

Implications for Altered Glutamate and GABA Metabolism in the Dorsolateral Prefrontal Cortex of Aged Schizophrenic Patients

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Objective: Pharmacological, clinical, and postmortem studies suggest altered γ -aminobutyric acid (GABA)-ergic and glutamatergic function in patients with schizophrenia. The dorsolateral prefrontal cortex is one key locus of abnormality. The precise neurochemical mechanisms underlying neurotransmitter alterations, such as hypoglutamatergia or GABA dysfunction, are not well understood. This study investigated key biochemical elements of GABA and glutamate metabolism in brain specimens from schizophrenic patients. The activities of nine principal GABA and glutamate-associated metabolic enzymes were measured concurrently in the dorsolateral prefrontal cortex of antemortem-assessed and neuropathologically characterized schizophrenic and comparison subjects.

Method: Postmortem dorsolateral prefrontal cortex specimens from schizophrenia, Alzheimer's disease, and normal non-psychiatric comparison subjects were assayed to determine activities of the principal glutamate and GABA-metabolizing enzymes glutamine synthetase, glutamate dehydrogenase, α -ketoglutarate dehydrogenase, phosphate-activated glutaminase, alanine aminotransferase, aspartate ami-

notransferase, glutamic acid decarboxylase, GABA-transaminase, and succinic semialdehyde dehydrogenase.

Results: Glutamic acid decarboxylase activities were twofold greater and phosphate-activated glutaminase activities were fourfold greater in the schizophrenic group than in the comparison group. Differences in postmortem interval, tissue pH, inhibition of phosphate-activated glutaminase, and medication effects could not account for the differences. Differences in phosphate-activated glutaminase and glutamic acid decarboxylase activities in equivalent specimens from Alzheimer's patients were not observed. The activities of the remaining enzymes were unchanged.

Conclusions: Greater phosphate-activated glutaminase and glutamic acid decarboxylase activities, specific to schizophrenia patients, provide additional biochemical evidence that dorsolateral prefrontal cortex glutamate and GABA metabolism is altered in schizophrenic subjects. These greater activities are consistent with models of a dysregulated glutamatergic/GABA-ergic state in schizophrenia.

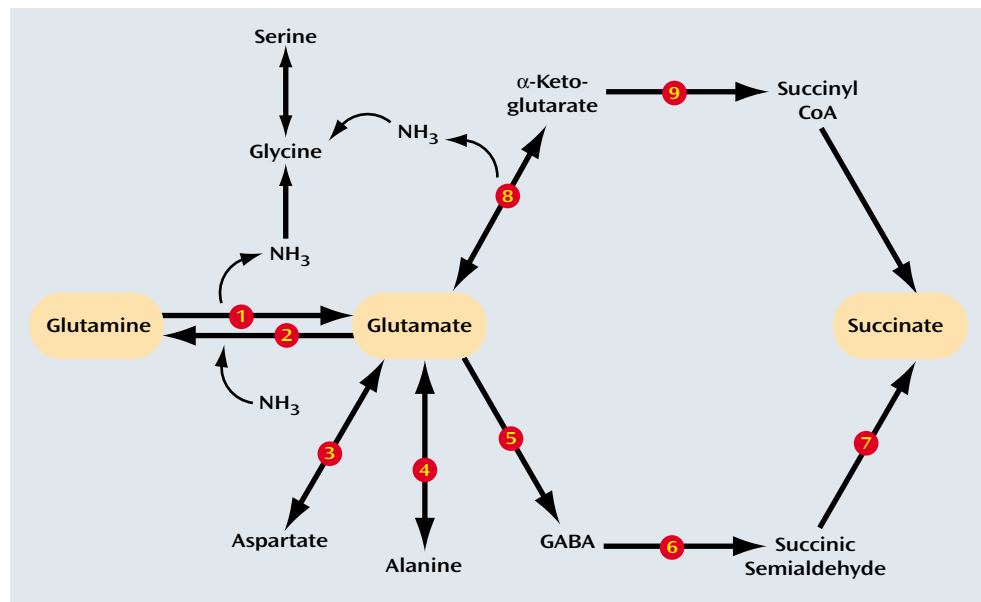
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Several neurochemical hypotheses have been proposed to account for the origin and symptoms of schizophrenia, including abnormal dopaminergic, γ -aminobutyric acid (GABA)-ergic, and glutamatergic neurotransmission (1–5). Evidence supporting an association between glutamatergic dysfunction and schizophrenia has come from pharmacological studies showing that *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine and ketamine, can induce many of the psychotic signs and symptoms of schizophrenia in normal comparison subjects or exacerbate them in patients with schizophrenia (6–8). Reports of abnormal glutamatergic neurotransmission in the hippocampus, entorhinal cingulate, and prefrontal cortices (2, 9–13) of schizophrenic patients and the involvement of the glutamatergic system in learning, memory, emotion, and behavior (14) have given further

credence to the hypothesis of the dysregulated glutamatergic state in schizophrenia.

Similarly, evidence for abnormal GABA-ergic neurotransmission in schizophrenia includes lower GABA uptake and release in the frontal cortex (15), lower glutamic acid decarboxylase activities and mRNA in some brain regions and neurons (16–18), greater [3 H]-muscimol and GABA_A receptor binding (19, 20), fewer small, putatively GABA-ergic neurons in the hippocampus and the anterior cingulate cortex (21–23), and less GABA-transporter-1 protein in axon terminals of chandelier neurons (24). However, evidence suggestive of greater laminar GABA-ergic activity has also been reported (25, 26). Thus, in addition to glutamatergic dysfunction, there are concurrent defects in GABA-ergic neurotransmission (20, 26, 27).

FIGURE 1. Enzymatic Pathways Involved in the Metabolism of Glutamine, Glutamate, and GABA in the Brain^a



^a Enzymes are indicated as follows: 1) phosphate-activated glutaminase, 2) glutamine synthetase, 3) aspartate aminotransferase, 4) alanine aminotransferase, 5) glutamic acid decarboxylase, 6) GABA transaminase, 7) succinic semialdehyde dehydrogenase, 8) glutamate dehydrogenase, 9) α -ketoglutarate dehydrogenase.

CNS glutamate and GABA metabolism entails a series of integrated synthetic and degradative pathways (Figure 1). Glutamate, in addition to its role in brain ammonia clearance, serves as an important energy source after its deamination by glutamate dehydrogenase and oxidative decarboxylation by α -ketoglutarate dehydrogenase. Furthermore, glutamate is integrally related to the synthesis of glutamine (by means of glutamine synthetase) and aspartate and alanine (through aspartate aminotransaminase [AST] and alanine aminotransaminase [ALT], respectively). Glutamate can be synthesized by glutamate dehydrogenase, AST, ALT, GABA-transaminase, and phosphate-activated glutaminase, a neuronal enzyme most directly involved in the generation of glutamate pools for neurotransmission. Glutamate is converted to GABA by glutamic acid decarboxylase. Finally, GABA is metabolized to succinate by the combined reactions of GABA-transaminase and succinic semialdehyde dehydrogenase.

Since GABA derives from glutamate and glutamate can derive from GABA, alterations in glutamate metabolism can effect GABA metabolism or vice versa. The overall aim of this study was to confirm that GABA and glutamate metabolism was abnormal in schizophrenic patients and to determine how these activities may be associated with one another. A panel of nine principal enzymes involved in glutamate and GABA metabolism (glutamate dehydrogenase, glutamine synthetase, phosphate-activated glutaminase, α -ketoglutarate dehydrogenase, AST, ALT, glutamic acid decarboxylase, GABA-transaminase, and succinic semialdehyde dehydrogenase) (Figure 1) was studied concurrently in the same brain region (dorsolateral prefrontal

cortex) in the same group of aged antemortem-assessed and diagnosed schizophrenia patients dying of natural causes and normal age-matched nonpsychiatric comparison subjects. The dorsolateral prefrontal cortex (28) was targeted for study because it has been implicated in neuroimaging studies (29), it shows significant shrinkage and greater packing density in schizophrenia patients (30), it has an important role in the mediation of working memory (29, 31), it is affected by long-term treatment with phenylcyclidine (32), and it is a site of interaction between glutamatergic receptors and the mesocortical dopamine system (33). To test the regional specificity of findings, specimens dissected from the occipital cortex (Brodmann's area 17) were also assessed in the same subjects.

Method

Postmortem Tissue

Frozen postmortem brain samples from subjects diagnosed with chronic schizophrenia with DSM-III-R criteria (N=27), normal comparison subjects (N=13), and comparison subjects with Alzheimer's disease (N=10) were obtained from the Department of Psychiatry, Mount Sinai/Bronx Veterans Administration Medical Center Brain Bank, New York. The mean age, postmortem interval, tissue pH, and sex distributions of the subjects are shown in Table 1. All schizophrenic subjects had been hospitalized for the long term at Pilgrim Psychiatric Center (New York) for many years, and complete medical charts were available for all patients. The diagnosis of schizophrenia was made prospectively (N=16) (34, 35) and by postmortem chart review (34). The diagnostic and assessment procedures have been described in detail (13). All assessment and postmortem evaluations and procedures were approved by the institutional review boards of Pilgrim Psychiatric Center, Mount Sinai School of Medicine, and the Bronx VA Medi-

TABLE 1. Postmortem Characteristics of Subjects With Schizophrenia, Normal Comparison Subjects, and Subjects With Alzheimer's Disease

Group	Male-Female Ratio	Age (years)		Postmortem Interval (hours)		pH Level of Tissue		Storage Interval (days)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Subjects with schizophrenia (N=27)									
Total	2:1	72.3	12.2	14.6	9.7	6.33	0.30	2,128.0	1,048
Matched for age	2:3	81.1	9.3	14.2	8.4	6.36	0.30	1,998.6	996
Normal comparison subjects (N=13)									
Total	5:8	82.8	10.0	8.0	5.5	6.29	0.31	1,759.4	1,006
Matched for age	1:4	81.5	11.1	8.0	5.7	6.23	0.33	1,815.9	1,130
Subjects with Alzheimer's disease (N=10)	2:3	79.8	9.9	10.1	9.3	6.27	0.36	2,198.9	676

cal Center. All patients had thorough neuropathologic characterization to rule out associated neurologic complications such as Alzheimer's disease, multi-infarct dementia, etc. (34). Normal comparison subjects had no history of any psychiatric or neurologic disorders and no discernible neuropathologic lesions. Nine schizophrenia subjects stopped taking neuroleptic medications for at least 6 weeks (range=0–124 weeks) before death.

Gray matter (50–100 mg wet weight) from the dorsolateral prefrontal cortex (Brodmann's area 46) (28) and visual cortex (Brodmann's area 17) were dissected from blocks of frozen brain (–80°C). The tissues were pulverized at –190°C and stored at –80°C until use.

Enzyme Measurements

Brain samples (~100 mg wet weight) were homogenized in four volumes of ice-cold 10.0 mM Trizma (Sigma Chemicals, St. Louis) hydrochloric acid buffer, pH 7.0. Homogenates were aliquoted into ice-cold individual tubes for each assay and kept frozen at –80°C until use. Measurements of glutamic acid decarboxylase activity were performed immediately after homogenization in phosphate buffer containing dithiothreitol and Triton-X (Sigma Chemicals, St. Louis). Protein concentrations used in the assays varied from 0.015 to 5.000 mg/ml. The methods for the measurement of the activities of glutamate dehydrogenase (36), phosphate-activated glutaminase (37), glutamine synthetase (38), ALT and AST (39), α -ketoglutarate dehydrogenase (40), glutamic acid decarboxylase (41), GABA-transaminase (42), succinic semialdehyde dehydrogenase (43), and citrate synthase (44) have been described in detail. *N*-Acetylaspartate and *N*-acetylglutamate were measured on a Waters/Millennium (Milford, Mass.) automated high-performance liquid chromatography system by using the methods described by Tsai et al. (3). All assays were run in triplicate at 37°C, except assays for citrate synthase and phosphate-activated glutaminase, which were run at 30°C. Protein measurements were performed by using the method of Lowry et al. (45), with bovine serum albumin used as a standard. Spectrophotometric assays were run by using a Hitachi 3010 (San Jose, Calif.) double-beam UV-visible spectrophotometer fitted with a 12-cell jacketed thermostatic temperature controller. Radioisotope assay measurements were run by using a LKB (Turku, Finland) rack-beta scintillation counter.

Measurement in Neuroleptic-Treated Rats

To assess the effects of neuroleptic exposure on phosphate-activated glutaminase and glutamic acid decarboxylase activities, groups of six male Sprague-Dawley rats (6–8 months of age) received daily subcutaneous injections of haloperidol (2 mg/kg) or saline vehicle for 21 days. Rats were sacrificed 24 hours after the last injection, and cortices were dissected and immediately frozen on dry ice. Phosphate-activated glutaminase and glutamic acid decarboxylase activities were assayed as described.

Statistical Analysis

Analysis of variance (ANOVA) and covariance (ANCOVA) followed by Newman-Keuls tests and *t* tests were used to analyze the results of these studies. Because homogeneity of slopes and variance assumptions were satisfied, ANCOVA was used for analyses that were based on the entire group. For these analyses, the age of the subject at the time of death was entered as the covariate. *T* tests were used to compare differences between groups when the groups had been matched for age at the time of death. Pearson's product-moment correlations were used to assess the relation between phosphate-activated glutaminase activities and the potentially confounding variables of age at the time of death, postmortem interval, and number of weeks neuroleptic free before death. Statistical analyses were performed by using Statistica for Windows (release 5.0, Statsoft, Inc., Tulsa, Okla.) or SPSS for Windows (version 10, SPSS Inc., Chicago).

Results

The schizophrenic subjects were younger as a group than the other groups ($F=4.03$, $df=2, 47$, $p<0.03$; Newman-Keuls test, $p=0.02$) (Table 1). Between-group differences in postmortem interval, tissue pH, and storage interval were not statistically significant (all $p>0.10$). Two approaches were taken to control for the group differences in age. First, in statistical analyses that involved comparisons of entire groups, age was entered as a covariate. Second, when statistically significant differences were observed between groups, *t* tests were repeated on subgroups of 10 normal comparison subjects and 10 schizophrenic subjects that were matched closely for age (Table 1).

Citrate synthase activities in the dorsolateral prefrontal cortex were calculated per mg of protein and subsequently expressed as units of activity (μ moles product formed/hour per mg of protein) for comparison with other enzymes (Table 2). No significant differences in citrate synthase activity were observed among the three groups ($F=0.96$, $df=2, 47$, $p=0.39$). Furthermore, there were no correlations between citrate synthase activity and postmortem interval, tissue pH, or storage time ($r<0.24$, $N=40$, $p>0.12$). Results of assays for mitochondrial enzymes were expressed in relation to both citrate synthase activity and to protein content.

Rates for glutamic acid decarboxylase, GABA-transaminase, and succinic semialdehyde dehydrogenase activity are shown in Table 2. There were no significant activity differences in GABA-transaminase and succinic semialde-

TABLE 2. Enzyme Activities in the Frontal Lobes of Deceased Subjects With Schizophrenia and Normal Comparison Subjects

Enzyme	Activity (μmol product formed/hour per mg protein cytosolic enzyme) ^a				Ratio of Activity to Citrate Synthase Activity			
	Normal Comparison Subjects (N=13)		Subjects With Schizophrenia (N=27)		Normal Comparison Subjects (N=13)		Subjects With Schizophrenia (N=27)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Citrate synthase (reference)	9.45×10^3	8.73×10^2	8.96×10^3	1.68×10^3				
GABA pathway								
Glutamic acid decarboxylase	0.026	0.014	0.040 ^a	0.017				
GABA transaminase	0.111	0.017	0.103	0.024	0.0122	0.002	0.0141	0.002
Succinic semialdehyde dehydrogenase	0.332	0.031	0.325	0.10	0.0363	0.003	0.0445	0.01
Glutamate pathway								
Glutamate dehydrogenase	2.13	0.55	2.11	0.38	0.150	0.03	0.166	0.02
Glutamine synthetase	1.34	0.38	1.55	0.28				
α -Ketoglutarate dehydrogenase	1.34	0.10	1.37	0.41	0.098	0.03	0.112	0.03
Aspartate aminotransferase	34.8	3.8	32.5	4.50	3.68	0.24	3.63	0.28
Alanine aminotransferase	0.604	0.10	0.591	0.09	0.064	0.01	0.067	0.01
Glutaminase	0.287	0.53	1.246 ^b	0.97	0.030	0.054	0.132 ^c	0.09

^a Significantly more (154%) than that of normal comparison subjects (Newman-Keuls test, $p=0.04$). Significantly more (206%) than that of age-matched normal comparison subjects ($t=-3.4$, $df=18$, $p=0.03$). Significantly more (192%) than that of normal comparison subjects matched for postmortem interval ($t=-2.39$, $df=11$, $p<0.03$).

^b Significantly more (433%) than that of normal comparison subjects (Newman-Keuls test, $p<0.03$).

^c Significantly more (438%) than that of normal comparison subjects ($t=-2.3$, $df=18$, $p=0.03$).

hyde dehydrogenase between schizophrenic and normal comparison subjects, whether the enzyme activities were expressed as units/mg of protein or per unit of citrate synthase activity. In contrast, there was significantly greater glutamic acid decarboxylase activity in the schizophrenic subjects ($F=3.61$, $df=2$, 46 , $p<0.04$) (Newman-Keuls test of normal comparison versus schizophrenic subjects, $p=0.04$) (154%) and in normal comparison versus age-matched schizophrenic subjects ($t=-3.4$, $df=18$, $p=0.03$) (206%). Differences in glutamic acid decarboxylase activities in the dorsolateral prefrontal cortex of identical specimens from Alzheimer's disease patients were not observed (94%). Because differences in the agonal state have been linked to variations in brain glutamic acid decarboxylase activity, it is important to note that tissue pH was not significantly different among the three groups ($F=0.39$, $df=2$, 46 , $p=0.67$).

The activities of α -ketoglutarate dehydrogenase, glutamate dehydrogenase, glutamine synthetase, and AST and ALT in the dorsolateral prefrontal cortex did not differ significantly ($p>0.10$) in comparisons of schizophrenic and normal comparison subjects as whole groups or when matched for age, whether the enzyme activities were expressed per mg of protein or per unit of citrate synthase activity (Table 2).

There was statistically significantly greater phosphate-activated glutaminase activity in the dorsolateral prefrontal cortex (Table 2) in schizophrenic versus normal comparison subjects when the entire groups were assessed (450%) ($F=4.85$, $df=2$, 46 , $p<0.02$; Newman-Keuls test of comparison versus schizophrenic subjects, $p=0.04$) or when they were assessed on an age-matched basis (438%) ($t=-2.3$, $df=18$, $p<0.04$).

Phosphate-activated glutaminase activity was virtually abolished (95%–100%, data not shown) in the presence of

2.0 mM of 6-diazo-5-oxo-L-norleucine, a selective inhibitor of phosphate-activated glutaminase (46), indicating that the observed activities derived from phosphate-activated glutaminase. To test for the disease specificity of elevated phosphate-activated glutaminase activities in the dorsolateral prefrontal cortex of schizophrenic subjects, the level of phosphate-activated glutaminase activity was determined in the dorsolateral prefrontal cortex of the Alzheimer's disease group. Phosphate-activated glutaminase activity in the group with Alzheimer's disease was not significantly different from that in the normal comparison subjects (Newman-Keuls test, $p=0.80$), but it differed significantly from the schizophrenia group (Newman-Keuls test, $p=0.03$). To determine whether the differences in phosphate-activated glutaminase activity in the schizophrenia group were specific to the dorsolateral prefrontal cortex, the activities of phosphate-activated glutaminase in the primary visual cortex (Brodmann's area 17) were compared in the same groups of schizophrenic and normal comparison subjects. In the visual cortex, there was significantly less phosphate-activated glutaminase activity than in the dorsolateral prefrontal cortex in both schizophrenic and comparison subjects (24.9% of the dorsolateral prefrontal cortex) ($t=13.9$, $df=37$, $p=0.0007$). Although whole-group phosphate-activated glutaminase activity in the visual cortex of the schizophrenic subjects was nominally greater than that in the visual cortex of the comparison subjects, this difference was not statistically significant (Newman-Keuls test, $p=0.10$). The activity of phosphate-activated glutaminase did not correlate significantly with postmortem interval, age, tissue pH, weeks free from neuroleptic exposure before death, or storage time ($r=-0.16$ to 0.24 , $N=40$, $p>0.10$).

There were significant correlations between glutamic acid decarboxylase and phosphate-activated glutaminase

activities in the dorsolateral prefrontal cortex of the entire study group ($r=0.78$, $N=40$, $p=0.00001$) as well as in the schizophrenic group when it was assessed alone ($r=0.80$, $N=27$, $p=0.00001$). In contrast, the activities of glutamic acid decarboxylase and phosphate-activated glutaminase did not correlate significantly with each other when assessed in the normal comparison group alone ($r=0.26$, $N=13$, $p=0.38$), which suggests that the significant correlation between phosphate-activated glutaminase and glutamic acid decarboxylase activity in the group as a whole was predominantly attributable to the relationship between glutamic acid decarboxylase and phosphate-activated glutaminase in the schizophrenia group.

N-Acetylaspartate and *N*-acetylaspartyl-glutamate levels in the dorsolateral prefrontal cortex of the subjects with schizophrenia, the comparison subjects with Alzheimer's disease, and the nonpsychiatric comparison subjects are shown in Table 3. No statistically significant differences in *N*-acetylaspartate or *N*-acetylaspartyl-glutamate levels were observed among the groups. The ratios of *N*-acetylaspartate to *N*-acetylaspartyl-glutamate levels were approximately 14:1, consistent with previous findings (3).

The activities of phosphate-activated glutaminase and glutamic acid decarboxylase did not correlate significantly with the number of weeks that each schizophrenic subject had been free of exposure to neuroleptic drugs ($r=0.10$ to -0.01 , $N=40$, $p>0.60$). Additionally, when the schizophrenic group was stratified into patients who had been exposed to neuroleptics until the time of death ($N=15$) versus those who had been neuroleptic free for 6 weeks or more ($N=9$), the activities of phosphate-activated glutaminase and glutamic acid decarboxylase did not differ significantly between the groups ($t<0.8$, $df=22$, $p>0.43$).

Differences in cortical phosphate-activated glutaminase activities in rats subchronically treated with haloperidol relative to normal control subjects receiving saline vehicle were not evident (haloperidol: $N=6$, mean=0.034 activity units/units of citrate synthase activity, $SD=0.005$; saline vehicle: $N=6$, mean=0.036 activity units/units of citrate synthase activity, $SD=0.005$) ($t=-0.8$, $df=10$, $p=0.44$). Similarly, cortical glutamic acid decarboxylase activity was not altered by haloperidol treatment (haloperidol treatment: $N=6$, mean=0.268 activity units, $SD=0.080$; saline-vehicle treatment: $N=6$, mean=0.256 activity units, $SD=0.110$) ($t=1.1$, $df=10$, $p=0.30$).

Comment

Of the nine enzymes involved in glutamate and GABA metabolism that we examined, the activities of two principal enzymes responsible for the synthesis of glutamate and GABA—phosphate-activated glutaminase and glutamic acid decarboxylase, respectively—were selectively higher in the cortices of schizophrenic subjects. These high activities were specific to schizophrenia insofar as

TABLE 3. Levels of *N*-Acetylaspartate and *N*-Acetylaspartyl-Glutamate in the Prefrontal Cortex of Deceased Subjects With Schizophrenia, Subjects With Alzheimer's Disease, and Normal Comparison Subjects

Group	nmol/mg of Protein			
	<i>N</i> -Acetylaspartate		<i>N</i> -Acetylaspartyl-Glutamate	
	Mean	SD	Mean	SD
Subjects with schizophrenia (N=27)	46.3	6.9	2.1	0.7
Subjects with Alzheimer's disease (N=10)	42.8	12.3	2.2	0.6
Normal comparison subjects (N=13)	47.1	14.2	2.4	0.7

they were not observed in identical brain regions derived from Alzheimer's disease patients. The greater phosphate-activated glutaminase and glutamic acid decarboxylase activities found were not attributable to group differences in peri- or postmortem parameters. These findings support the hypotheses that glutamate and GABA neurotransmission are altered in the prefrontal cortex of schizophrenic subjects.

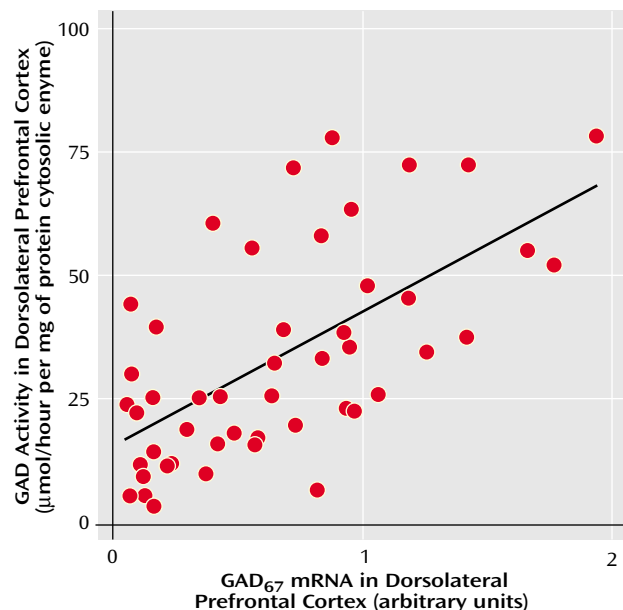
Higher Activity Levels

The activity of phosphate-activated glutaminase, the major enzyme responsible for the conversion of glutamine to glutamate, was significantly greater in the dorsolateral prefrontal cortex of schizophrenic subjects but not in the occipital cortex. Phosphate-activated glutaminase activity was nearly completely inhibited by 6-diazo-5-oxo-L-nor-leucine (46), indicating the specificity of the assay and suggesting that it arose from the mitochondrial phosphate-activated-specific enzyme.

The activity of glutamic acid decarboxylase was greater by nearly twofold in the dorsolateral prefrontal cortex of schizophrenic subjects and correlated significantly and positively with the activity of phosphate-activated glutaminase—especially in the schizophrenia group—making it unlikely that disease-independent influences on phosphate-activated glutaminase and glutamic acid decarboxylase activities would lead to disease-specific correlations between the activities of the two enzymes. Similarly, since the *N*-acetylaspartate, *N*-acetylaspartyl-glutamate, and GABA-transaminase data reproduced the findings of others (3, 42), it is unlikely that the higher activity of glutamic acid decarboxylase was artifactual. There are two isoforms of glutamic acid decarboxylase (glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇), which are encoded by different genes (47). Both isoforms are expressed in GABA-ergic neurons, and both participate in GABA synthesis. The enzymatic assays performed did not distinguish between these isoforms.

However, in a recent study of the same brain regions and the same group of subjects as those described here (48), significantly greater glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇ gene expression was observed when using real-time reverse-transcription

FIGURE 2. Correlation of Glutamic Acid Decarboxylase (GAD) Activity in the Dorsolateral Prefrontal Cortex With GAD₆₇ mRNA Expression in Sister Tissue Aliquots Among Combined Subjects With Schizophrenia or Alzheimer's Disease and Normal Comparison Subjects^a



^a $r=0.56$, $N=40$, $p=0.0001$.

polymerase-chain-reaction techniques. Comparisons of glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇ mRNA expression and glutamic acid decarboxylase activity in the dorsolateral prefrontal cortex revealed significant correlations (glutamic acid decarboxylase activity versus glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇ mRNA expression, respectively: $r=0.54$, $N=39$, $p=0.0005$, and $r=0.56$, $N=40$, $p=0.0001$) (Figure 2). Similar significant correlation coefficients resulted when glutamic acid decarboxylase activity and glutamic acid decarboxylase mRNA expression levels were assessed in the schizophrenia group only. Additionally, the expression of both glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇ mRNA was found to be higher in a recent microarray study (49) that used a subset (12 of 27 schizophrenic and 12 of 13 comparison subjects) of the group of subjects described here. Glutamic acid decarboxylase activity was greater by 154% in the current study, while glutamic acid decarboxylase₆₅ and glutamic acid decarboxylase₆₇ mRNA expression were greater by 219% and 245%, respectively, in the reverse-transcription polymerase-chain-reaction studies and by 144% and 169% in the microarray study. These results taken as a whole suggest that abnormalities in the expression of both genes may have contributed to the greater glutamic acid decarboxylase activity measured in the current study.

Unchanged Metabolizing Enzymes

Measurements of five glutamate-associated (glutamate dehydrogenase, glutamine synthetase, AST, ALT, and α -ke-

tooglutarate dehydrogenase) and two GABA-associated enzymes (GABA-transaminase and succinic semialdehyde dehydrogenase) in the dorsolateral prefrontal cortex showed no significant activity differences between the schizophrenic and comparison groups. Similar results (regarding GABA-transaminase) have been reported by Sherif et al. (42). Thus, the abnormalities noted in glutamic acid decarboxylase and phosphate-activated glutaminase activity are not attributable to a global disruption of metabolic pathways, subject selection bias, or other uncontrolled experimental artifacts.

Experimental Artifacts

Glutamic acid decarboxylase and phosphate-activated glutaminase activities have been reported to be sensitive to, and negatively affected by, antemortem and postmortem conditions such as coma and hypoxia (41, 50, 51). Care was taken to minimize these sources of artifact; they are unlikely to have contributed strongly to the results reported here. First, subjects who were comatose for more than 12 hours before death were excluded. Second, subjects were matched closely for brain pH, which is widely believed to be reflective of agonal state (52, 53). Third, only subjects with relatively short postmortem intervals (8–14 hours) were included in the study. Last, the activities of glutamic acid decarboxylase and phosphate-activated glutaminase in schizophrenic subjects were higher than those of both normal comparison subjects and comparison subjects with Alzheimer's disease, indicating that the higher levels were limited to subjects with schizophrenia. Therefore, although the contribution of antemortem and postmortem factors cannot be excluded in any postmortem study, their influence was as closely controlled as possible in the present investigation.

Postmortem studies of schizophrenia must always take into consideration the influence of long-term neuroleptic exposure. A number of analyses with comparison subjects have suggested that neuroleptic exposure may not have contributed significantly to the observed changes in this study. The activities of phosphate-activated glutaminase and glutamic acid decarboxylase did not correlate significantly with the number of weeks (range=0–124) that different subjects had been free of neuroleptic drugs. There were no differences in the activities of phosphate-activated glutaminase and glutamic acid decarboxylase between the schizophrenic subjects exposed to neuroleptics until the time of death and those who had been neuroleptic free for 6 weeks; this suggests that acute or residual neuroleptic effects (54) did not influence phosphate-activated glutaminase and glutamic acid decarboxylase activity. Finally, the activities of phosphate-activated glutaminase and glutamic acid decarboxylase were not altered in the cortices of rats exposed to a haloperidol-dosing regimen that is known to affect dopaminergic receptor expression and binding (55). In addition, a detailed review of all medications received by the subjects during the 2 years

preceding death failed to reveal exposure to other drug classes that were consistently or uniquely associated with the schizophrenia group.

The Hypotheses of the Hypoglutamatergic State

The greater activities of phosphate-activated glutaminase and glutamic acid decarboxylase observed in this study are in good agreement with the findings of Benes et al. (25), who found a nearly 80% greater glutamate immunoreactivity in the vertical axons of the cingulate cortex of schizophrenic subjects, and with the results of Daviss and Lewis (26), who reported a selectively greater population of calbindin-positive GABA-ergic neurons in the dorsolateral prefrontal cortex of schizophrenic subjects. However, at first glance, greater glutamatergic tone, as reflected by greater glutamate immunoreactivity and greater activity of phosphate-activated glutaminase, conflicts with the hypoglutamatergic hypothesis of schizophrenia. Greater activity of phosphate-activated glutaminase may reflect a compensatory upregulation of the synthetic mechanisms for the conversion of glutamine to glutamate, analogous to the greater compensatory tyrosine hydroxylase activity found in hypodopaminergic states (56). Additionally, it is noteworthy that the hypoglutamatergic state hypothesis in schizophrenia is based in large part on the observation that NMDA receptor antagonists mimic some of the symptoms of schizophrenia (6–8). Recent observations (57) have suggested that, consistent with the current results, the psychotomimetic effects of the NMDA antagonist ketamine are more likely a result of glutamate hyperactivity than glutamate hypoactivity.

The greater activity of glutamic acid decarboxylase observed here and in the reports of larger populations of calbindin-positive GABA-ergic neurons (26) is also superficially incongruent with some observations of lower glutamic acid decarboxylase mRNA expression in the dorsolateral prefrontal cortex of some schizophrenic subjects (17, 58) (but not others [48]), fewer GABA uptake sites (15), lower numbers of GABA-transporter immunoreactive chandelier-cell axon terminals in the dorsolateral prefrontal cortex (24), lower numbers of small interneurons in the cingulate gyrus and hippocampus (21–23), and larger numbers of GABA_A receptors in the cingulate cortex and hippocampus (19, 20, 59).

It is difficult to reconcile the reported lower levels of glutamic acid decarboxylase mRNA in these latter studies with the current findings of greater activity of glutamic acid decarboxylase. Differences in the overall age of the subjects, the region of frontal cortex used in the studies, the selection of antemortem-assessed subjects with stringent inclusion/exclusion diagnostic criteria, and the exclusion of any patients dying of unnatural causes or after prolonged agonal states likely account for the differences in the glutamic acid decarboxylase data. It is possible that the biochemical changes detected in the brains of elderly schizophrenic subjects who had a severe and unremitting

lifetime course of illness are different from the changes that are present in midlife and after a less severe course.

Additionally, results from homogenate-based assays, such as those reported here, are insensitive to changes in specific neuronal populations and do not preclude significantly lower levels of enzymes in some cells and significantly higher levels in others. It is also noteworthy that the reportedly lower GABA-ergic parameters have not generally reflected an overall smaller number of GABA-ergic neurons but have applied to only a subset of them (17, 20, 24, 27, 59). Given the heterogeneity of GABA-ergic neurons in the cortex (25), the greater glutamic acid decarboxylase activity observed in the tissue homogenates used in this study could readily reflect the larger population of calbindin-positive GABA-ergic cortical neurons observed previously by Daviss and Lewis (26).

The apparent incongruity of the reportedly smaller GABA uptake and fewer GABA transporter sites on chandelier cell terminals and the greater glutamic acid decarboxylase activity observed in the current study can be viewed as internally consistent and reflective of compensatorily higher glutamic acid decarboxylase activity. Because a significant proportion of releasable GABA is derived from its reuptake, an impaired GABA reuptake mechanism could force greater de novo GABA synthesis.

These results, taken together with those published previously, all support the hypothesis of impaired glutamatergic/GABA-ergic function in subjects with schizophrenia leading to over- or dysregulated inhibition of some neurons and overexcitation of other neurons. Given the complexity of the glutamatergic and GABA-ergic systems of the brain and the high likelihood that both neurochemical systems subserve a multiplicity of functions at the macro- and microcircuit level, specific hypotheses regarding the functional implications of these changes must await more detailed and expansive studies.

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