Background: Patients with stiff-person syndrome (SPS) have circulating antibodies against glutamic acid decarboxylase, the rate-limiting enzyme responsible for the synthesis of γ-aminobutyric acid (GABA). Although the patients’ symptoms of stiffness and unexpected spasms can be explained on the basis of reduced or impaired inhibitory neurotransmitters, such as GABA, it is unclear whether the level of GABA in the brains of these patients is reduced and, if so, whether the reduction is due to anti–glutamic acid decarboxylase antibodies.

Objective: To measure GABA levels in the brains of patients with SPS.

Design: Prospective case-control study.

Setting: National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md.

Patients: Eight patients with SPS with high titers of circulating anti–glutamic acid decarboxylase antibodies and typical clinical symptoms of SPS and 16 control subjects.

Main Outcome Measures: Results of brain magnetic resonance imaging and magnetic resonance spectroscopy, which measures GABA levels in specific brain regions.

Results: No abnormalities were noted on brain magnetic resonance images. A prominent and significant decrease in GABA level was, however, observed in the sensorimotor cortex and a smaller decrease in the posterior occipital cortex but not in the cingulate cortex or pons.

Conclusions: The reduction of brain GABA in patients with SPS supports the clinical symptoms and indicates that the inhibitory GABAergic pathways are involved in the disease. Regardless of the responsible autoantigens, in SPS autoantibodies block the function of GABAergic neurons and interfere with the synthesis of GABA but do not cause structural changes in the brain.

Arch Neurol. 2005;62:970-974

TIFF-PERSON SYNDROME (SPS) is an often-overlooked disabling disorder characterized by muscle rigidity and episodic spasms involving axial and limb musculature.1-3 The hallmark sign of SPS is the co-contraction of agonist and antagonist muscles, with continuous motor unit firing at rest documented electrophysiologically.4,6,7

Up to 65% of patients with SPS have antibodies against glutamic acid decarboxylase (GAD-65), the rate-limiting enzyme for the synthesis of γ-aminobutyric acid (GABA) at the GABAergic nerve terminals.8,9 In vitro, anti-GAD antibodies inhibit GAD activity,10 but whether these antibodies exert the same effect in vivo and are of pathogenic relevance in patients with SPS remains unclear.11 Of interest, anti-GAD antibodies are produced intrathecally and the level of GABA appears reduced in the cerebrospinal fluid of these patients,12 but whether there is also reduction of GABA in certain brain regions is unknown. Demonstrating reduced GABA in the brain will strengthen the view that GABAergic pathways are affected, and will be consistent with the impairment of inhibitory pathways as the main mechanism of the patients’ symptoms of stiffness and spasms.9,13

In this study, we measured regional brain GABA levels in patients with SPS and in healthy control subjects, using a novel method of 2-dimensional (2D) J-resolved magnetic resonance imaging that allows simultaneous quantification of multiple metabolites in defined brain regions within a single acquisition.14,15

METHODS

SUBJECTS

We studied 8 patients with SPS (mean ± SD age, 43.2 ± 9.3 years; 5 men and 3 women) and 16 age-matched healthy volunteers as control subjects (mean ± SD age, 43.3 ± 16 years; 8 men and...
8 women). All controls had normal neurologic examinations and normal MR images. Informed consent was obtained for each subject under a protocol approved by the institutional review board of the National Institute of Neurological Disorders and Stroke, Bethesda, Md. Patients selected for the study fulfilled the clinical criteria for diagnosis of SPS. The mean ± SD duration of symptoms since onset of the disease was 6.6 ± 2.1 years (range, 1–17 years). At the time of the MR examination, clinical symptoms included characteristic muscular stiffness involving the lower extremities and lower back, and episodic spasms. The serum from all of our patients had very high titers of circulating anti-GAD antibodies, as determined by enzyme-linked immunosorbent assay. Western blot, and immunostaining of GABAergic neurons in the rat brain, as described previously. For each patient, MR scanning was repeated at monthly intervals during a 3-month period to obtain average baseline values and thereby minimize the known episodic variations in this disease. Of the 8 patients, 3 were receiving long-term treatment with benzodiazepines, 2 with baclofen, and 3 with both medications. In all patients, drug treatment was maintained at the lowest possible dosage beginning no later than 1 month before the study, and these dosages remained unchanged during the baseline evaluation period.

REGIONS OF INTEREST

The MR images of the brain were obtained by means of a 1.5-T MR imager (Signa; GE Medical Systems, Milwaukee, Wis) equipped with self-shielded gradients and a quadrature head coil. Each subject was placed midline in the head coil lying in a supine position on the gantry with the head immobilized by large Styrofoam pads pressed comfortably to either side of the head in the area of the ears. Conventional MR imaging included T1-weighted sagittal and axial spin-echo acquisitions (repetition time, 400 milliseconds; echo time, 15 milliseconds), spin density and T2-weighted axial fast spin-echo acquisitions (repetition time, 4000 milliseconds; echo times 15 and 105 milliseconds), 5-mm section thickness, 24-cm field of view, and matrix size of 256 × 256. From the conventional images, regions of interest (ROIs) were identified on the conventional scans in sensorimotor cortex regions corresponding to the extremities and trunk bilaterally, and at the midline in the posterior occipital cortex, cingulate cortex, and pons (Figure 1). Selection of these regions was based on the clinical symptoms of SPS as well as previous observations regarding motor cortex and brainstem excitability. The ROIs measured 2.5 × 2.5 × 2.0 cm (Figure 1). This ROI size was selected to provide adequate signal-to-noise ratios for GABA concentrations in the physiologic range of 1 mmol/L, while acquisition times were minimized to maintain sufficiently rapid temporal sampling rates.

MR SPECTROSCOPY

Localized 2D J-resolved spectra were acquired by means of a modification of the standard point-resolved spectroscopy (PRESS) pulsing sequence, also known as a 2D J-PRESS sequence. Acquisition parameters included a repetition rate of 2000 milliseconds, echo time of 35 milliseconds, digital resolution of 1024 complex points, and sweep width of 1000 Hz. During each scan, evolution time progressively increased from 35 milliseconds in 64 increments with 10-millisecond steps and 2 averages per increment, resulting in a total acquisition time of 6 minutes per ROI spectrum. Global and local shimming and chemical shift-selective water suppression were performed for each acquisition.

Figure 1. Regions of interest (ROIs) on axial (A) and sagittal (B) magnetic resonance images of a patient’s brain. The white squares show the location of the ROIs. A, The sensorimotor ROIs were centered on the medial sensorimotor regions bilaterally, corresponding to lower extremities and trunk. B, Additional ROIs were positioned at the midline in the posterior occipital region, cingulate cortex, and pons.

DATA ANALYSIS AND METABOLITE MEASUREMENTS

Spectroscopic data analysis was performed on a workstation (Sun Microsystems, Santa Clara, Calif) using software developed at the National Institutes of Health and designed to analyze decoupled metabolite peaks. Homonuclear decoupled proton spectra from each ROI acquisition were postprocessed by means of locally developed conventional algorithms that included convolution solvent filtering, cosine-squared bell apodization, zero-filling twice, Fourier transform, and modulus calculation. The analysis of preselected spectral regions with known peak assignment was hypothesis driven, and metabolites were identified on the 2D spectra from their peak intensities. Metabolite peak intensities were measured by means of modulus peaks occurring along the J = 0 axis (J0 subspectrum) at the characteristic chemical shifts assigned to the largest resonances of N-acetyl aspartate (NAA) (CH3 at 2.02 ppm), creatine (Cre) (CH3 at 3.04 ppm), and choline (Cho) (CH3 at 3.2 ppm). The α-CH2 GABA peak (at 2.31 ppm) was chosen because it is the largest peak that does not overlap with either the NAA or Cre peaks. Similarly, the γ-CH2 glutamate (Glu) peak (at 2.36 ppm) was evaluated. Additional postprocessing included tilting, symmetrization, and baseline correction. Metabolite peak intensities were obtained directly from the tilted unsymmetrized 2D spectrum at the J0 axis to take advantage of the greater signal-to-noise ratios for the peaks occurring at J0 compared with the side peaks, and to avoid introducing errors from further data manipulation introduced by procedures such as symmetrization of the side peaks. Peak intensity measurement was performed by automated computer determination of the peak height at the characteristic assigned resonance of each metabolite. All signal-to-noise ratios were measured and calculated as ratios of the measured metabolite peak intensities to the root mean squared values of the measured background noise levels, the latter being the signal variance of background 2D spectral regions with few detectable metabolite peaks. For each patient, mean metabolite values were then calculated by averaging the respective metabolite results obtained from the baseline evaluation scans.

METABOLITES

N-acetylaspartate is a compound localized exclusively in neurons, and its changes may reflect local neuronal loss or neuronal injury. The concentration of Cho reflects cellular density and total membrane content and varies with the relative amounts of gray to white matter and of glial cells to neu-
changes in other brain regions were not statistically significant. (29%-36%) and to a lesser extent in posterior occipital cortex (12%); cortex bilaterally in patients with SPS relative to healthy control subjects (mean ± SEM). The GABA/Cre ratios are significantly decreased in motor different regions of interest (Figure 1) and are expressed as ratios to Cre peaks were identified and measured. Levels of GABA, Glu, NAA, Cho, and Cre were expressed as ratios relative to levels of Cre in controls and patients and compared with the t statistic (2-tailed).

## RESULTS

Conventional MR imaging studies in the patient group were unremarkable. The NAA, Cho, and Glu ratios to Cre did not show any significant differences in the different brain regions compared with the controls (Table). However, decreases in the GABA levels in several brain regions were observed in the patient group relative to the

### Table. GABA Levels in Different Brain Regions

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Region</th>
<th>Control Subjects</th>
<th>Patients With SPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cre</td>
<td>RM</td>
<td>1.87 ± 0.06</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>1.84 ± 0.04</td>
<td>1.66 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>1.89 ± 0.04</td>
<td>1.80 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>1.83 ± 0.06</td>
<td>1.82 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.79 ± 0.08</td>
<td>1.85 ± 0.08</td>
</tr>
<tr>
<td>NAA/Cho</td>
<td>RM</td>
<td>2.10 ± 0.11</td>
<td>1.88 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>1.97 ± 0.15</td>
<td>1.77 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>2.53 ± 0.12</td>
<td>2.23 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>1.58 ± 0.08</td>
<td>1.40 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.37 ± 0.09</td>
<td>1.27 ± 0.03</td>
</tr>
<tr>
<td>Cho/Cre</td>
<td>RM</td>
<td>0.91 ± 0.05</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>1.02 ± 0.08</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>0.70 ± 0.02</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>1.19 ± 0.08</td>
<td>1.31 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.37 ± 0.12</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>GABA/Cre</td>
<td>RM</td>
<td>0.24 ± 0.03</td>
<td>0.17 ± 0.01†</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>0.22 ± 0.03</td>
<td>0.14 ± 0.01‡</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>0.19 ± 0.01</td>
<td>0.16 ± 0.006†</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>0.21 ± 0.02</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.23 ± 0.02</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Glu/Cre</td>
<td>RM</td>
<td>0.20 ± 0.02</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>LM</td>
<td>0.21 ± 0.02</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>OC</td>
<td>0.18 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>0.18 ± 0.01</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.24 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
</tbody>
</table>

Abbreviations: Cho, choline-containing compounds; CN, cingulate cortex; Cre, creatine-phosphocreatine; GABA, γ-aminobutyric acid; Glu, glutamate; LM, left motor cortex; NAA, N-acetyl-containing compounds (primarily N-acetyl aspartate); OC, occipital cortex; P, pons; RM, right motor cortex; SPS, Stiff-person syndrome.

*The GABA levels were obtained with magnetic resonance spectroscopy in different regions of interest (Figure 1) and are expressed as ratios to Cre (mean ± SEM). The GABA/Cre ratios are significantly decreased in motor cortex bilaterally in patients with SPS relative to healthy control subjects (29%-36%) and to a lesser extent in posterior occipital cortex (12%); changes in other brain regions were not statistically significant.

†P<.03.‡P<.01.

controls (Table, Figure 2, and Figure 3). The GABA/Cre ratios were significantly decreased in motor cortex bilaterally in patients with SPS relative to controls (29%-36%), and to a lesser extent in the posterior occipital cortex (12%). Mild reductions in GABA/Cre ratios were observed in the cingulate and pontine ROIs in patients compared with controls, but these were not statistically significant. No other differences in any metabolite level were found in patients or in controls.

Among the group of patients with SPS, 2 had the most severe symptoms and were wheelchair bound or bedridden. Their mean ± SEM motor cortex GABA/Cre levels (0.111 ± 0.006) were 32% less than those of other patients (0.162 ± 0.011; P<.004) and 52% less than those of controls (0.231 ± 0.025; P<.002).
Our study shows that in patients with SPS there are decreased GABA levels in several brain regions. The finding is consistent with impairment of GABAergic transmission as an explanation for the patients’ symptoms and supports the view that SPS is a central disorder.

The previous in vitro and in vivo experiments and studies demonstrate that the spectroscopy technique used in this study is capable of measuring GABA with appropriate resolution and accuracy. Some variability in the measurements may arise from the inherent limitations of spatial resolution in our study. To obtain ROIs, voxel size had to be large enough to obtain sufficient signal-noise ratios, and ROIs were maintained in the same brain regions during repeated scans to minimize variations. Possible sources of variability in our measurements may be related to the clinical status of the patient. Changes in the degree of impairment of GABA levels at the time of the MR examination may reflect the fluctuation of symptoms seen in SPS. Several patients were being treated with benzodiazepines before admission and may have had differences in residual drug effects. Repeating the MR spectroscopy examinations monthly for 3 consecutive months helped to reduce these effects.

Although the number of patients was small, the significant correlation between the decrease in GABA levels and the severity of symptoms in 2 patients suggests that inhibition of GABA function in the brains of patients plays a pivotal role in the clinical symptoms of SPS.

In SPS, a regional decrease in GABA levels in the motor cortex may impair the action of inhibitory pathways, leading to muscle rigidity and spasms. In turn, the increased excitation to the spinal cord causes excessive firing by α-motoneurons as discussed previously. Pharmacologic data are consistent with the view that a reduced level of GABA may be responsible for the patients’ stiffness. Drugs that enhance GABA activity or affect GABAergic transmission, such as diazepam, vigabatrin, gabapentin, or baclofen, help alleviate the symptoms of SPS. Transcranial magnetic stimulation studies in patients with SPS also indicate that the motor cortex is hyperexcitable, thereby reflecting a loss of intracortical inhibition by GABAergic neurons of the cerebral cortex.

An observation from the present MR spectroscopy study is that GABAergic neurons do not appear to be uniformly affected in various brain regions of patients with SPS because the decrease in GABA levels was significant in the sensorimotor cortex and, to a lesser extent, in the occipital cortex. Whether these differential variations are due to statistical factors owing to our limited number of samples, or are inherently related to the disease process, is unclear. For example, differences in the antigenic determinants among regional neurons or in their accessibility to the circulating antibodies may make some populations of GABAergic neurons more vulnerable to immunologic attack. If GAD is the putative antibody, the proportions of GAD isoforms are not uniformly expressed in different GABAergic neurons and may have different cellular localization. The accessibility of different GAD antigens to antibodies may also differ depending on GAD antigen exposure on the cell surface during GAD exocytosis. Most importantly, other antigenic targets that have different properties and distribution may be responsible in SPS.

The theory of reduced brain GABA levels is also supported by a previous observation that GABA levels are also reduced in the cerebrospinal fluid of patients with SPS. Regardless of the responsible antigen and the type of pathogenic antibodies, it is clear that in SPS the symptoms can be explained on the basis of impaired GABAergic function resulting in impaired inhibitory neurotransmission. It is very likely that in SPS the responsible antibodies block function on still-intact cells rather than cause structural changes in GABAergic neurons, as supported by the lack of abnormal neurologic signs other than increased muscle tone, the absence of structural lesions on MR images, and the reversibility of the findings by successful immunotherapy.

In patients with SPS, MR spectroscopy of the brain provides a tool to evaluate regional changes in the levels of brain GABA, correlate it with disease severity, and examine whether GABA levels change with response to therapies.

Accepted for Publication: November 5, 2004.
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Author Contributions: Study concept and design: Levy, Levy-Reis, and Dalakas. Acquisition of data: Levy, Levy-Reis, and Fujii. Analysis and interpretation of data: Levy. Drafting of the manuscript: Levy, Fujii, and Dalakas. Critical revision of the manuscript for important intellectual content: Levy, Levy-Reis, Fujii, and Dalakas. Statistical analysis: Levy. Administrative, technical, and material support: Levy-Reis, Fujii, and Dalakas. Study supervision: Levy and Dalakas.

Acknowledgment: The authors thank major collaborators, including Mian Li, MD, Sherry Thomas-Vorbach, Joan Kyhos, BSN, and Beverly McElroy, CRN. We thank Mark Hallett, MD, for his support. We are indebted to Giovanni DiChiro, MD (deceased), for setting up the new spectroscopy program at the National Institutes of Health.

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