Published in final edited form as:

Biol Psychiatry. 2010 October 1; 68(7): 667–670. doi:10.1016/j.biopsych.2010.05.016.

ELEVATED GAMMA-AMINOBUTYRIC ACID LEVELS IN CHRONIC SCHIZOPHRENIA

Dost Öngür^{1,2}, Andrew P. Prescott³, Julie McCarthy¹, Bruce M. Cohen^{1,2}, and Perry F. Renshaw³

¹ McLean Hospital, Belmont, MA

² Harvard Medical School, Boston, MA

³ University of Utah Medical School, Salt Lake City, UT

Abstract

Background—Despite widely-replicated abnormalities of gamma-aminobutyric acid (GABA) neurons in schizophrenia *postmortem*, few studies have measured tissue GABA levels *in vivo*. We used proton magnetic resonance spectroscopy to measure tissue GABA levels in participants with schizophrenia and healthy controls in the anterior cingulate cortex (ACC) and parieto-occipital cortex (POC).

Methods—21 schizophrenia participants effectively treated on a stable medication regimen (mean age 39.0, 14 male) and 19 healthy controls (mean age 36.3, 12 male) underwent a proton magnetic resonance spectroscopy scan using GABA-selective editing at 4 Tesla after providing informed consent. Data were collected from two 16.7cc voxels and analyzed using LCModel.

Results—We found elevations in GABA/Cr in the schizophrenia group compared with controls ($F(1,65)=4.149$, $p=0.046$) in both brain areas (15.5% elevation in ACC, 11.9% in POC). We also found a positive correlation between GABA/Cr and Glu/Cr which was not accounted for by %GM or brain region.

Conclusions—We found elevated GABA/Cr in participants with chronically treated schizophrenia. *Postmortem* studies report evidence for dysfunctional GABAergic neurotransmission in schizophrenia. Elevated GABA levels, whether primary to illness or compensatory to another process, may be associated with dysfunctional GABAergic neurotransmission in chronic schizophrenia.

Keywords

MRS; MEGAPRESS; GABA; glutamate; anterior cingulate cortex; psychosis

Corresponding Author: Dost Öngür, AB 320, McLean Hospital, 115 Mill St., Belmont, MA 02478, Phone: (617) 855 3922, Fax: (617) 855 2895, dongur@partners.org.

Financial Disclosure: Dr. Renshaw is a consultant to Novartis, GlaxoSmithKline, Kyowa Hakko, and has received research support from Eli Lilly, GlaxoSmithKline, and Roche. None of the other authors reported any biomedical financial interests or potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Clinical manifestations of schizophrenia are proposed to arise from abnormal information processing in the cerebral cortex(1). Because coordination of activity in pyramidal neurons is modulated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), abnormal GABAergic neurotransmission may lead to compromised cortical processing and symptom expression(1–3). Indeed, a large literature implicates GABAergic interneuron abnormalities in schizophrenia(4,5). *Postmortem* studies report reduced message and expression of GAD67 (the synthetic enzyme for GABA), and of GAT1, the transporter that clears synaptic GABA, as well as an apparent compensatory upregulation in postsynaptic GABA_A receptors in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC)(4,6). The literature on tissue GABA measurements in schizophrenia is more discrepant, however, with reports of normal(7) and reduced brain GABA levels(8). Cerebrospinal fluid (CSF) studies report normal (7), reduced (9), or elevated (10,11) GABA in schizophrenia.

Despite great interest in GABAergic dysfunction in schizophrenia few published studies have examined brain GABA levels *in vivo*. Goto et al recently used proton magnetic resonance spectroscopy (MRS) at 3 Tesla in participants with early schizophrenia and reported GABA/Creatine (Cr) reductions in the striatum but not two cortical regions(12). In this study, we used proton MRS to measure brain GABA/Cr levels in a cohort of chronically ill, stably treated participants with schizophrenia at 4 Tesla. We used a GABA-selective editing MRS sequence termed MEGAPRESS(13,14). We collected data from the anterior cingulate cortex (ACC), where GABAergic abnormalities are reported in schizophrenia(5,15) and the parietal occipital cortex (POC) as a control region. Based on reports of abnormal GABAergic interneurons in the ACC *postmortem*, we hypothesized reduced GABA/Cr in the ACC but normal GABA/Cr in the POC in schizophrenia.

METHODS

Participants

Following approval by McLean Hospital IRB, 19 control and 21 schizophrenia participants provided informed consent (NC and SZ groups, respectively). All participants with schizophrenia were chronically ill and receiving antipsychotic treatment (except one). Participants with significant neurological or medical problems, current substance abuse or history of substance dependence (except tobacco smoking) were excluded. All participants had negative urine toxicology. Female participants were in the luteal phase of the menstrual cycle. Participants with schizophrenia were assessed using the Structured Clinical Interview for DSM-IV (SCID), Young Mania Rating Scale (YMRS), Montgomery-Asberg Depression Rating Scale (MADRS), and Positive and Negative Syndrome Scale (PANSS) on scan day. Three participants in the SZ group met criteria for schizoaffective disorder; these participants were chronically psychotic, not currently in a mood episode. Demographic and clinical variables are in Table S1 in the Supplement.

MRI/MRS Procedures

Proton MRS acquisitions were conducted on a 4 Tesla MR scanner (Varian/UnityInova, Varian Inc., Palo Alto, CA), using a 16-rung, single-tuned, volumetric-birdcage coil (XLR Imaging Inc, London, Ontario, Canada (www.xlrimaging.com). See the Supplement for details of anatomical imaging, MRS voxel positioning, and the MEGAPRESS sequence. Briefly, 23x22x33mm (16.7cc) voxels were placed on the ACC or POC, and MEGAPRESS(13) was implemented for GABA editing. Total time in magnet was under 75 minutes. Using the same procedures, we also carried out a test-retest study to determine reliability of our GABA/Cr measures as described in the Supplement.

MRS processing was blinded to diagnosis. For each voxel, the 'off' scans were subtracted from the 'on' scans to yield a difference-edited MEGAPRESS spectrum. In addition, the 'off' scans were averaged to yield a standard PRESS (TE = 68 ms) proton MRS spectrum. We used LCModel (version 6.0–1)(16) with a MEGAPRESS-specific phantom-acquired basis set (see Supplement). Out of 80 possible voxels, 71 usable datasets were obtained. There were no group differences in Cr levels and Cr was used as an internal reference to reduce subject-specific sources of variance in the MRS signal.

Statistics

Demographic and clinical variables were compared across groups using ANOVA, t-tests, and chi-square tests (Table S1 in the Supplement). Given our *a priori* hypothesis, we considered the GABA/Cr ratio as our primary outcome measure. We also calculated GABA/NAA and GABA/Cr* using a T2-corrected Cr resonance magnitude at 68ms based on our reported T2 relaxation times(17). Diagnosis effects were investigated using MANOVAs. We calculated parallel statistics for Glu/Cr and NAA/Cr levels and also examined medication effects on metabolite levels using one-way ANOVAs with medication status (e.g. taking or not taking benzodiazepines) as the between-subjects variable. All p-values are 2-tailed.

RESULTS

GABA/Cr levels showed a significant main effect of diagnosis ($F(1,65)=4.149$, $p=0.046$) and main effect of brain region ($F(1,65)=40.617$, $p<0.001$) but no brain region x diagnosis interaction ($F(1,65)=0.147$, $p=0.702$). GABA/Cr was elevated by 15.5% (ACC) and 11.9% (POC) in SZ (Table 1). Two sample t-tests within each brain region did not yield significant GABA/Cr differences ($t=1.523$, $p=0.137$ in ACC; $t=1.214$, $p=0.234$ in POC). Three participants with schizoaffective disorder had GABA/Cr values (0.113 ± 0.019 in the ACC and 0.080 ± 0.309 in the POC) similar to the SZ group as a whole. Both the GABA/NAA analysis (16.3%/12.9% elevation in ACC/POC; $F(1,65)=4.334$, $p=0.042$) and the T2 relaxation time corrected GABA/Cr analysis (13.7%/8.2% elevation in ACC/POC; $F(1,65)=3.033$, $p=0.087$) showed a similar pattern (Table 1); the latter analysis was significant at a trend level.

There were no significant main effects of brain region or diagnosis and no brain region x diagnosis interaction on Glu/Cr (not shown) (Table 1). There was a significant main effect of brain region on NAA/Cr ($F(1,65)=33.279$, $p<0.001$) but no diagnosis main effect and no brain region x diagnosis interaction (not shown) (Table 1). See the Supplement for correlation analyses.

CPZ equivalents were not correlated with GABA/Cr (Pearson's $R=-0.074$, $p=0.669$), Glu/Cr, or NAA/Cr (not shown). In a series of one-way ANOVAs, GABA/Cr levels were no different between participants who were/were not taking second generation antipsychotics ($F(19,1)=0.079$, $p=0.781$), first generation antipsychotics ($F(19,1)=0.921$, $p=0.348$), benzodiazepines ($F(19,1)=0.913$, $p=0.350$), anticonvulsants ($F(19,1)=3.122$, $p=0.091$) or lithium ($F(19,1)=0.458$, $p=0.506$). When we repeated our primary analysis excluding schizophrenia participants taking anticonvulsants, the diagnosis effect on GABA/Cr was not significant ($F(1,46)=0.229$, $p=0.634$) (Figure S3 in the Supplement).

DISCUSSION

We found elevated GABA/Cr levels in a group of chronically ill, stable participants with schizophrenia using proton MRS at 4 Tesla. The abnormality existed in both brain areas studied, suggesting that it extends outside the ACC. Interestingly, this finding is the reverse of our *a priori* hypothesis. We did not observe NAA/Cr or Glu/Cr abnormalities in this study

although NAA/Cr reductions are widely reported in schizophrenia(18). Potential explanations for the discrepancy include clinical status or small sample size.

Strengths of this study include high magnetic field, metabolite-selective data acquisition and analysis, and focus on two distinct brain regions. We evaluated our GABA quantification in a test-retest study where our reliability measures were comparable to previous reports(19). The spectral signal-to-noise ratios and CRLBs were similar across the two study groups, suggesting comparable overall spectral quality and fitting. By collecting data from two brain regions, we showed that GABAergic abnormalities in schizophrenia are not selective to the ACC. This is consistent with *postmortem* reports of GABAergic abnormalities in multiple cortical regions in schizophrenia(20). The ACC and POC are functionally linked through the default mode network, however, and other regions may show different patterns.

The major limitation of this study is that all participants except one were taking medications on a stable regimen. Many psychotropic medications have prominent effects on GABAergic neurotransmission and we cannot rule out medication effects on GABA/Cr measures in our study. We made attempts to examine medication effects in our data: there was no correlation of CPZ equivalents and GABA/Cr, and no evidence that lithium, anticonvulsants, or benzodiazepines impact GABA/Cr measures. Anticonvulsants can affect GABA levels and there was a trend towards anticonvulsant effect on GABA/Cr levels in our data. When we repeated our analysis excluding participants on anticonvulsants, the diagnosis effect on GABA/Cr was no longer significant, suggesting that we cannot rule out a role for anticonvulsant medication effects in our GABA/Cr findings. These subgroup analyses are underpowered due to small N, and well-designed medication-effect studies are ultimately needed to examine this issue.

Although Cr's utility as internal reference has been questioned because Cr levels may change in disease states, internal referencing to Cr did not skew our results because there were no group differences in Cr. One alternative to internal referencing with Cr would be using the water resonance but we did not acquire useable water unsuppressed spectra.

Our findings provide evidence for abnormalities in cortical GABAergic neurotransmission in schizophrenia. The literature on *postmortem* and CSF GABA levels in schizophrenia is equivocal with reductions, no change, or elevations in GABA CSF in chronically treated participants(10,11). One MRS study showed reduced GABA/Cr in the basal ganglia(12) but little information was provided regarding voxel location/GM-WM-CSF composition/spectral quality, thus precluding direct comparison with our study. Many sources of noise including medication/substance effects contribute to discrepancies in the literature; large well-controlled studies are needed to reconcile the multiple findings. We propose that an abnormality in GABAergic neurotransmission in schizophrenia may be associated with elevated brain GABA levels. Thus, there may be an uncoupling of neurotransmitter tissue levels and efficacy of neurotransmission in some circumstances.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by 5K23MH079982 (DO) from the National Institute of Mental Health, and sponsored in part by the Counter-Drug Technology Assessment Center, an office within the Office of National Drug Control Policy, via Contract Number DABT63-99-C awarded by the Army Contracting Agency. The content of the information does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred. This project also was sponsored in part by NIH grant S10 RR13938.

References

1. Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* 2008;31(5):234–242. [PubMed: 18395805]
2. Blum BP, Mann JJ. The GABAergic system in schizophrenia. *Int J Neuropsychopharmacol* 2002;5(2):159–179. [PubMed: 12135541]
3. Carlsson A. The neurochemical circuitry of schizophrenia. *Pharmacopsychiatry* 2006;39 (Suppl 1):S10–14. [PubMed: 16508890]
4. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci* 2005;6(4):312–324. [PubMed: 15803162]
5. Benes FM. Altered glutamatergic and GABAergic mechanisms in the cingulate cortex of the schizophrenic brain. *Arch Gen Psychiatry* 1995;52(12):1015–1018. discussion 1019–1024. [PubMed: 7492253]
6. Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M. Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. *Proc Natl Acad Sci U S A* 2007;104(24):10164–10169. [PubMed: 17553960]
7. Perry TL, Hansen S, Jones K. Schizophrenia, tardive dyskinesia, and brain GABA. *Biol Psychiatry* 1989;25(2):200–206. [PubMed: 2564786]
8. Korpi ER, Kaufmann CA, Marnela KM, Weinberger DR. Cerebrospinal fluid amino acid concentrations in chronic schizophrenia. *Psychiatry Res* 1987;20(4):337–345. [PubMed: 2885877]
9. van Kammen DP, Sternberg DE, Hare TA, Waters RN, Bunney WE Jr. CSF levels of gamma-aminobutyric acid in schizophrenia. Low values in recently ill patients. *Arch Gen Psychiatry* 1982;39(1):91–97. [PubMed: 7055411]
10. Zimmer R, Teelken AW, Meier KD, Ackenheil M, Zander KJ. Preliminary studies on CSF gamma-aminobutyric acid levels in psychiatric patients before and during treatment with different psychotropic drugs. *Prog Neuropsychopharmacol* 1980;4(6):613–620. [PubMed: 7220676]
11. McCarthy BW, Gomes UR, Neethling AC, Shanley BC, Taljaard JJ, Potgieter L, Roux JT. gamma-Aminobutyric acid concentration in cerebrospinal fluid in schizophrenia. *J Neurochem* 1981;36(4):1406–1408. [PubMed: 7264636]
12. Goto N, Yoshimura R, Moriya J, Kakeda S, Ueda N, Ikenouchi-Sugita A, Umene-Nakano W, Hayashi K, Oonari N, Korogi Y, Nakamura J. Reduction of brain gamma-aminobutyric acid (GABA) concentrations in early-stage schizophrenia patients: 3T Proton MRS study. *Schizophr Res* 2009;112(1–3):192–193. [PubMed: 19464152]
13. Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R. Simultaneous in vivo spectral editing and water suppression. *NMR Biomed* 1998;11(6):266–272. [PubMed: 9802468]
14. Hasler G, van der Veen JW, Geraci M, Shen J, Pine D, Drevets WC. Prefrontal Cortical Gamma-Aminobutyric Acid Levels in Panic Disorder Determined by Proton Magnetic Resonance Spectroscopy. *Biol Psychiatry*. 2008
15. Carter CS, MacDonald AW 3rd, Ross LL, Stenger VA. Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: an event-related fMRI study. *American Journal of Psychiatry* 2001;158(9):1423–1428. [PubMed: 11532726]
16. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993;30(6):672–679. [PubMed: 8139448]
17. Öngür D, Prescot AP, Jensen JE, Rouse ED, Cohen BM, Renshaw PF, Olson DP. T2 relaxation time abnormalities in bipolar disorder and schizophrenia. *Magnetic Resonance in Medicine*. (In Press).
18. Ongur D, Jensen JE, Prescot AP, Stork C, Lundy M, Cohen BM, Renshaw PF. Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. *Biol Psychiatry* 2008;64(8):718–726. [PubMed: 18602089]
19. Marjanska, M.; Henry, PG.; Auerbach, EJ.; Franc, D.; Mueller, B.; Ugurbil, K.; Lim, KO. Reproducibility of In Vivo GABA Quantification in Anterior Cingulate at 3 Tesla. 2007. p. 1398
20. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry* 2008;165(4):479–489. [PubMed: 18281411]

Figure 1.

(A) Midsagittal (left) and axial (right) views demonstrate ACC and POC voxel placement on a canonical individual T1-weighted image – not individual subject data. (B) Sample MEGAPRESS spectra from the ACC in a control subject (left) and subject with schizophrenia (right). The actual data are shown in black, with LCMoel fit overlaid in red, and the residual tracing on top of the panel. Individual metabolite basis sets contributing to the LCMoel fit are stacked at the bottom panels.

Table 1

Metabolite levels (Mean±SD)

	Cr	GABA/Cr	GABA/Cr*	GABA/NAA	Glu/Cr	NAA/Cr	Cho/Cr	ml/Cr
ACC								
NC	3286±627	0.103±0.032	0.103±0.032	0.087±0.025	0.804±0.152	1.176±0.121	0.537±0.338	0.249±0.033
SZ	3171±603	0.119±0.029	0.117±0.029	0.101±0.026	0.889±0.210	1.185±0.127	0.595±0.490	0.265±0.044
POC								
NC	1996±327	0.064±0.016	0.064±0.016	0.047±0.013	0.772±0.081	1.372±0.109	0.178±0.032	0.227±0.023
SZ	2016±345	0.071±0.018	0.069±0.018	0.053±0.012	0.769±0.085	1.341±0.099	0.200±0.032	0.240±0.031

Abbreviations are as in the text. GABA/Cr* refers to ratios using T2-corrected Cr resonance magnitude.