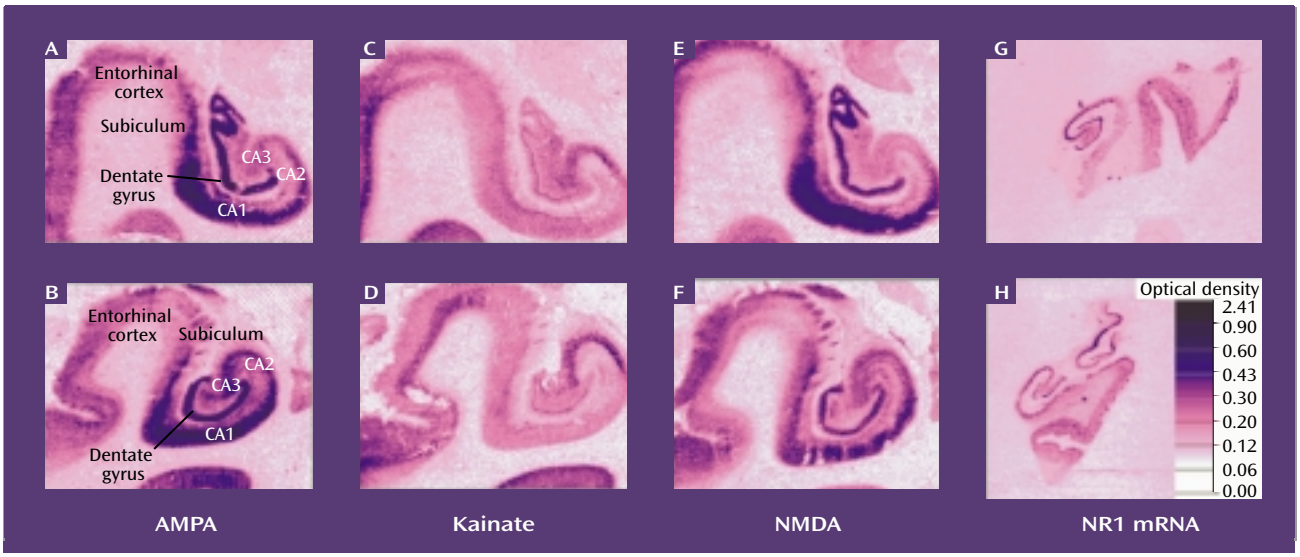
After the collection of tissue, two experienced research clinicians carried out an extensive review of the case histories of the subjects with a provisional diagnosis of schizophrenia by using a structured instrument (32). Diagnoses of schizophrenia were confirmed by consensus of the clinicians according to DSM-III-R criteria. Demographic characteristics associated with the tissue are presented in Table 1. The most recent doses of antipsychotic drugs recorded in the case histories were noted and equivalence calculated (33).

Chronic Administration of Antipsychotic Drugs in Rats

Because antipsychotic treatment is nearly ubiquitous in persons with schizophrenia, we tested whether the density of glutamate receptors in schizophrenia could be confounded by effects of antipsychotic treatment on the receptors. We measured the effects of chronic administration of haloperidol on the density of glutamate receptors and NMDA subunits in rat brain.

We treated Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA,) with water containing haloperidol or no drug for 6 months and compared ionotropic glutamate receptor density in hippocampus in both groups of rats to suggest any change associated with chronic antipsychotic treatment in the

FIGURE 1. Autoradiograms of Postmortem Brain Sections Showing NMDA, Kainate, and AMPA Receptor Binding and Level of mRNA for NMDA Subunit NR1 in Hippocampal Subregions of Normal Comparison Subjects and Subjects With Schizophrenia[a]



[a] Panels A, C, E, and G: tissue from normal comparison subjects; panels B, D, F, and H: tissue from subjects with schizophrenia.

measures we assessed in human tissue. The rats were given drinking water containing either no drug or haloperidol (34) and housed in 12-hour light/dark conditions with ad libitum food and water. Haloperidol was dissolved in 10% glacial acetic acid. The solution was diluted with distilled water and the pH was adjusted to 5.5–6.0 by using 10 N sodium hydroxide to give a stock solution of 0.25 mg/ml. This solution was further diluted with distilled water to 0.025 mg/ml every 7 days. The dose of haloperidol in the drinking water was modified according to the measured daily water intake of the rats. Trunk blood was collected at the time of sacrifice at 6 months, and the plasma was frozen at –20°C until analysis. Haloperidol was quantified by using a modification of the method of Bianchetti and Morsel in which chlorohaloperidol was used as the internal standard and reduced haloperidol was simultaneously determined. Quantification of haloperidol was done in the laboratory of Thomas Cooper (Nathan S. Kline Institute, Orangeburg, N.Y.).

Rats were sacrificed by decapitation at the end of the period of chronic drug administration. The brains were rapidly removed, frozen by immersion in –40°C isopentane, mounted using embedding matrix, and stored at –80°C. Coronal sections (20 µm thick) were cut in a cryostat from several coronal levels, thaw-mounted onto Probe-On microscope slides, and dried at room temperature under an airflow. The slides were kept desiccated at –80°C until they were used in the binding experiments.

Receptor Autoradiography

All receptor and message analysis was carried out in the Maryland Psychiatric Research Center laboratory. For glutamate receptor binding, all sections were initially washed for 30 minutes and preincubated for 10 minutes in appropriate buffer at 0–4°C to eliminate endogenous glutamate. For NMDA receptor binding, slides were incubated for 45 minutes at 0–4°C with 100 nM ^{3}H-glutamate in the presence of 2.5 µM quisqualate and 1 µM kainate in 50 mM Tris acetate buffer at pH 7.0. For nonspecific binding, 500 µM NMDA was used. For AMPA receptor binding, slides were incubated for 45 minutes at 0–4°C with 37 µM ^{3}H AMPA in the presence of 2.5 mM $CaCl_2$ and 100 mM potassium thiocyanate in 50 mM Tris HCl buffer at pH 7.2. For nonspecific binding, 1 mM glutamate was used. For kainate receptor binding, slides were incubated for 30 minutes at 0–4°C with 50 nM ^{3}H-kainate in 50 mM Tris citrate buffer at pH 7.0. For nonspecific binding, 100 µM kainate was used. All sections were rinsed, then blown dry under a stream of warm air. Dried sections were placed in X-ray cassettes along with ^{3}H standards (Amersham) for 5 days (AMPA) or 2 weeks (NMDA and kainate) at 4°C. Quantitative analysis of autoradiograms were performed by using a Compaq computer-based densitometer and image analyzer (Loats Inc., Westminster, Md.).

In Situ Hybridization

For quantification of the NMDA receptor subunit NR1, the following oligo probe sequence was used: 5′-GCCATCTGCCACCAGGTGCACCTCGTAGGTGAAGTTCATGGTCCGTGCCAGCTTGATGAGCAGGTC-3′ (35).

For NR2A, the oligo probe sequence was 5′-AGAAGGCCCGTGGGAGCTTTCCCTTTGGCTAAGTTTC-3′ (36).

For NR2B, the oligo probe sequence was 5′-GGGCCTCCTGGCTCTCTGCCATCGGCTAGGCACCTGTTGTAACCC-3′ (36).

In situ hybridization experiments, including probe labeling, hybridization, and posthybridization washing, were performed as previously described (37). Briefly, the labeled probes were prepared with ^{35}S dATP and terminal transferase (United States Biochemical Corp., Cleveland). The sections were hybridized overnight at 37°C; the hybridization buffer consisted of 50% deionized formamide, 4 saline sodium citrate buffer, 1 Denhardt's solution, 10% dextran sulfate, 250 µg/ml yeast tRNA, 500 µg/ml single-strand salmon DNA, and 100 mM dithiothreitol. After the overnight hybridization, the sections were rinsed four times for 15 minutes each in 2 saline sodium citrate buffer containing 50% formamide at 46°C, two times for 30 minutes each in 1 saline sodium citrate buffer at room temperature, and then 2 minutes each in 70%, 90%, and 100% ethanol, and air dried. For autoradiography, sections were apposed to Hyperfilm B_{max} (Amersham) along with ^{14}C microscales standards at 4°C and exposed for 2 weeks. Films were developed using Kodak D-19. Films of the sections were digitized and their optical densities read in anatomical regions of interest by using quantitative densitometry on an Inquiry System (Loats, Inc.).

TABLE 2. Binding of NMDA, Kainate, and AMPA Receptors in Hippocampal Subregions in Postmortem Brain Tissue From Subjects With Schizophrenia and Normal Comparison Subjects

Subregion and Study Group	NMDA Receptor Binding (fmol/mg)			Kainate Receptor Binding (fmol/mg)			AMPA Receptor Binding (fmol/mg)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Entorhinal cortex									
Schizophrenia	13	50.0	15.8	25	64.7	26.8	16	183.7	60.4
Comparison	15	58.7	25.1	22	67.5	27.8	15	182.0	65.2
Dentate gyrus									
Schizophrenia	15	71.3	34.3	22	57.7	28.4	17	324.6	105.1
Comparison	17	72.7	21.4	20	56.4	23.2	13	344.8	130.2
CA3									
Schizophrenia	16	49.2	25.4	23	75.6	28.7	18	285.9	125.9
Comparison	14	54.7	15.2	20	76.1	24.9	13	294.6	119.1
CA2									
Schizophrenia	12	50.8	25.9	22	65.0	39.5	17	285.6	127.8
Comparison	14	58.3	16.8	19	71.2	40.4	12	334.4	151.1
CA1									
Schizophrenia	18	78.3	21.6	27	58.3	37.8	19	310.7	129.3
Comparison	19	85.3	22.3	22	62.2	37.5	15	324.0	142.6
Subiculum									
Schizophrenia	15	40.8	17.3	15	31.5	9.4	6	141.3	43.9
Comparison	15	48.0	16.2	16	38.0	10.7	6	155.6	38.9

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze demographic characteristics associated with the three sets of postmortem tissue samples. Analysis of variance was used to analyze the relationship between tissue set (Maryland Brain Collection, Melbourne Brain Collection 1, Melbourne Brain Collection 2) and diagnosis (normal or schizophrenia) for each dependent variable (level of NR1, NMDA, and kainate) for which data were available from all three tissue sets. For variables with data for two tissue sets (AMPA), a two-by-two ANOVA was used. Independent t tests were used for variables with data for one tissue set (NR2A, NR2B) and to compare data for rats that were given haloperidol or no drug. Significant differences by tissue set were found for postmortem interval ($F=41.32$, $df=2, 63$, $p=0.0001$) and gender ($F=15.83$, $df=2, 63$, $p=0.0001$) (Table 1). However, no diagnosis or interaction effects with diagnosis were present.

Results

Glutamate Receptor Binding

NMDA-sensitive glutamate receptor binding did not differ by diagnosis (schizophrenia tissue versus normal comparison tissue) in any subregions of the hippocampal or parahippocampal cortex where it was assessed (Table 2). The three tissue sets differed in the level of NMDA-sensitive glutamate receptor binding in entorhinal cortex ($F=4.47$, $df=1, 24$, $p=0.05$), but there was no interaction of tissue set with site or diagnosis. Representative autoradiograms for NMDA receptor binding for schizophrenia tissue and normal comparison tissue are shown in Figure 1 (panels E and F).

Kainate receptor binding did not differ by diagnosis in hippocampus (Table 2). The three sets of tissue samples differed in the level of kainate receptor binding in CA1 ($F=41.48$, $df=1, 45$, $p=0.0001$), CA2 ($F=27.25$, $df=1, 37$, $p=0.0001$), CA3 ($F=20.51$, $df=1, 39$, $p=0.0001$), dentate gyrus ($F=9.90$, $df=1, 38$, $p=0.003$), and entorhinal cortex ($F=9.45$, $df=1, 43$, $p=0.004$), but there was no interaction of tissue set with site and diagnosis nor of tissue set and site. Unlike previous studies (38, 39), our analysis did not show a lower level of kainate binding in CA2 in schizophrenia than in normal comparison tissue ($F=1.38$, $df=1, 37$, $p=0.25$). Representative autoradiograms for kainate receptor binding are shown in Figure 1 (panels C and D).

The level of AMPA receptor binding was similar in the schizophrenia tissue and the normal comparison tissue in all subregions analyzed, except for a small but significant difference in CA2, where there was less AMPA receptor binding in schizophrenia ($F=5.62$, $df=1, 25$, $p=0.03$). The three sets of tissue samples differed in the level of AMPA receptor binding in CA1 ($F=36.75$, $df=1, 30$, $p=0.0001$), CA2 ($F=64.33$, $df=1, 25$, $p=0.001$), CA3 ($F=33.45$, $df=1, 27$, $p=0.0001$), dentate gyrus ($F=30.98$, $df=1, 26$, $p=0.0001$), and entorhinal cortex ($F=55.79$, $df=1, 27$, $p=0.0001$), but there were no interactions of tissue set with any other variable (Table 2). Representative autoradiographs for AMPA receptor binding are shown in Figure 1 (panels A and B).

Glutamate Receptor Binding and Chronic Antipsychotic Administration

NMDA, kainate, and AMPA receptor binding in hippocampal subregions of rat brain were similar in rats that received the typical antipsychotic haloperidol for 6 months and in control rats (Table 3). The mean plasma level of haloperidol when rats received 1.5 mg/kg/day of the drug was 6.8 ng/ml (SD=1.1) in trunk blood at sacrifice in our laboratory (34).

NMDA Subunit mRNA

The level of mRNA for the NR1 subunit was significantly lower in the dentate gyrus in the schizophrenia tissue than in the normal comparison tissue ($F=4.86$, $df=1, 42$, $p=0.03$) (Table 4, Figure 2). In addition, the level of NR1 mRNA in CA3 was 25.2% lower in the schizophrenia tissue than in the normal comparison tissue, although the difference was not significant ($F=3.35$, $df=1, 39$, $p=0.07$). The three sets of tissue samples differed in the level of NR1 mRNA in CA1 ($F=5.92$, $df=1, 49$, $p=0.02$), but there were no interac-

TABLE 3. Binding of NMDA, Kainate, and AMPA Receptors Binding in Hippocampal Subregions in Rats Given Haloperidol-Treated Water and in Control Rats Given Untreated Water

	NMDA Receptor Binding (fmol/mg)				Kainate Receptor Binding (fmol/mg)				AMPA Receptor Binding (fmol/mg)			
	Haloperidol		Control		Haloperidol		Control		Haloperidol		Control	
Subregion	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dentate gyrus	91.4	16.9	98.0	21.2	65.6	15.2	61.7	12.5	333.3	56.0	341.8	46.3
CA3	38.5	10.4	48.2	13.8	110.0	10.9	106.6	10.7	255.3	34.8	278.8	38.4
CA2	38.6	11.5	49.8	14.9	21.5	4.8	20.8	3.0	276.0	34.1	292.1	41.9
CA1	90.0	19.5	92.6	22.0	23.7	6.8	22.6	5.4	377.1	26.9	372.9	36.8
Subiculum	22.4	7.9	24.8	8.5	51.7	19.8	50.7	17.2	250.8	42.3	283.3	31.8

TABLE 4. Level of mRNA of NMDA Receptor Subunits NR1, NR2A, and NR2B in Hippocampal Subregions in Postmortem Brain Tissue From Subjects With Schizophrenia and Normal Comparison Subjects

	NR1 mRNA (nCi/g)						NR2A mRNA (nCi/g)						NR2B mRNA (nCi/g)					
	Schizophrenia			Comparison			Schizophrenia			Comparison			Schizophrenia			Comparison		
Subregion	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Entorhinal cortex	21	36.5	11.4	22	44.6	8.4	5	7.7	2.7	7	9.9	3.5	4	9.7	1.8	6	9.8	1.4
Dentate gyrus	25	71.1[a]	21.1	21	85.4	20.2	—	—	—	—	—	—	—	—	—	—	—	—
CA3	23	50.9[b]	16.6	20	68.0	15.5	8	9.4	3.6	4	10.1	7.7	8	10.5[b]	1.3	4	8.8	1.3
CA2	22	46.6	10.6	20	54.2	9.8	8	9.6	3.7	4	8.0	7.3	8	10.5[a]	1.2	4	7.5	1.2
CA1	27	37.1	8.0	26	41.3	9.6	9	11.3	2.4	8	8.7	4.7	9	9.8	1.9	8	9.7	2.2
Subiculum	23	32.9	9.5	26	38.7	5.9	6	8.8	5.2	7	9.5	6.6	5	10.1	2.5	5	9.2	2.0

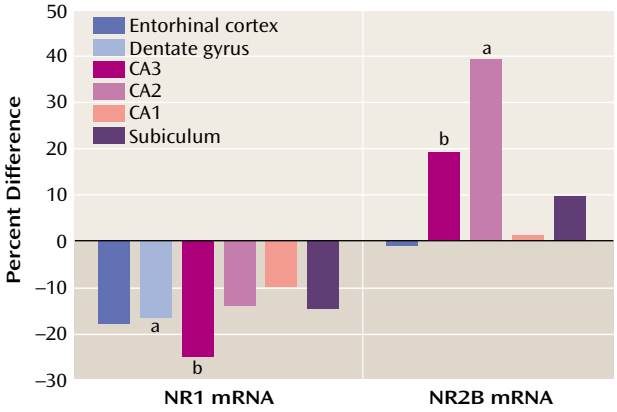
[a] Significant difference between schizophrenia and comparison groups ($p<0.05$).
[b] Difference between schizophrenia and comparison groups at $p<0.10$ level.

tions of tissue set with diagnosis. In all tissue sets, any differences in level of NR1 mRNA by diagnosis, even if not significant, showed lower levels in schizophrenia tissue.

The level of mRNA for the NR2A subunit showed no differences between the schizophrenia tissue and the normal comparison tissue. However, the level of NR2A mRNA in CA2 was 20% lower in the schizophrenia tissue than in the normal comparison tissue, although the difference was not significant (t=0.52, df=10, p=0.62) (Table 4). For this analysis, only tissue from the Maryland Brain Collection was available.

The level of mRNA for the NR2B subunit in CA2 was 39.6% higher in the schizophrenia tissue than in the normal comparison tissue, a significant difference (t=4.00, df=10, p=0.003) (Table 4, Figure 2). In CA3 the level of NR2B mRNA was 19.2% higher in schizophrenia tissue, although the difference was not significant (t=2.11, df=10, p=0.06). For this analysis, only tissue from the Maryland Brain Collection was available. The differences in NR2B mRNA between schizophrenia tissue and normal comparison tissue characteristically showed a higher level in schizophrenia; the nonsignificant differences in NR2A mRNA also showed a higher level in schizophrenia, but the difference in NR1 mRNA showed a lower level in schizophrenia.

FIGURE 2. Percentage Difference From Normal Comparison Levels of mRNA for NMDA Receptor Subunits NR1 and NR2B in Hippocampal Subregions of Subjects With Schizophrenia



[a] Significant difference between level in schizophrenia and normal level ($p<0.05$).
[b] Nonsignificant difference between level in schizophrenia and normal level ($p<0.10$).

Discussion

The hippocampus has been a consistent focus of biologic study in schizophrenia, yielding several replicable observations of abnormality. This emphasis has been encouraged by observations of behavioral disruptions in the illness that suggest hippocampal dysfunction (40, 41). The behavioral actions of PCP in healthy humans and in schizophrenia have implicated the blockade of glutamate transmission at the NMDA receptor in psychosis (22, 24 , 25, 30, 42). Moreover, specific anatomic data exist (reviewed below) that support hippocampal pathology related to glutamate in the illness.

Several alterations in neurochemical measures of glutamatergic and related transmitter function in the postmortem hippocampus and/or the related dorsoventral temporal cortex in schizophrenia have been identified. Because the glutamate system has several different receptor families, receptor configurations, transmitters/modulators, and modulating receptor sites, the task of focusing a search for a pathologic site involving glutamate neu-

rotransmission has been challenging. Although there appears to be no difference in the density of hippocampal NMDA glutamate receptors in schizophrenia and in normal tissue (39, 43, 44), a lower level of kainate binding, particularly in CA2, has been found (38, 39, 45), but not consistently (46). Lower levels of non-NMDA receptor binding (39) and lower concentrations of non-NMDA receptor mRNA (47) have both been reported in CA3 in schizophrenia. Moreover, lower levels of the glutamate receptor subunit mRNAs for GluR2, KA2 and GluR6 (kainate and AMPA subunits) have been found selectively in hippocampus in schizophrenia (48), and a higher flip/flop isoform ratio has been identified for AMPA receptors (49). However, in one study, the level of GluR1–GluR7 mRNA subunits appeared no different in schizophrenia than in normal tissue in all brain regions evaluated (50), whereas another study found a lower level of GluR1 mRNA in the parahippocampal gyrus and a lower level of GluR2 mRNA in several hippocampal subregions in schizophrenia (51). These results suggest possible, but inconsistently found, differences in the kainate and AMPA receptor complexes in schizophrenia.

A higher level of binding to NR1/NR2B receptors has been reported in superior temporal cortex in schizophrenia (52). The level of aspartate binding, putatively to presynaptic elements, has been reported to be either higher (46) or no different (45) in temporal cortex in schizophrenia than in normal tissue. Reports of lower levels of hippocampal synaptophysin are consistent with a lower density of synapses in hippocampus in schizophrenia (53). Some evidence supports higher glutamate concentrations within temporal cortex, but not in hippocampus, in schizophrenia (54). Release of glutamate and release of γ-aminobutyric acid (GABA) may be lower in schizophrenia tissue (55). Differences in $GABA_A$ receptor density, in GABA release, and in glutamate-related transmitters and their enzymes in hippocampus have been reported in the illness (30, 45, 56). Often, more pronounced or significant differences have been found within the left compared to the right hemisphere of the temporal cortex (57). Although these findings taken together do not support a single, simple interpretation of glutamatergic involvement in schizophrenia, they do indicate potential instability in ionotropic glutamate transmission in hippocampus in this illness. The data reported here fit into a growing published literature on the complexity of glutamatergic transmission in brain, its distinctive regional characteristics, and our still evolving understanding of the primary elements of the system (58).

In the study reported here, the hippocampal block dissections were not entirely standardized with respect to their position along the anterior-posterior axis; thus, the pathologic findings could not be localized along the long axis of the hippocampus with any precision. On the basis of segregated afferent and efferent pathways and the now suggested anterior-posterior hippocampal differences in schizophrenia (59), it has become apparent that this localization may be important. Consequently, we have begun a new examination of the whole hippocampus in schizophrenia using a specialized tissue collection technique to check if these reported abnormalities are regionally localized within hippocampus.

The hypothesis underlying the study reported here was based on the possibility that PCP-induced psychosis and schizophrenic psychosis might share a common biologic mechanism. The mechanism of PCP-induced psychosis, inferred from animal studies done in this laboratory (27, 28), is reduced glutamatergic transmission at the NMDA receptor within the trisynaptic hippocampal glutamatergic pathway (25). These findings suggest the simplistic idea that schizophrenia tissue would evidence the same up-regulation of the NMDA receptor (as a marker of glutamatergic transmission at those hippocampal synapses) as the rat PCP preparation (28). The rat studies of chronic haloperidol administration showed no difference in these parameters with antidopaminergic actions, suggesting that findings for NMDA receptors could be distinguished from the effects of chronic drug treatment.

The initial hypothesis, that we would find NMDA receptor up-regulation in hippocampus, particularly in CA1 or CA3, secondary to an internal disruption of hippocampal glutamatergic transmission, was not confirmed by this study. Using levels of receptor density as an index of an alteration in glutamate mediated transmission in hippocampus, the results suggest no differences between the schizophrenia tissue and the normal comparison tissue. These results are consistent with reports from other laboratories (39, 43, 44).

Subsequent analysis of the expression of NMDA receptor subunits was indicated to fully examine the a priori hypothesis. The NR1 receptor subunit of the multimeric NMDA receptor is the critical subunit for the ionophore function of gating calcium ions. The NR1 subunit has multiple isoforms that have distinct pharmacologic properties (60); consequently considerable diversity exists among brain NMDA receptors. The NR2 family of NMDA subunits, which are also found in hippocampus, have four members: NR2A, NR2B, NR2C, and NR2D. The NMDA receptor on hippocampal pyramidal cells is thought to be composed of NR1, NR2A, and NR2B subunits, with the NR2A and NR2B subunits present in only moderate concentrations. The NR2C and NR2D subunits are expressed at the lowest levels in hippocampus and are thought to combine with the NR1 subunit located preferentially on the hippocampal interneurons. Experimentally constituted receptors, lacking the NR1 subunit, fail to generate a functioning NMDA ionophore that can gate ionic calcium flow (36). NMDA receptors composed of NR1, NR2A, and NR2B subunits demonstrate significant pharmacologic, functional, and anatomic differences from NMDA receptors composed of NR1, NR2C, and NR2D subunits (60). For example, in recombinant NMDA receptor studies in

vitro, the NR1-NR2A-NR2B receptors increased the elevation of calcium that is modulated by protein kinase C and mediated by NMDA receptors, whereas the NR1-NR2C-NR2D receptors decreased this intracellular calcium measure (62). In this paper, we have evaluated the subunit message that purportedly directly modulates hippocampal pyramidal neuronal activity, i.e., the mRNA of the NR1, NR2A, and NR2B subunits.

A lower level of NR1 expression and a higher level of NR2A or NR2B expression, as reported here, are consistent with the idea that the composition of at least some of the hippocampal NMDA receptors—those that reside on pyramidal neurons—may be abnormal in schizophrenia. A linkage analysis of schizophrenia in an African tribal family suggested that alterations in the NMDA receptor may provide a genetic predisposition to the illness (63), although this observation needs further replication. Molecular techniques have provided mouse models of regionally reduced NR1-composed NMDA receptors using antisense (64), adenovirus (65), or customized recombination systems (66). These mouse preparations show behavioral alterations in the whole animal (67). Such animal models may provide critical preclinical systems for understanding the biologic consequences of lower levels of NR1. Indeed, transgenic mice with a lower than normal expression of the NR1 NMDA receptor subunit have some characteristics similar to those observed in pharmacologically induced animal models of schizophrenia (68).

An NMDA receptor lacking the NR1 subunit would be unable to gate calcium and thus would not be fully functional, but it could demonstrate full ligand binding. One might tentatively suggest, based on this evidence, that several subregions of the hippocampus may have a lower number of functioning NMDA receptors in schizophrenia, compared with normal tissue, and may thus be "hypoglutamatergic." This altered receptor mechanism would reduce NMDA-mediated neural transmission not through receptor blockade but by abnormal receptor composition and consequently abnormal function. The outcome of this difference in receptor composition would be reduced transmission at the NMDA receptor. Perhaps any mechanism that compromises hippocampal NMDA-sensitive glutamate-mediated neurotransmission in an analogous fashion would produce psychosis. These speculations would be further supported by the demonstration of lower levels of NR1 protein and higher levels of NR2A and NR2B protein in hippocampal subregions in schizophrenia and by documentation of NR2C and NR2D levels, efforts that are now ongoing in our laboratory.

These early studies suggest an abnormality of the composition of the NMDA-sensitive glutamate receptor in hippocampus in schizophrenia. Preliminary data from other laboratories show NMDA subunit alterations in thalamus (69) and in superior temporal cortex (52) in schizophrenia, suggesting that such differences in subunits might not be limited to hippocampus. Moreover, higher levels of NR2B subunit protein in hippocampus in schizophrenia has recently been reported from another laboratory, suggesting that higher levels of NR2B mRNA will translate into higher levels of NR2B protein (70). Although the interpretation of the data remains tentative, it is consistent with considerable evidence documenting glutamate-related abnormalities of limbic cortex and pathology in hippocampus in schizophrenia.

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