The Effect of Gabapentin on Brain Gamma-aminobutyric Acid in Patients with Epilepsy

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Gabapentin has come into clinical use as adjunctive therapy in the treatment of epilepsy. Designed to mimic gamma-aminobutyric acid (GABA), its mechanism of action remains elusive. In vivo measurements of GABA in human brain were made using ^{1}H magnetic resonance spectroscopy. We used a 2.1-T magnetic resonance imager-spectrometer and an 8-cm surface coil to measure a 13.5-cm^3 volume in the occipital cortex. GABA levels were measured in 14 patients enrolled in an open-label trial of gabapentin. GABA was elevated in patients taking gabapentin compared with 14 complex partial epilepsy patients, matched for antiepileptic drug treatment. Brain GABA levels appeared to be higher in patients taking high-dose gabapentin (3,300–3,600 mg/day) than in those taking standard doses (1,200–2,400 mg/day). Gabapentin appears to increase human brain GABA levels.

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in human cortex and plays a pivotal role in suppressing the origin and spread of seizure activity [1–3]. Animal models indicate that GABA synaptic function decreases in many seizure states. Significant reductions in cerebrospinal fluid (CSF) concentrations of GABA are seen in patients with various epileptic syndromes [4]. The level of GABA in synaptic terminals and in the extracellular fluid depends on the functioning of a metabolic cycle between neurons and glia. The effectiveness of the class of antiepileptic drugs that target GABA metabolism hinges on the elevation of GABA concentration [5].

Gabapentin has come into clinical use as adjunctive therapy in the treatment of epilepsy [6, 7]. Designed to mimic GABA, its mechanism of action remains elusive [5, 8]. Recent reports suggested that it promotes the release of GABA from neurons and glia [9], perhaps by the reversal of the GABA transporter [10, 11]. This action on glia would tend to increase extracellular GABA not only within the seizure focus, but also in the surrounding volume of brain [11–13].

We reported in vivo measurements of GABA in human brain using ^{1}H nuclear magnetic resonance spectroscopic editing techniques [14–17]. Initial observations of the effect of the novel antiepileptic drug vigabatrin showed that human brain GABA levels increased twofold associated with improved seizure control [15, 17, 18]. This article reports our initial observation using gabapentin at standard (1,200–2,400 mg/day) and high doses (3,300–3,600 mg/day).

Materials and Methods

Nine men and 16 women with complex partial seizures were studied. All patients had been evaluated extensively by the Yale Epilepsy Program and were determined to have complex partial seizures [19]. None showed an occipital focus on electroencephalograms. Repeated brain GABA measurements were made in 5 healthy volunteers on no medications over a period of 2 years. The median age of this group of 3 men and 2 women was 36 years (range, 26–43 years). All subjects gave informed consent for the study, which was approved by the Yale Human Investigations Committee.

Six men and 8 women with a median age of 35 years (94% confidence interval [CI], 25–38; range, 21–45 years) were studied without gabapentin. The median time of the last seizure before spectroscopy was 7.5 days (94% CI, 3–47; n = 14). Three of 14 patients were examined before the addition of gabapentin. Two patients were drug free during the GABA measurements. The other 9 patients were matched with the patients taking gabapentin on the basis of antiepileptic medications. Of the 14 patients, 10 were on monotherapy—carbamazepine (6 patients), valproate (2),

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phenytoin (1), and primidone (1). Two patients were on polytherapy with carbamazepine and valproate.

Five men and 9 women with complex partial seizures receiving gabapentin (median dose, 32 mg/kg/day; 94% CI, 26–56; range, 15–72 mg/kg/day) were studied. The median age of the patients was 36 years (94% CI, 29–43; range, 25–54 years). The median time of the last seizure before spectroscopy was 10.5 days (94% CI, 3–24; n = 14), not significantly better than the control group. Three patients were on gabapentin monotherapy. Other medications included carbamazepine (5 patients), valproate (1), phenytoin (1), carbamazepine and valproate (3), and carbamazepine and phenobarbital (1).

Studies were performed at Yale Medical School with a 2.1-T Oxford Magnet Technologies 1-m bore magnet equipped with an extensively modified Biospec I spectrometer (Bruker Instruments) and Oxford Magnet Technologies shielded gradients and power supplies. Subjects were supine on a pallet with the occipital cortex apposed to an 8-cm distributed capacitance radiofrequency surface coil tuned to the ^{1}H nuclear magnetic resonance (NMR) frequency of 89.43 MHz. Prior to the NMR measurement a T1-weighted, gradient-echo magnetic resonance image (MRI) of the subject's brain was obtained. From the image a 1.5 × 3.0 × 3.0-cm (13.5-cm^3) volume in the occipital cortex was chosen for spectroscopic measurements. More than 95% of the acquired signal was derived from this volume centered 2.1 cm deep from the dura. An automated shimming routine was used to optimize the B_0 field homogeneity in the sensitive volume. Homonuclear editing of the 3.0 ppm C4 GABA resonance at 2.1 T was performed using the J-editing pulse sequence described previously [14, 15, 17]. Spectral editing detects signals from hydrogen atoms on adjacent carbon atoms in the same molecule. In this case, the spin-spin, "J" editing selects the GABA C4 triplet resonance at 3.0 ppm coupled to the GABA C3 multiplet resonance at 1.9 ppm. Two primary spectra averaging 128 signal acquisitions each were subtracted to obtain a difference spectrum. The localization techniques were three-dimensional image selected in vivo spectroscopy (3D-ISIS) sequence, outer volume suppression, selective excitation, and surface spoiler coil. Spectral acquisition conditions were repetition time (TR) of 3.39 seconds, echo time (TE) of 68 msec, sweep width of 2,500 Hz, and acquisition time of 410 msec. A chemical shift selective 80-msec hyperbolic secant pulse followed by an inversion recovery delay and a binomial 22 refocusing pulse were used for water suppression. Spectral editing of the GABA C4 resonance at 3.0 ppm was achieved by applying a delays alternating with nutations for tailored excitations (DANTE) pulse train to selectively invert the 1.9 ppm C3 resonance. The 26.5-msec editing pulses were applied symmetrically in time about the center of the sequence. The free induction decay (FID) was zero filled to 32K and a 3-Hz exponential filter was applied before Fourier transformation. The GABA signal was integrated over a 0.30-ppm bandwidth at 3.00 ppm. The creatine signal was integrated over a 0.20-ppm bandwidth at 3.00 ppm in the GABA nulled spectrum. The following equation was used to calculate the GABA concentration:

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

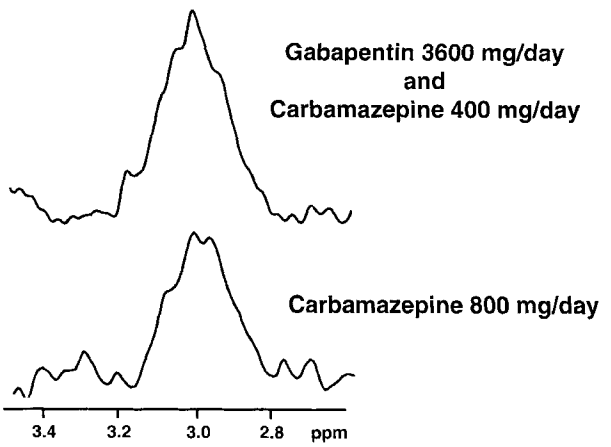
where G* is the integral in the edited spectrum, Cr* is the creatine integral, M is the contribution to the edited GABA spectrum from edited macromolecule resonances at 3.00 ppm [15, 17, 20], ICF is the correction for the limited integral bandwidths determined from localized edited spectra of solutions of GABA and creatine line-broadened to match the in vivo processed line widths [15], EE is the correction for loss of intensity due to imperfect editing efficiency [15], 3/2 is the creatine to GABA proton ratio, and [Cr] is 9 mmol/kg—the creatine concentration in human cortex [21, 22].

Student's *t* test two-tailed probability distribution tables were used to calculate 95% confidence interval of a group mean difference (95% CI). The 95% CI for the group mean assumed a normal distribution. Binomial probability tables were used to find CIs for medians (i.e., 94% CI for sample size of 14) [23].

Results

Figure 1 shows ^{1}H spectra of occipital lobe GABA from 2 patients with complex partial epilepsy. Brain GABA levels were elevated with high-dose (3,600 mg/day) gabapentin and carbamazepine compared with carbamazepine alone. The women had seizures 1 and 2 days before spectroscopy.

Fig 1. ^{1}H spectra of GABA in 2 patients with complex partial epilepsy—one on carbamazepine monotherapy (below) *and the other on carbamazepine and high-dose gabapentin* (above). *The GABA C4 resonance at 3.0 ppm was increased with 3,600 mg of gabapentin per day (50 mg/kg/day). Occipital lobe GABA levels increased from 1.07 to 1.53 mmol/kg of wet-weight brain. The chemical shift scale (spectral frequency) in parts per million (ppm) is shown below. Spectral editing detects signals from hydrogen atoms on adjacent carbon atoms in the same molecule. In this case, the editing technique selects the GABA C4 triplet resonance at 3.0 ppm coupled to the GABA C3 multiplet resonance at 1.9 ppm. Two primary spectra averaging 128 signal acquisitions each were subtracted to obtain each difference spectrum.*



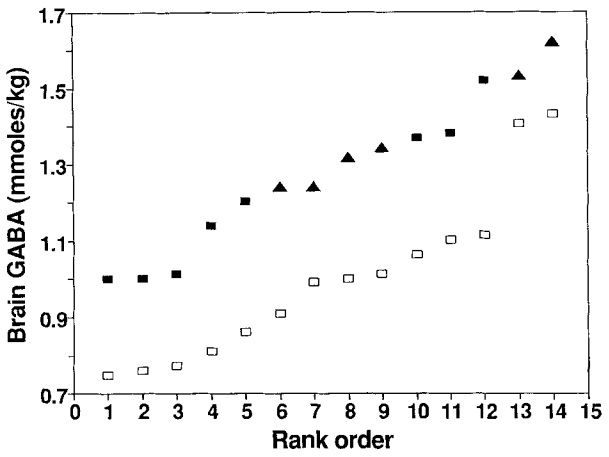


Fig 2. Human brain GABA is increased in patients taking gabapentin. Brain GABA was elevated in both groups taking gabapentin (filled squares *and* triangles), *compared with 14 patients matched for other antiepileptic drugs* (open squares). *The difference of the medians and the means was the same, 0.28 mmol/kg. On average, patients taking high-dose gabapentin* (filled triangles) *had higher brain GABA levels than did those on standard doses* (filled squares).

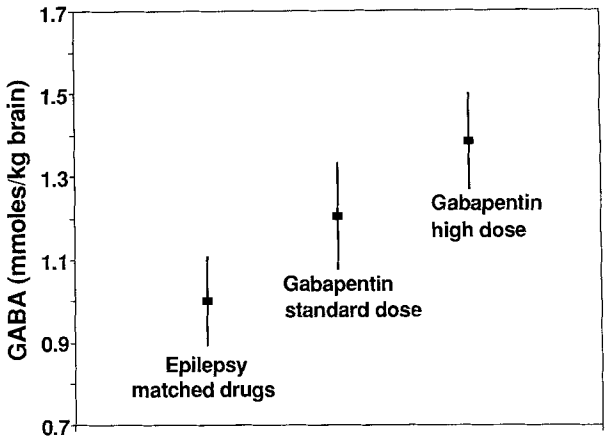


Fig 3. Human brain GABA increases with gabapentin dose. Brain GABA was elevated in both groups taking gabapentin, compared with 14 patients matched for antiepileptic drugs. Patients taking high-dose gabapentin (5 patients taking 3,600 mg/day, 1 on 3,300 mg/day) had higher brain GABA levels (mean with 95% confidence interval) than did those on standard doses (5 patients on 1,800 mg/day, 1 each at 1,200, 1,400, and 2,400 mg/day).

Figure 2 shows that brain GABA levels were higher in patients taking gabapentin than in those who were not taking the drug, by 0.28 mmol/kg (difference of medians). Mean occipital lobe GABA levels in patients taking gabapentin (1.28 mmol/kg; 95% CI, 1.18–1.38; n = 14) were increased compared with those in epilepsy patients (1.00 mmol/kg; 95% CI, 0.89–1.11; n = 14) matched for other medications ($p < 0.001$; mean difference, 0.28 mmol/kg; 95% CI, 0.13–0.44; *df*, 26).

Gabapentin monotherapy increases brain GABA levels. Brain GABA levels were increased (1.4, 1.2, and 1.2 mmol/kg) in 3 patients on gabapentin monotherapy compared with 2 epilepsy patients (0.8 and 0.7 mmol/kg) on no medications. The 3 patients were taking gabapentin at doses of 1,800, 3,600, and 1,800 mg/day, respectively. Brain GABA levels were as high in 3 patients on monotherapy as in those on gabapentin polytherapy.

Serial measurements before and after the addition of gabapentin (1,200 and 1,800 mg/day) showed that GABA levels increased by 0.13 and 0.11 mmol/kg in the 2 patients whose seizure control improved with gabapentin. Brain GABA decreased by 0.23 mmol/kg in 1 patient whose seizure control worsened.

Figure 3 shows that patients taking high-dose gabapentin (3,300–3,600 mg/day; median, 3,600) had higher brain GABA levels (mean, 1.38 mmol/kg; 95% CI, 1.27–1.50, n = 6) than did those on standard-dose gabapentin (1,200–2,400 mg/day; median, 1,800; mean GABA, 1.20 mmol/kg; 95% CI, 1.07–1.33; n = 8). Linear regression showed that brain GABA increased by 0.11 mmol/kg for each gram increase in gabapentin (95% CI, 0.05–0.16; *df*, 26) as gabapentin increased to 3.6 gm/day.

To assess the precision of the brain GABA measurements, 24 studies were performed on 5 nonepileptic volunteers. The mean brain GABA concentration was 1.17 mmol/kg (variation among subjects, 95% CI, 1.11–1.22; n = 5). The precision of repeated measurements had a mean standard deviation of 0.08 mmol/kg and a mean 95% CI of 0.15. Brain GABA levels in patients not taking gabapentin were lower than those in epilepsy-free volunteers. Patients taking gabapentin had GABA levels in the normal range or higher.

Discussion

Gabapentin increases GABA turnover up to twofold in some areas of the rat brain [24]. Our results suggest that occipital lobe concentrations of GABA are raised in epilepsy patients taking gabapentin. Increased GABA synthesis stimulated by gabapentin would explain both results.

The mechanism by which gabapentin increases GABA concentrations of human brain is unknown. GABA is formed from the alpha-decarboxylation of glutamate by glutamic acid decarboxylase (GAD). In vitro, gabapentin stimulates GAD at a concentration of 1.0 to 2.5 mM [24–26]. At higher concentrations (23–25 mM), it inhibits GABA-transaminase, the enzyme that catabolizes GABA [25, 26]. In a rat model, an intravenous bolus of 25 mg/kg of gabapentin raises

brain gabapentin levels to 0.07 mM [27]. Chronic therapy with standard doses (plasma concentration, 6.75 μg/ml) results in a human cortex gabapentin concentration of 0.03 mM [28]. Even at high doses with plasma levels of 12.5 μg/ml, the human brain concentration of gabapentin would appear too low to significantly stimulate GAD or inhibit GABA-transaminase [26, 29].

The activity of GAD is believed to be primarily responsible for regulating the steady-state concentration of GABA in vivo through the interconversion of holoenzyme (active) and apoenzyme (inactive) forms [30–32]. The activation of GAD (to holoenzyme) is stimulated by inorganic phosphate (Pi) and inhibited (increased level of apoenzyme) by ATP, GABA, glutamate, and aspartate [31, 32]. New studies revealed that GAD is composed of two major isoforms (65-kd and 67-kd proteins), which are the products of two different genes [32–34]. GAD65 also comprises the major pool of apoenzyme and may be involved in short-term changes in GABA synthesis flux and GABAergic function [32, 35].

In vitro assays of GAD may not reflect in vivo activity. Serial in vivo ^{13}C NMR spectroscopic studies measuring GABA concentrations and rates of turnover would address these issues directly [36–40]. Measuring the rate of GABA synthesis using in vivo ^{13}C NMR spectroscopy would determine whether the increase in human brain GABA with gabapentin is associated with an increase in GAD activity.

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