Reductions in Occipital Cortex GABA Levels in Panic Disorder Detected With $^{1}$H-Magnetic Resonance Spectroscopy

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**Background:** There is preclinical evidence and indirect clinical evidence implicating γ-aminobutyric acid (GABA) in the pathophysiology and treatment of human panic disorder. Specifically, deficits in GABA neuronal function have been associated with anxiogenesis, whereas enhancement of GABA function tends to be anxiolytic. Although reported peripheral GABA levels (eg, in cerebrospinal fluid and plasma) have been within reference limits in panic disorder, thus far there has been no direct assessment of brain GABA levels in this disorder. The purpose of the present work was to determine whether cortical GABA levels are abnormally low in patients with panic disorder.

**Methods:** Total occipital cortical GABA levels (GABA plus homocarnosine) were assessed in 14 unmedicated patients with panic disorder who did not have major depression and 14 retrospectively age- and sex-matched control subjects using spatially localized $^{1}$H-magnetic resonance spectroscopy. All patients met DSM-IV criteria for a principal current diagnosis of panic disorder with or without agoraphobia.

**Results:** Patients with panic disorder had a 22% reduction in total occipital cortex GABA concentration (GABA plus homocarnosine) compared with controls. This finding was present in 12 of 14 patient-control pairs and was not solely accounted for by medication history. There were no significant correlations between occipital cortex GABA levels and measures of illness or state anxiety.

**Conclusions:** Panic disorder is associated with reductions in total occipital cortex GABA levels. This abnormality might contribute to the pathophysiology of panic disorder.

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YSREGULATION in brain γ-aminobutyric acid (GABA) neuronal function might contribute to the pathophysiology of human panic disorder. For example, lowered brain GABA levels are associated with anxietylike behaviors in animals, and elevated brain GABA levels tend to be associated with anxiolysis. Although clinical studies of GABA levels in patients with panic disorder have shown normal plasma and cerebrospinal fluid GABA levels, to date there have been no in vivo studies, to our knowledge, evaluating brain GABA levels in this patient population. Other components of the GABA system, such as the benzodiazepine (BZD) receptor, have been implicated in the pathophysiology of panic. For instance, impaired brain GABA/BZD receptor functioning has been directly linked to neophobic behaviors in mice, behaviors that resemble human agoraphobia. Furthermore, a generalized cortical reduction in BZD receptor binding in patients with panic disorder was recently observed using a positron emission tomographic technique to test whether total occipital cortex GABA levels (GABA plus homocarnosine) are abnormally reduced in panic disorder. In this study, we chose to evaluate GABA levels in an occipital cortex region of interest (ROI) with effects being most pronounced in the right orbitofrontal and insular cortices, although, subsequently, other groups also using positron emission tomography did not detect these abnormalities. In addition, regional cortical reductions in BZD receptor binding have been identified with single-photon emission computed tomographic techniques in frontal, temporal, left hippocampal, precuneus, and occipital areas of patients with panic disorder.

We hypothesized, based on the previously mentioned observations, that there are deficits in GABA neuronal functioning in panic disorder. We therefore executed a study using a novel $^{1}$H-magnetic resonance spectroscopic (MRS) technique to test whether total occipital cortex GABA levels (GABA plus homocarnosine) are abnormally reduced in panic disorder. In this study, we chose to evaluate GABA levels in an occipital cortex region of interest (ROI) because researchers have developed a reliable method to measure GABA in this location and have used it successfully to detect GABA abnormalities in other neu-
PARTEICIPANTS AND METHODS

PARTICIPANTS

This study was conducted at the Yale Anxiety Clinic and the Yale Magnetic Resonance Center, New Haven, Conn. Most patients (12 of 14) responded to paid advertisements in local newspapers and on television; patient 3 was self-referred and patient 9 was a clinic referral. After a psychiatric evaluation performed by a research psychiatrist (A.W.G. or A.A.), patients were informed of the study rationale and procedures. All patients gave their written informed consent to participate and received their own copy of the Yale institutional review board–approved informed consent document.

We studied 14 outpatients with panic disorder (8 women and 6 men; mean±SD age, 37±10 years) who were moderately ill judging by their mean±SD total prescan Panic Disorder Severity Scale (PDSS)24 score (13±4; n=13). The PDSS samples 7 symptom domains (each scored on a scale from 0-4) relevant to panic disorder, including frequency of panic symptoms, distress during panics, phobic symptoms, anticipatory anxiety, and functioning (see Shear et al20 for a review of psychometric properties). All patients had a weekly panic attack frequency of 1 or more in the month before study entry. Baseline mean±SD scores were as follows: Hamilton Anxiety Rating Scale (HAM-A),21 17±8 (n=14); 25-item Hamilton Depression Rating Scale (HAM-D),22 20±10 (n=14); 17-item HAM-D,23 14±6; and Clinician-Rated Anxiety Scale (CRAS) (contains 37 items, each rated on a scale from 0-4, covering panic attacks, phobias, and many symptoms of generalized anxiety),24,25 31±16 (n=14). All patients had normal physical examination findings and normal results on follow-up tests, including urine toxicology, urinalysis, electrocardiogram, serum electrolytes and glucose, liver and thyroid function tests, blood cell count and serum gonadotrophin levels (for women), and human immunodeficiency virus testing. Of the women, 1 was menopausal, 1 was perimenopausal, 3 were at the end of their menstrual cycle just before the scan, 1 was midcycle, 1 was in the first half of the cycle, and 1 was in the second half of the cycle. Patients met DSM-IV criteria26 for a current principal diagnosis of panic disorder with or without agoraphobia. The panic diagnosis was confirmed using a semistructured interview (either the Anxiety Disorders Interview Schedule DSM-IV version27 or the Structured Clinical Interview for DSM-IV28) administered by experienced research personnel under the supervision of the principal investigator (A.W.G.).

Patients with a lifetime history of a psychotic disorder, a bipolar disorder, major depressive disorder, obsessive-compulsive disorder, an eating disorder, posttraumatic stress disorder, alcohol dependence, or a major personality disorder were excluded. In addition, patients were excluded if they had had a substance abuse disorder within 6 months of the diagnostic interview. Patients 2, 9, and 14 (Table) were smokers (>10 cigarettes per day). Patient 11 had a probable comorbid somatoform disorder (conversion disorder), and patient 8 carried an additional diagnosis of social phobia—specific subtype. Of 14 patients studied, 9 were medication naive (patients 1, 4, 5, 7, 10, 11, 12, 13, and 14). Of the remaining 5 patients, 2 had discontinued medication use 3 months before study entry (patient 9 was taking desipramine hydrochloride and clonazepam and patient 6 was taking sertraline hydrochloride and clonazepam) and 3 were taking occasional as-needed doses of short-acting BZD medications (patients 3 and 10 were taking 0.25- and 0.5-mg tablets of alprazolam, respectively, and patient 2 was taking one half of a 0.5-mg tablet of clonazepam). The 3 patients who had taken medications as needed were completely medication free for at least 1 week before the first MRS scan.

Control subjects (in good physical health and medication free) were part of the Yale Magnetic Resonance Center’s control database of 30 subjects. They had no lifetime history of psychiatric illness by clinical assessment. Structured Clinical Interview evaluations were not conducted on controls. Controls were paired with patients retrospectively based on sex and age. Complete sex matching was accomplished, and we attempted to ensure that patient-control pairs were close in age (mean±SD age difference in the 14 patient-control pairs, 4±4 years). The mean±SD time between matched control and patient scans was 6±4 months, with control scans generally occurring before patient scans. Recruitment and assessment procedures for controls and patients remained constant during MR data acquisition (27 months). Controls were recruited from flyers placed in the Yale Medical Center.

SPECTROSCOPIC AND IMAGING PROCEDURES

We used a parallel-group design to test whether unmedicated patients with panic disorder had lower occipital cortical total GABA levels (cortical GABA plus homocarnosine, a GABA-containing dipeptide) than retrospectively age- and sex-matched controls. Each patient and control subject underwent an MRS scan (lasting approximately 1.5 hours). The concentration of GABA was measured by comparing the integrated GABA resonance from the MRS edited spectrum with the integrated creatine resonance obtained during the same scan.

A trained research assistant or registered nurse under supervision of the principal investigator accompanied the patient throughout the MRS test (approximately 1.5 hours).

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The imaging and spectroscopy work was conducted at Yale Magnetic Resonance Center using a 2.1-T, 1-m bore magnet (Oxford Magnet Technologies, Oxford, England) with a spectrometer (Bruker Avance Biospec; Bruker Instruments, Billericay, Mass) and actively shielded magnetic field gradients (Oxford Magnetic Technologies). A workstation (Silicon Graphics Inc, Chippewa Falls, Wis) was used for image and spectroscopic analysis.

Before spectroscopy, T1-weighted, gradient echo magnetic resonance images were taken to select a 13.5-cm³ \((1.5 \times 3.0 \times 3.0-\text{cm})\) volume in the occipital cortex for MRS. The 1.5-cm dimension was along the axis that was perpendicular to the surface coil plane, and the volume was centered 1.5-cm deep to the dura mater. For each ROI, approximately 95% of the nuclear magnetic resonance signal was derived from the voxel selected. The occipital ROI was centered on the midline, included the visual cortex (on the left and right sides), and was identical to the ROI used in previous works.\(^{16,17}\) Participants lay supine on a pallet with their occiput resting next to an 8-cm radiofrequency surface coil tuned to the \(^1\text{H}\)-nuclear magnetic resonance frequency of 89.43 MHz. An automated shimming protocol was used to maximize B\(_0\) field uniformity in the ROI.\(^{24}\) Three-dimensional localization of the sensitive volume was accomplished by means of an image-selected in vivo spectroscopy sequence (comprising 8-millisecond phase-swept, hyperbolic secant inversion pulses, \(\mu=5\); bandwidth, 2000 Hz). Water suppression was achieved by an 80-millisecond hyperbolic secant-selective inversion pulse and a semiselective refocusing pulse (90° pulse; duration, 120 microseconds).\(^{16}\) Other spectral acquisition parameters for collection of GABA data included a sweep width of 2500 Hz, an acquisition time of 510 milliseconds, a repetition time of 3.39 seconds, and an echo time of 68 milliseconds.

GABA Editing Procedure

A homonuclear J-editing procedure was used to separate the GABA C4 triplet resonance at 3.0 ppm from overlapping resonances. This was done by applying a 26.5-millisecond DANTE (Delays-Alternating with Nutations-for Tailored Excitation) inversion pulse to the 1.9-ppm C3 GABA multiplet resonance.\(^{16}\) Subtraction of a spectrum acquired with the DANTE pulse from one in which the DANTE pulse was not applied provided the edited spectrum that reflected total cortical GABA levels.

Cortical GABA Measurement

The C4 GABA resonance from the edited spectrum was integrated and compared with an integrated creatine resonance (3.03 ppm) obtained during the same acquisition. In vivo time domain data were zero filled to 32K and multiplied by a 3-Hz exponential function before Fourier transformation. In the edited spectrum, the C4 GABA resonance was integrated over a 0.30-ppm bandwidth centered over 3.0 ppm. The creatine signal was integrated over a 0.2-ppm bandwidth centered at 3.0 ppm of the GABA-inverted spectrum. Cortical GABA concentrations were calculated from the following formula, which compares the integrated GABA resonance from the MRS edited spectrum with the integrated creatine resonance\(^{16}:\)

\[
[GABA] = \frac{(G^* / Cr^* - M / Cr^*) \times (ICF) \times (EE) \times (3/2)}{\times (Cr)}
\]

where \(G^*\) is the GABA integral in the edited spectrum, \(Cr^*\) is the creatine integral, \(M\) is the contribution of macromolecule resonances at 3.0 ppm, ICF is a correction factor for the limited integral bandwidths determined from localized edited spectra of solutions of GABA and creatine line broadened to match the in vivo processed line widths, EE is a correction factor for loss of signal intensity during the editing procedure, \(3/2\) is the creatine-GABA proton ratio, and \([Cr]\) is the concentration of creatine in the human occipital cortex (average cortical concentration, 9 mmol/kg).

The correction factors ICF and EE were obtained by subjecting a GABA solution in an 11.3-cm bottle to the same localization and editing procedure used in vivo. The GABA signal from the cortex was also calibrated by comparison with phantoms containing known solutions of GABA and creatine (the phantom studies were designed to simulate in vivo coil loading).\(^{16}\) Integration of GABA over a 0.3-ppm bandwidth was based on the assumption that the GABA line shape was constant. The assumption was validated based on the creatine line width, which was measured to vary by less than 1 Hz between studies. Measurements of pure GABA and creatine levels in solution show that small changes in line width have a minimal effect on the relative integrals.

STATISTICAL ANALYSIS

Nonparametric statistical procedures were used for all analyses. Paired tests were performed for all between-group analyses because the control sample had been carefully age and sex matched to the patient sample. The primary analysis, testing for a patient-control difference in cortical GABA levels, used the Wilcoxon signed rank test. Other subgroup analyses comparing groups on some clinical and demographic characteristics also used this test. Within-group Spearman correlational analyses were performed to determine whether cortical GABA levels were associated with measures of clinical illness severity, such as the HAM-A, PDSS, HAM-D, and CRAS, as well as to examine whether age correlated with cortical GABA levels in either group. The \(\alpha\) level for all statistical analyses was set at .05, and all tests were 2-tailed. Values are expressed as mean±SD.

However, women panickers vs controls (GABA level, 1.39±0.43 vs 1.89±0.38 mmol/kg; \(W=−30, n=8\) pairs; \(P=.04\)) had a statistically significant reduction in occipital cortex GABA concentration compared with men vs controls (GABA level, 1.35±0.32 vs 1.61±0.26 mmol/kg; \(W=−11, n=6\) pairs; \(P=.31\)). Age did not correlate with cortical GABA levels in either patients (\(n=14; r=−.09; P=.8\)) or controls (\(n=14; r=−.28; P=.34\)). A statistically significant reduction in patient GABA levels...
relative to controls remained (W = −60, n = 12 pairs; P < .02) despite removal of 2 patient-control pairs (pairs 4 and 5) from the Wilcoxon analysis who were not closely age matched. Inspection of a subgroup of medication-naïve patients with panic disorder (patients 1, 4, 5, 7, and 10-14) indicated that 7 of 9 had lower GABA levels compared with controls (1.39 ± 0.47 vs 1.85 ± 0.4 mmol/kg; W = −33, n = 9 pairs; P = .055).

OTHER CLINICAL VARIABLES AND TOTAL CORTICAL GABA LEVELS

To examine possible associations between cortical GABA levels and some illness severity measures (HAM-A, HAM-D, PDSS, and CRAS), we performed Spearman correlations on the patient data. The following correlation coefficients were observed: for GABA levels and the HAM-A, r = 0.35, n = 14, P = .23; the HAM-D, r = 0.29, n = 14, P = .32; the PDSS, r = 0.28, n = 13, P = .36; and the CRAS, r = 0.27, n = 14, P = .34. A modest positive correlation was observed between cortical GABA concentration and degree of agoraphobia, as measured on PDSS item 4 (r = 0.56; n = 13; P = .048). However, this finding did not remain statistically significant after Bonferroni correction. Finally, we found no significant association between prescan state anxiety (as measured on a visual analogue scale of anxious mood from 0-100 mm) and cortical GABA levels (r = −0.03; n = 13; P = .9).

MEASUREMENTS OF THE REFERENCE METABOLITE, CREATINE

We did not systematically collect additional short echo spectra for analysis of creatine, water, and other metabolites in our study sample. However, we have these data for patients 8 and 12 and controls 4, 13, and 14. Creatine values of 9.0 mmol/kg were observed in each case. Thus, these limited data suggested that creatine levels were similar between groups and similar to those reported in the literature.30

COMMENT

We observed abnormally reduced total occipital cortex GABA levels in a sample of unmedicated patients with panic disorder who did not have major depression, adding support to preclinical and clinical evidence suggesting that deficits in GABA function contribute to the pathophysiologic process of panic. The finding was relatively consistent, with 12 of 14 patients having lower GABA levels than their respective matched controls. The result was not fully explained by previous medication exposure. Women with panic disorder seemed to have more pronounced reductions in cortical GABA levels than men in our sample, although the significance of this finding is uncertain because it might be more related to sample size.

There are several limitations of the present study that merit additional comment. First, we used a retrospective control group, which limited our ability to match for variables such as age and, in females, phase of the menstrual cycle, both of which might affect central nervous system GABA levels.31,32 Follow-up studies should more carefully control for these variables by assessing control groups prospectively.

Second, we obtained data from a single occipital ROI and therefore cannot say at this point whether our observation is limited to certain cortical regions or present throughout the cortex.

Third, in most of our sample, we did not apply a segmentation procedure to adjust our GABA measurements based on the percentage of gray matter per voxel of interest. However, we obtained this information systematically for the last 3 patient-control pairs using a method devised by our group.33 The mean percentage of gray matter per voxel in these patient scans was 61% compared with 63% in controls (as determined from quantitative images of the T1 relaxation constant of tissue water). Thus, these pilot data suggest that the reduction in GABA is not due to reduced cortical gray matter content. However, subsequent studies are benefiting from the systematic application of segmentation protocols.
Fourth, the related compound, homocarnosine (GABA plus a histidine residue), is coenzyme with GABA and was not assessed in this study. Thus, the observed changes could be related to changes in the central nervous system level of homocarnosine in panic. Homocarnosine is of particular interest because of its potential neuromodulatory role in the central nervous system.

Fifth, we determined the concentration of cortical GABA by reference to total creatine level (creatinine plus phosphocreatine). Although this is a common method of quantification in MRS, changes in creatine levels would alter the GABA measurements. However, total cortical GABA levels determined by our MRS technique compare favorably to standard chemical assays of postmortem brain tissue and brain biopsy tissue in animals and humans. The GABA transaminase inhibitor vigabatrin produces marked amplification of the GABA MRS signal in animals and humans, as expected. Magnetic resonance spectroscopic measurements of occipital cortex GABA levels in healthy humans performed by the University of Alabama group, with a highly sensitive 4-T magnet, compared favorably with the data our group has already generated. Further validity and reliability studies are ongoing.

If replicated, the low occipital cortex GABA finding is likely to have implications for our understanding of the relationship between panic and other neuropsychiatric disorders. Recently, abnormally low occipital cortex GABA levels were observed in depressed patients. Therefore, the low cortical GABA concentration observed in this study might be a nongenetic finding reflecting a history of neuropsychiatric disease. However, it is notable, in this regard, that our group has not observed low occipital cortex GABA levels in schizophrenia (W. Abi-Saab, MD, unpublished data, 2000) or in patients with bipolar depression. Alternatively, low cortical GABA concentration could be a traitlike abnormality that predisposes to a variety of behavioral disturbances (depression, panic disorder, and alcoholism). Another possibility is that low cortical GABA levels are associated with distinct pathophysiological processes (eg, panic disorder, depression, epilepsy, and alcoholism). Follow-up investigations are indicated to discriminate among these possibilities. Attention to the prescan medication-free period (>4 weeks; including no as-needed medications), to protect against the potentially confounding effects of medication withdrawal syndromes, and the within-scan acquisition of other informative metabolite measurements (eg, creatine, choline, N-acetylaspartate, glutamate, and homocarnosine) will add to the future studies.

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