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7T Proton Magnetic Resonance Spectroscopy of Gamma-Aminobutyric Acid, Glutamate, and Glutamine Reveals Altered Concentrations in Patients With Schizophrenia and Healthy Siblings

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ABSTRACT

BACKGROUND: The *N*-methyl-D-aspartate receptor hypofunction model of schizophrenia predicts dysfunction in both glutamatergic and gamma-aminobutyric acidergic (GABAergic) transmission. We addressed this hypothesis by measuring GABA, glutamate, glutamine, and the sum of glutamine plus glutamate concentrations in vivo in patients with schizophrenia using proton magnetic resonance spectroscopy at 7T, which allows separation of metabolites that would otherwise overlap at lower field strengths. In addition, we investigated whether altered levels of GABA, glutamate, glutamine, and the sum of glutamine plus glutamate reflect genetic vulnerability to schizophrenia by including healthy first-degree relatives.

METHODS: Proton magnetic resonance spectroscopy at 7T was performed in 21 patients with chronic schizophrenia who were taking medication, 23 healthy first-degree relatives of patients with schizophrenia, and 24 healthy nonrelatives. Glutamate, glutamine, and GABA were measured cortically and subcortically in bilateral basal ganglia and occipital cortex.

RESULTS: Patients with schizophrenia had reduced cortical GABA compared with healthy relatives and the combined sample of healthy relatives and healthy nonrelatives, suggesting that altered GABAergic systems in schizophrenia are associated with either disease state or medication effects. Reduced cortical glutamine relative to healthy control subjects was observed in patients with schizophrenia and the combined sample of healthy relatives and patients with schizophrenia, suggesting that altered glutamatergic metabolite levels are associated with illness liability. No group differences were found in the basal ganglia.

CONCLUSIONS: Taken together, these findings are consistent with alterations in GABAergic and glutamatergic systems in patients with schizophrenia and provide novel insights into these systems in healthy relatives.

Keywords: First-degree relatives, GABA, Glutamate, Glutamine, Magnetic resonance spectroscopy, Schizophrenia

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that mediate excitatory postsynaptic potentials. Hypofunction of NMDARs is argued to play a key role in the pathogenesis of schizophrenia (1,2). This hypothesis was formulated based on the psychotomimetic effects of NMDAR antagonists (3–6) and has garnered support from drug (7), postmortem (8), and genetic (9) studies. In awake animals, NMDAR blockade leads to an increase in glutamate release (10) and pyramidal neuron spiking (11), potentially via NMDAR hypofunction on fast-spiking gamma-aminobutyric acidergic (GABAergic) interneurons that regulate pyramidal neuron activity (12). NMDAR antagonists preferentially reduce the firing rate of these interneurons, leading to disinhibition of pyramidal neurons (13,14).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive method for measuring metabolite concentrations in living tissue, and GABA, glutamate (Glu), and glutamine (Gln) concentrations can be quantified. Gln is an amino acid synthesized from Glu taken up by astrocytes in the presynaptic terminal. Because Glu and Gln peaks partially overlap, particularly at lower field strengths, the sum of Glu and Gln (Glx) is often reported as a measure of glutamatergic metabolite concentrations. Using MRS, NMDAR antagonists have been shown to increase prefrontal Glu (15) and Gln (16) in humans and to increase the ratio of Gln to Glu (17,18) and decrease GABA (18) in rodents.

Findings from previous ^{1}H-MRS studies in schizophrenia investigating glutamatergic metabolites are mixed, and results vary across illness stage and brain region (19–22). In high-risk patients and antipsychotic-naïve patients with a first episode of schizophrenia, increased Gln in the frontal cortex and

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thalamus (23–26), reduced Glu in the thalamus (26–29), and increased striatal Glu and Glx have been reported (30–33). In patients with chronic schizophrenia who are taking medication, glutamatergic metabolites are typically equivalent (34–45) or reduced (46–49) [however, see Chang *et al.* (50)]. In the largest study to date, however, increased Gln and no difference in Glu in frontal cortex in patients with chronic schizophrenia who are taking medication were reported (51). To our knowledge, only two studies have investigated glutamatergic metabolites in unaffected adult siblings of patients with schizophrenia. In one study, no Glu or Glx differences were observed in siblings (52). In the other study, reduced Glu was observed across brain regions in healthy co-twins (48).

Fewer studies have measured GABA in patients with schizophrenia (53), largely because concentrations are small, and the peak overlaps with more dominant metabolites. Using editing techniques, reduced GABA has been observed in patients with chronic schizophrenia who are taking medication (33–35,47,54,55), consistent with NMDAR hypofunction, although increased GABA has been observed in unmedicated patients (56). To our knowledge, no ^{1}H-MRS studies have investigated GABA in healthy relatives of patients with schizophrenia.

Despite general patterns emerging in the levels of glutamatergic and GABAergic metabolites in schizophrenia, there is an enormous amount of variability in findings across studies even after considering clinical factors. Differences in methodology are likely culprits. In particular, at higher magnetic field strengths, better separation between individual metabolites can be achieved, and concentration of these metabolites can be more reliably quantified.

Our aims in the current study were twofold. First, we investigated group differences in Glu, Gln, and GABA at an ultra-high magnetic field strength of 7T. Additionally, we investigated the ratio of Gln to Glu, as this has been found to be increased in patients with schizophrenia (20,51), and the ratio of GABA to Glx (57). Our second aim was to investigate the degree to which potentially altered concentrations of GABA, Glu, and Gln reflect genetic vulnerability to schizophrenia by measuring these metabolites in unaffected siblings of patients with schizophrenia and comparing them with both patients with schizophrenia and healthy nonrelatives.

Data were acquired in the occipital cortex and the basal ganglia. Although the occipital cortex is not traditionally implicated in the pathogenesis of schizophrenia, we measured in the occipital cortex because alterations in early visual processing in patients with schizophrenia (58–62) and healthy first-degree relatives (63) support functional alterations in this region. Moreover, most of the MRS literature from healthy subjects reports on the occipital cortex because of the high signal-to-noise ratio and spectral resolution that can be achieved here. Furthermore, we chose to measure in the basal ganglia given its reported dysfunction in schizophrenia, specifically, altered striatal dopamine transmission (64). Based on evidence for glutamatergic and GABAergic dysfunction in schizophrenia from animal models, postmortem work, and prior ^{1}H-MRS studies, we expected increased Gln and reduced GABA in patients with schizophrenia. We further expected reduced Glu based on previous studies in patients with chronic schizophrenia.

METHODS AND MATERIALS

Participants

Procedures were approved by the Medical Ethical Committee of the University Medical Center Utrecht. All subjects gave written informed consent and were compensated for participation.

The study was completed by 68 participants. There were 21 patients with schizophrenia or schizoaffective disorder (SZP group) recruited from the Genetic Risk and Outcome in Psychosis study (65) and from treatment-seeking patients at a hospital in The Netherlands. Also from the Genetic Risk and Outcome in Psychosis study, 23 healthy siblings of patients with schizophrenia (REL group) were recruited. Through community advertisements, 24 healthy nonrelatives as healthy control subjects (HC group) were recruited. Diagnoses of subjects in the SZP and REL groups were based on DSM-IV criteria and determined by clinicians using the Comprehensive Assessment of Symptoms and History interview (66) or Schedules for Clinical Assessment for Neuropsychiatry version 2.1 (67). All patients were taking antipsychotic medication, and five participants were taking benzodiazepines or mood stabilizers. Chlorpromazine-equivalent antipsychotic dosages were calculated for each patient (68). Subjects in the REL and HC groups were excluded if they had any current DSM-IV-TR Axis I disorder. Subjects in the HC group having a first-degree relative with a DSM-IV-TR Axis I disorder were also excluded. Exclusion criteria for all subjects were history of significant head trauma, history of neurologic illness, or substance abuse or dependence within 6 months before the study. Subjects 18–55 years old were included.

Clinical symptoms were assessed in patients only using the Positive and Negative Syndrome Scale (69). Social and occupational functioning was assessed using the Social Functioning Scale (70). The Dutch equivalent of the National Adult Reading Test (71) was used to estimate premorbid IQ. The Edinburgh Handedness Scale was used to measure handedness (72). Demographic data are presented in Table 1. Groups were matched on sex, handedness, IQ, and smoking status. Subjects in the SZP group were significantly older than subjects in the REL group, so age was taken into account as a covariate in between-group analyses.

Magnetic Resonance Studies

For each participant, one scan session was performed in a 7T whole-body magnetic resonance (MR) scanner (Philips Medical Systems, Cleveland, OH) to acquire ^{1}H-MRS and anatomic images. A second session was performed in a 3T scanner to acquire a high-resolution T1-weighted anatomic image that was later used for segmenting the ^{1}H-MRS voxel into different tissue types. The 3T images generally have more homogeneous image contrast, allowing for superior tissue classification.

Acquisition. In the 7T MR scanner, a birdcage transmit head coil (Nova Medical, Inc., Wilmington, MA) with two independent transmit channels (dual transmit) was used in combination with a 32-channel receive coil (Nova Medical, Inc.). A T1-weighted magnetization prepared rapid acquisition