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Localized ^{1}H NMR measurements of γ-aminobutyric acid in human brain *in vivo*

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ABSTRACT **Localized ^{1}H NMR spectroscopy in conjunction with *J* editing was used to measure the concentration of γ-aminobutyric acid (GABA) in the occipital lobe of four control human volunteers and four epileptic volunteers who were receiving the drug vigabatrin. The GABA concentration measured in four nonepileptic subjects was 1.1 ± 0.1 μmol/cm^3 of brain, which is in good agreement with previous values measured in surgically removed human cortex. A dose-dependent elevation of GABA concentration was measured in patients receiving the GABA transaminase inhibitor vigabatrin, with the maximum measured level of 3.7 μmol/cm^3 of brain measured at the highest dose (6 g per day) studied. ^{1}H NMR measurements of GABA in those patients receiving GABA-elevating agents such as vigabatrin will be of importance in establishing the relationship between seizure suppression and the concentration of brain GABA.**

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain and in human cortex (1, 2). The GABAergic system is believed to have an important role in the origin and spread of seizure activity (3). Anticonvulsant properties have been shown for compounds that act to reduce the uptake of GABA from the extracellular space (4–6), enhance the activity of the GABA–benzodiazapine receptor complex, and block the degradation of GABA by GABA transaminase (7–9). Vigabatrin (4-aminohex-5-enoic acid) is a highly specific inhibitor of GABA transaminase (10). The drug binds to neuronal and glial GABA transaminase with high affinity and irreversibly inhibits the enzyme markedly, raising GABA concentrations *in vitro* and *in vivo* (11–13). Vigabatrin has been shown to be a safe and efficacious antiepileptic medication in human studies (14–17). It is well absorbed after oral administration with predictable pharmacokinetics (18, 19).

The involvement of the GABAergic system in the pathogenesis and treatment of epileptic disorders suggests that an *in vivo* method of assessing GABA metabolism would be of value. ^{1}H NMR has the potential to provide regional measurements of brain GABA concentrations and its rate of turnover. The use of ^{1}H NMR to study GABA is complicated by the proton resonances of GABA being overlapped by the larger resonances of creatine, glutamate, and *N*-acetylaspartate (20–22) as well as resonances from macromolecules (22, 23). In 8.5-tesla (T) studies of rat brain, a homonuclear *J* coupling-based editing pulse sequence was used to resolve the C4 and C2 GABA proton resonances (21). Increased GABA levels have been measured by ^{1}H NMR at 8.5 T in rat brain after administration of the irreversible GABA transaminase inhibitors gabaculine and vigabatrin (24, 25). The lower available B_0 fields and the need to perform spatial localization in human brain studies have limited the application of spectral editing to lactate (26, 27) and glutamate (28, 29). We present here localized homonuclear *J*-edited ^{1}H NMR measurements of GABA in the occipital lobe of nonepileptic human subjects and of epileptic subjects receiving vigabatrin.

METHODS

General Protocol. Subjects were positioned supine on a patient bed in a 2.1-T 1-m bore Oxford Magnet Technologies (Oxford) magnet with the head positioned in an adjustable holder so that the 8-cm ^{1}H transceiver coil was subjacent to the occipital lobe. A multislice inversion recovery image using an adiabatic hyperbolic secant pulse for B_1-insensitive contrast was obtained to select the volume for spectroscopy (30). For localized spectroscopy, a volume in the occipital lobe of ≈2 × 4 × 4 cm^3 (*y*, *x*, *z*) was chosen from the image in which the *y* dimension was parallel to the coil axis. The B_0 homogeneity in the selected volume was mapped and optimized with an automated shimming routine (31). Spectra were obtained with an extensively modified Biospec I spectrometer (Bruker Instruments, Billerica, MA) with Oxford Magnet Technologies (Oxford) shielded gradients and power supplies using the pulse sequence described below.

Subjects. Four men and four women volunteered for the study. The mean age of the four, nonepileptic control subjects, two men and two women, was 31 years (range 28–36). One of the control subjects was studied five times. Four patients with epilepsy, whose mean age was 31 years (range 24–35) were studied while taking vigabatrin. Three patients, two men and one woman, were studied while receiving vigabatrin for various periods of time as part of a phase-three trial. The first patient was taking vigabatrin (6 gm/day) and carbamazepine (1.6 gm/day), while patient two was treated with vigabatrin (6 gm per day), phenytoin (400 mg/day), phenobarbital (150 mg/day), and an antihypertensive. Patient three was initially studied taking vigabatrin (3 gm/day), valproate (1.5 gm/day), and carbamazepine (1.6 gm/day). Additional measurements were made as vigabatrin was tapered over 7 weeks. A fourth patient received a prescription for vigabatrin for compassionate use from the United Kingdom. She was studied while taking valproate (3 gm per day) prior to starting vigabatrin. Serial spectra were obtained as the vigabatrin dose was increased to 2, 4, and 5 g/day over 8 weeks. Valproate was maintained at 3 g/day. All patients had been evaluated extensively by the Yale Epilepsy Program and were determined to have complex partial seizures (32–34). The occipital lobe appeared lesion-free on magnetic resonance imaging. None showed an occipital focus on electroencephalogram. All subjects gave informed consent for the study, which was approved by the Yale Human Investigations Committee.

Solution Measurements. Solution measurements were obtained from volumes at similar locations relative to the coil as in the *in vivo* studies using a 11.5-cm diameter cylindrical bottle. The bottle was filled with either 20 mM GABA/15 mM

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Abbreviations: GABA, γ-aminobutyric acid; DANTE, delays alternating with nutations for tailored excitation; 1-D, 2-D, and 3-D, one-, two-, and three-dimensional.

glycine/50 mM phosphate/69 mM K^+, pH 7.0, or 10 mM creatine/10 mM *N*-acetylaspartate/10 mM lactate (≈50 mM K^+).

Localized Spectroscopy. The localization portion of the sequence has been described (35, 36). Briefly three-dimensional (3-D) localization was achieved by using the 3-D-image-selected *in vivo* spectroscopy (ISIS) (37) sequence with 8-msec phase-swept hyperbolic secant inversion pulses (30) (μ=5; bandwidth=2000 Hz). Maximum pulse power was 350 W. A curved surface-spoiler (38) gradient with 1.5-cm wire separation was pulsed at 8 amps to provide *x* and *y* outer volume suppression. Additional outer volume suppression was achieved with a selective pulse (39) in an *x* gradient. A $\theta/3$ depth pulse (40) followed by a dephasing gradient (28, 35) was used to reduce signal from high flux regions near the surface coil. The surface coil rf magnetic field (B_1) profile was sufficient to cut off outer volume signal in *z*. A 5-msec five-lobe sinc ($\frac{\sin x}{x}$-shaped pulse truncated at the fifth zero crossing) in a 2.35-mT/m (1000 Hz/cm for ^{1}H) *y* gradient was used for spatially selective excitation (35, 36, 41). Crusher *x* gradient pulses of 8-msec duration and 7.52-mT/m strength were used in the spin-echo sequence to eliminate nonrefocusing magnetization. All gradient pulses had a 10 mT/m·msec rise rate. An 80-msec hyperbolic secant-selective inversion pulse (30) and a semiselective refocusing pulse (42) calibrated to give a 90-degree pulse duration of 120 μsec were used for water suppression. The power of the sinc and refocusing pulse was calibrated by maximizing the signal between 3.5 and 3.7 cm from coil center on a 1-D profile of the pulse sequence along the coil center with a *z* cossinc to form a 2-D column. Pulse power varied by <10% between subjects. There was an ≈3-fold variation in B_1 strength from the center of the coil to the center of the localized volume. To avoid excessive tissue heating from the surface coil rf field, the holder kept all points of the head of the subject at least 0.075 of a coil diameter (0.6 cm) away from the coil wire. Regional rf heating was calculated by assuming a uniformly conducting medium using both a Faraday loop and a magnetic vector potential model to be <1 W/kg in the brain. *In vivo* data were acquired with an acquisition time of 410 msec, a sweep width of 2500 Hz, and a repetition rate of 3.39 sec.

Pulse Sequence for GABA Editing. Homonuclear editing of the 3.0-ppm C4 GABA proton resonance at 2.1 T was performed by using the *J*-editing pulse sequence diagrammed in Fig. 1. The volume localization portion of the sequence is not shown (see above). The editing sequence consists of a 68-msec spin echo (TE) with a 22 semiselective refocusing pulse (42) with the carrier placed at the C3 GABA resonance at 1.91 ppm. The semiselective refocusing pulse creates a null at 1.91 ppm, which inhibits *J* modulation of the C4 GABA resonance (3.0 ppm) due to *J* coupling to the C3 GABA resonance. To selectively induce *J* modulation, a 26.5-msec 48-pulse DANTE (delays alternating with nutations for tailored excitation) pulse train (44, 45), which is time symmetrical about the center of the semiselective delay to minimize phase distortion, is applied to invert the C3 GABA resonance and cause the outer sidebands of the C4 GABA triplet to invert at the TE time of 1/2 *J* (TE = 68 msec). Subtraction of a spectrum obtained with the DANTE inversion from a spectrum obtained without the DANTE inversion eliminates signals that are not *J*-coupled to resonances at 1.9 ppm and gives the edited GABA spectrum. Phase cycling is used to eliminate residual dispersive refocusing magnetization due to the sequence not being perfectly time symmetric (43). The first pulse and last pulse of the DANTE train are reduced in length by half to improve selectivity (46).

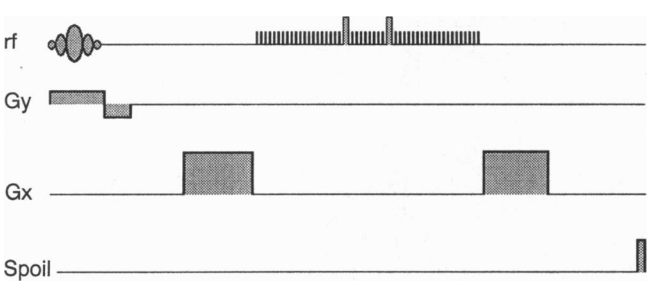

FIG. 1. Pulse sequence used for the *J* editing of GABA (see text). Spectra are obtained either with or without a 26.5-msec DANTE inversion pulse applied to the 1.9-ppm C3 GABA resonance to induce *J* modulation of the C4 resonance. The DANTE and 22 pulses are phase-cycled as a single pulse train (43). Subtraction of a spectrum obtained with the DANTE pulse from a spectrum obtained without the DANTE pulse gives the edited spectrum. Prior to the editing sequence, 3-D image-selected *in vivo* spectroscopy with outer volume suppression is used to localize the volume from which the signal is obtained. Gx, *x* gradient; Gy, *y* gradient; Spoil, spoiler gradient.

Quantitation of GABA. To estimate the concentration of GABA, the integral of the C4 GABA resonance in the edited spectrum was compared with the integral of the creatine resonance (3.03 ppm) in the subspectrum obtained with the DANTE pulse. *In vivo* time domain data were zero-filled to 32 K and multiplied by a 3-Hz exponential function prior to Fourier transformation. In the spectra obtained *in vivo*, the half-height linewidth of the 3.03 creatine resonance ranged from 7 to 9 Hz after 3-Hz linebroadening. In the edited spectrum, the C4 GABA resonance was integrated over a 0.30-ppm bandwidth centered about 3.00 ppm. To reduce distortion from overlapping resonances, the intensity of the creatine resonance was integrated over a 0.20-ppm bandwidth. The concentration of GABA was estimated by using the following formula:

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

where G* is the integral in the edited spectrum, Cr is the creatine integral, M is the contribution to the edited GABA spectrum from edited macromolecule resonances at 3.00 ppm, ICF is the correction for the limited integral bandwidths determined from localized edited spectra of solutions of GABA and creatine linebroadened to match the *in vivo* processed linewidths, EE is the correction for loss of intensity due to imperfect editing efficiency, 3/2 is the creatine-to-C4 GABA proton ratio, and [Cr] is the assumed creatine concentration in human occipital lobe. No correction was made for transverse relaxation time (T_2) differences between GABA and creatine, and it was assumed that the repetition rate was sufficiently long to eliminate differential longitudinal magnetization saturation.

RESULTS AND DISCUSSION

Determination of Editing Efficiency and Selectivity. Editing efficiency, which is defined here as the ratio of the C4 GABA resonance intensity in the edited spectrum to the intensity in the subspectrum obtained without the DANTE pulse (modulation inhibited), was determined as a function of DANTE pulse frequency offset from the center (1.91 ppm) of the C3 GABA multiplet. Measurements were obtained from the 11.5-cm bottle containing the GABA and glycine solution with the localized volume centered at 3.5 cm above the coil plane. Edited spectra of C4 GABA obtained with the sequence are shown in Fig. 2. After exponential line broadening to 7.5 Hz to match the linewidths in the *in vivo* spectra after apodization, the integral of the C4 GABA resonance in the edited spectrum gave 99% of the intensity measured in the spectrum without the DANTE pulse. An additional 3% of the

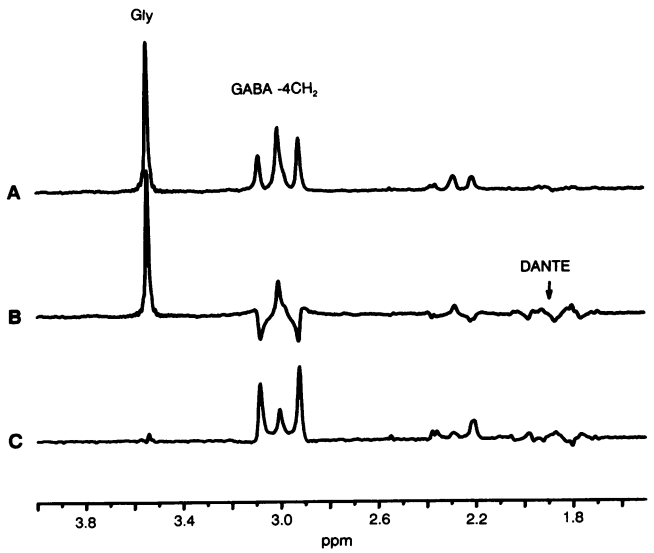

FIG. 2. Localized edited spectra of a solution containing GABA and glycine. Spectra: A, without DANTE; B, with DANTE at 1.91 ppm; C, Difference (edited) spectrum between spectra A and B. In spectrum A the *J* modulation of the C4 GABA triplet at 3.0 ppm (GABA-4CH_2) is inhibited by the semiselective refocusing pulse null at the 1.9-ppm C3 GABA resonance. In contrast, in spectrum B, when the DANTE pulse inverts the C3 GABA resonance, the outer sidebands of the C4 GABA triplet invert. Subtraction of the two spectra eliminates the nonmodulating resonance of glycine at 3.56 ppm (Gly peak) resulting in an edited spectrum (spectrum C) of the C4 GABA resonance at 3.0 ppm.

C4 GABA resonance intensity (measured relative to the glycine resonance, which is a singlet) was lost because of strong coupling effects. Loss of refocusing magnetization at 3.0 ppm due to imperfect DANTE selectivity was measured in a similar manner from the solution that contained 10 mM creatine. For a volume 3.5 cm above coil center, a 7% intensity correction was needed at the creatine 3.0-ppm frequency to correct for the loss of refocusing magnetization due to the DANTE pulse relative to the spectrum obtained without the DANTE. To obtain a complete selectivity profile of the sequence, a 1-D gradient profile was obtained from a 10-mm NMR tube containing water by using a homogenous transmitter coil with the B_1 strength adjusted to match the center of the localized volume in the human brain studies. In the profile, a 7% intensity correction was sufficient to achieve signal cancellation between 2.9 and 3.2 ppm in agreement with the value measured with the surface coil.

GABA Editing Studies in Normal Human Brain at 2.1 T. Fig. 3 shows localized edited GABA spectra obtained in the occipital lobe of three volunteers. Imperfect DANTE selectivity was compensated for by increasing the intensity of the subspectrum obtained with the DANTE inversion by 7% prior to subtraction to eliminate residual creatine intensity. In all spectra the resonance at 3.0 ppm was measured to be consistent with the linewidth and chemical shift of the C4 GABA resonance measured in solution. However, as shown below, there is a contribution to this resonance from mobile brain proteins so that the edited resonanace will be referred to as GABA*. To confirm that the resonance was not due to incomplete creatine cancellation, the frequency of all pulses was shifted by 17 Hz so that the effect of the DANTE pulse on the singlet resonanace of trimethylamine groups at 3.2 ppm was the same as for the 3.0-ppm creatine resonance in the GABA-edited spectra. The 3.2-ppm resonance was chosen based on 2-D NMR studies of rat brain (22), which have shown that there are no significant *J* connectivities between 2.1 and 3.2 ppm. Shifting the frequency of the DANTE yielded no intensity at 3.2 ppm (Fig. 3), an indication that the 3.0 ppm resonance in the GABA-edited spectrum is being selected based on *J* coupling and chemical shift. The substantial reduction in the intensity of the edited resonance at 3.0 ppm is consistent with the existence of a *J* connectivity between 3.0 and 1.9 ppm; which agrees with the chemical shifts of the *J*-coupled C4 and C3 GABA resonances measured in solution. In eight measurements on four nonepileptic subjects taking no medications a GABA*-to-creatine intensity ratio of 0.16 (SD, 0.01; range, 0.15–0.18) was measured. Five measurements made in the same subject over a period of 6 months showed a mean value of 0.16 (SD, 0.01; range, 0.15–0.18).

Editing of Cerebral GABA in Epileptic Subjects During Vigabatrin Administration. The sensitivity of the NMR method to changes in GABA concentration was assessed in four epileptic subjects receiving the GABA transaminase inhibitor vigabatrin. Fig. 4 shows GABA-edited spectra obtained on a single subject before administration of vigabatrin and during a dosage of 4 g per day. The GABA*-to-creatine ratio increased from 0.13 before vigabatrin to 0.31 at the 4-g dose. Serial measurements of GABA in another subject showed a decline from 0.28 to 0.19 as the vigabatrin dosage

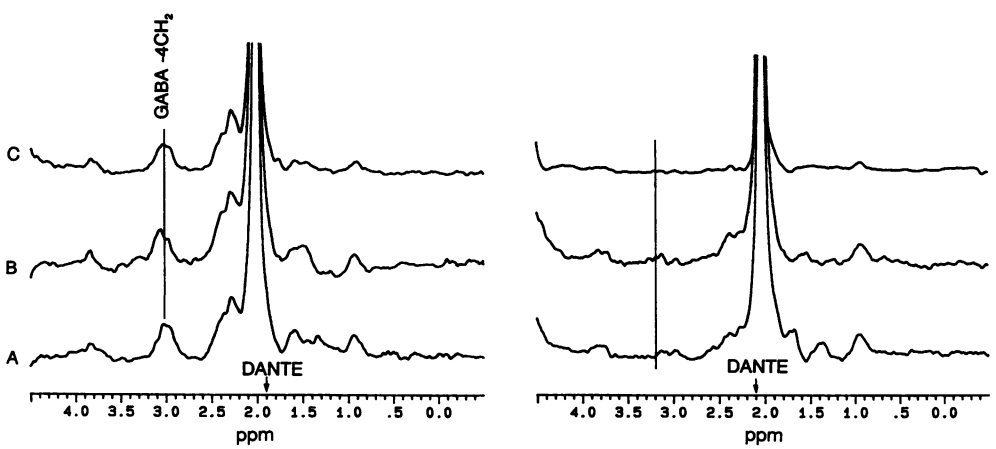

FIG. 3. Localized GABA edited and control edited ^1H NMR spectra from three nonepileptic subjects (A, B, and C). (*Left*) GABA edited spectra obtained with the DANTE placed at 1.9 ppm. (*Right*) Control edited spectra obtained with the DANTE placed at 2.1 ppm. In the GABA edited spectra, a resonance is present at 3.0 ppm that corresponds in chemical shift and linewidth to the resonance of C4 GABA (GABA-4CH_2). In the control edited spectra, no resonance intensity is observed at 3.2 ppm, indicating that the 3.0 ppm resonance in the GABA edited spectra is selected on the basis of *J* coupling to a resonance at 1.9 ppm. The large resonance at 2.0 ppm is from *N*-acetylaspartate, which is inverted by the DANTE pulse.

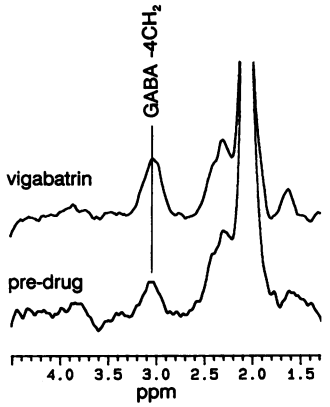

FIG. 4. GABA edited spectra measured in an epileptic subject before and during vigabatrin administration. Spectra: lower, before vigabatrin; upper, during treatment with 4 g per day of vigabatrin. The intensity of the edited C4 GABA resonance at 3.0 ppm (GABA-$4CH_2$) is increased by 2.3 times over the intensity in the spectrum obtained before vigabatrin administration.

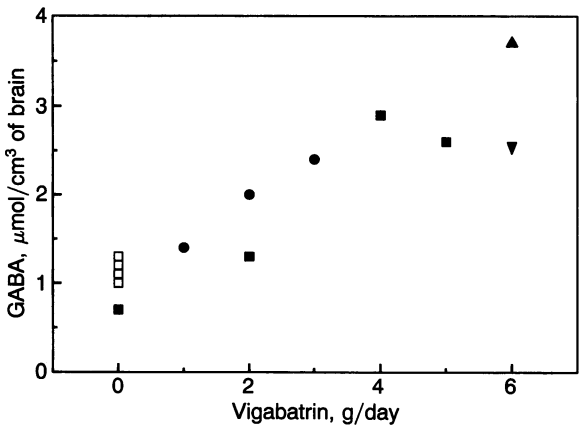

FIG. 5. The GABA concentration measured in the occipital lobe of control and epileptic subjects plotted versus vigabatrin dosage. □, Eight measurements on four control subjects (mean 1.1 ± 0.1 μmol/cm³ of brain); ■ and ●, two epileptic subjects from which multiple measurements were obtained; ▲ and ▼, measurements obtained from separate epileptic subjects. A general increase in GABA concentration with vigabatrin dosage was observed.

was tapered from 3 g per day to 1 g per day. In two other subjects who were both receiving 6 g per day of vigabatrin, the GABA*-to-creatine ratio was measured to be 0.38 and 0.29. In a ^{1}H NMR spectrum at 500 MHz of a solution of vigabatrin in buffered deuterium oxide, no resonance at 3.0 ppm was observed (resonance positions 1.9, 2.05, 2.3, 3.8, 5.45, and 5.85 ppm), indicating that vigabatrin does not interfere with the *in vivo* measurement.

Macromolecule Resonances at 3.0 ppm. Studies of rat brain tissue have indicated that macromolecules, primarily proteins, contribute signals to the *in vivo* ^{1}H spectrum of normal brain (23). Of particular relevance to GABA studies is the coupling between resonances at 3.0 and 1.7 ppm. The macromolecule resonance at 3.0 ppm may contribute as much as 60% of its full on-resonance editing intensity to the *in vivo* GABA-edited spectrum based on measurements of off-resonance editing efficiency performed on the GABA solution. The contribution of macromolecules to the edited spectra was assessed on two nonepileptic subjects by measuring the intensity of the 3.0-ppm resonance with the DANTE optima at 1.9 and at 1.7 ppm. The macromolecule-to-creatine ratio in the GABA-edited spectrum was calculated from this data by assuming that the off-resonance editing efficiency of the macromolecule resonance was the same as for GABA, which yielded an average macromolecule-to-creatine ratio of 0.07 (range, 0.06-0.07; n=3). The contribution of macromolecular resonances to the GABA-edited spectrum was measured in two epileptic subjects receiving vigabatrin and found to be no greater than in the control subjects. The macromolecule contribution to the *in vivo* spectrum can be reduced in principle by use of editing pulses with improved selectivity or by performing ^{1}H NMR measurements at higher B_0 fields.

Cerebral GABA Concentrations. Fig. 5 shows all of the measurements of GABA concentration in control and epileptic subjects. The GABA concentration was estimated from the ratio of the integral of the GABA and creatine resonances (see above). A mean creatine concentration of 8 μmol/cm³ of brain was assumed based on previous measurements of biopsied human occipital lobe (47–50). The integrated intensity of the GABA-edited spectrum was corrected for coedited macromolecules by subtracting the mean macromolecule-to-creatine ratio of 0.07. The GABA concentration in the eight measurements on control subjects was 1.1 μmol/cm³ of brain (SD, 0.1; range, 1.0–1.3). If the mean of the measurements on the control subject studied multiple times is included as a single value, the group mean and standard deviation are unchanged (mean, 1.1 μmol/cm³ of brain, SD, 0.1). The ^{1}H NMR measurement is in good agreement with reported measurements of GABA concentration in biopsied temporal and frontal lobes, which ranged from 0.5 to 1.4 μmol/cm³ of brain (47, 51–53) when delays between tissue excision and freezing were kept to a minimum. Serial ^{1}H NMR measurements in a single patient ranged from 0.7 μmol/cm³ of brain before vigabatrin was started to 2.9 on a dose of 4 g per day. In another patient GABA levels decreased from 2.4 to 1.4 μmol/cm³ of brain, as vigabatrin was tapered from 3 g to 1 g per day.

Previous human studies of vigabatrin have been limited to measurements of GABA concentration in blood and cerebrospinal fluid (54–56). Lumbar cerebrospinal fluid GABA concentrations ranged from 0.004 to 0.005 μmol/ml prior to vigabatrin treatment to 0.012–0.014 after 1–3 months of standard dose therapy. If one assumes cerebrospinal fluid levels are the same order of magnitude as extracellular fluid levels, the ^{1}H NMR measurement accurately reflects intracellular GABA levels. In general, these studies have demonstrated a correlation between increased GABA concentrations in the cerebrospinal fluid with improved seizure control. Therefore, it is of interest that, in the present study, the patient whose seizure control was most improved on vigabatrin treatment was measured to have the highest cerebral GABA level, 3.7 μmol/cm³ of brain, which is >3 times the average level measured in nonepileptic control subjects.

SUMMARY AND CONCLUSIONS

The results of this study show that *in vivo* ^{1}H NMR spectroscopy allows the repeated noninvasive measurement of regional cerebral GABA levels. The assignment of the resonance at 3.0 ppm in the edited spectrum to GABA is based on the following considerations: (*i*) the chemical shift (3.0 ppm) and linewidth of the edited resonance agree with the C4 GABA resonance measured in solution, (*ii*) the maximum intensity of the C4 GABA resonance in the *in vivo* edited spectrum was obtained when the DANTE was placed at 1.9 ppm, which agrees with the C3 GABA resonance chemical shift measured in solution, (*iii*) the GABA concentration measured in nonepileptic control subjects is in good agreement with previous values measured in surgically removed human cortex when postexcision delays before freezing were minimized, and (*iv*) a dose-dependent elevation of GABA concentration was measured in patients receiving the GABA transaminase inhibitor vigabatrin, which has been shown in

previous animal studies to cause a severalfold increase in GABA levels. Occipital lobe GABA concentrations measured *in vivo* increased above the control mean value of 1.1 ± 0.1 mol/cm^3 of brain to a maximum of 3.7 μmol/cm^3 of brain at the highest vigabatrin dose studied. ^{1}H NMR GABA measurements in those patients receiving GABA-elevating agents such as vigabatrin will be of importance in establishing the relationship between seizure suppression and the concentration of brain GABA.

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