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Autoantibodies to glutamic acid decarboxylase in patients with epilepsy are associated with low cortical GABA levels

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Summary

Antibodies to glutamic acid decarboxylase (GAD), the major pathway for the synthesis of γ-aminobutyric acid (GABA) in humans, are found at elevated levels in a sub-group of patients with chronic epilepsy. To test whether the antibodies were associated with changes in cortical GABA levels we used magnetic resonance spectroscopy. Four patients with epilepsy and high serum GAD antibody levels (107-6,200 units/ml) and 10 healthy controls were recruited. A 3T GABA-optimized spectrum was obtained from a reproducible voxel in the cortex. Compared to the control group, the patient group had significantly lower GABA concentrations within the cortex. Demonstration of an association between high serum GAD antibodies and low cortical GABA levels in patients with epilepsy suggests that GAD antibodies are, at least, a marker of a specific disease process and support a role for immune-mediated GABAergic dysfunction.

Keywords

γ-Aminobutyric acid; Glutamic acid decarboxylase; Epilepsy; Magnetic resonance spectroscopy; Autoantibodies

Over recent years, a variety of serum autoantibodies have been identified in conjunction with a number of neurologic diseases. However, it is not always clear whether they play a functional role in the pathogenesis of these diseases, are markers for a specific disease process, or are merely epiphenomena. Serum autoantibodies to one enzyme in particular, glutamate acid decarboxylase (GAD), are found in a number of conditions, including stiff person syndrome (SPS) (Levy et al., 2005) and in some patients with refractory epilepsy (McKnight et al., 2005).

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Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. The authors declare no conflicts of interest.

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GAD is the primary enzyme responsible for the synthesis of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA), and its downregulation is associated with increased seizure rates and epilepsy (Mathews, 2007). Anti-GAD positive serum applied to hippocampal cells in culture led to an increase in spontaneous activity (Vianello et al., 2008), and an injection of anti-GAD-positive immunoglobulin G (IgG) into rats led to an increase in spontaneous firing of the neurons (Manto et al., 2007).

In SPS, localized decreases in GABA are seen (Levy et al., 2005), but this has not been studied in patients with other GAD antibody-associated syndromes. In this study, we used magnetic resonance spectroscopy (MRS) to measure GABA and glutamate levels in patients with anti-GAD-antibody positive epilepsy, compared with healthy controls, in order to investigate possible immune-mediated GABAergic dysfunction in this patient group.

Methods

Six patients with elevated anti-GAD antibody levels were identified from our database. Two declined to participate; the details for the remaining patients are shown in Table 1. Ten healthy controls were recruited [all female; mean age 34 years (range 25–57 years)]. All subjects gave their informed consent and the study was carried out with prior approval from the Local Ethics Committee.

A 3T Siemens (Erlangen, Germany)/Varian (Palo Alto, CA, U.S.A.) magnetic resonance imaging (MRI) system was used. A 2 × 2 × 2 cm voxel of interest was placed by hand over the left precentral knob within the primary sensorimotor cortex (SMC) (Fig. 1B). The MEGA-PRESS sequence was used to allow simultaneous spectral GABA editing, three-dimensional voxel localization, and water suppression (Mescher et al., 1998). For full details see Data S1.

Analysis was performed using the jMRUI software package version 2.2 (http://www.mrui.uab.cat/mrui). The GABA and the glutamate/glutamine (Glx) resonances were both fitted with two Gaussian peaks. The amplitudes of both GABA peaks were summed to give a total value for GABA; summing was similarly performed for the Glx peak (a composite measure of glutamate and glutamine). Analysis was conducted by two independent observers blind to the disease status of the subject. The interobserver reliability was calculated using an interrater reliability coefficient at $\alpha = 0.8801$.

Sera were acquired on the day of scanning. Antibodies to GAD were measured using a radioimmunoassay kit (RSR Ltd, Cardiff, United Kingdom). The results were compared to known standards (1–300 units GAD/ml). Sera with values >10 units/ml (U/ml) were considered positive and tested at serial dilutions to obtain accurate values (Table 1). The sera were negative for antibodies to voltage-gated calcium channels, voltage-gated potassium channels (for methods, see McKnight et al., 2005), and also for N-methyl-D-aspartate (NMDA) receptors and glycine receptors by routine diagnostic assays. Cerebrospinal fluid (CSF) was not available for testing.

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Results

A summary of the clinical information, treatments, and antibody titers for each patient is shown in Table 1. A representative spectrum from the SMC is shown in Fig. 1A.

The group of four patients with elevated anti-GAD antibody titers had GABA levels lower than the control group [patients GABA-to-N-Acetylaspartate (NAA) ratio (mean ± standard error, SE) 0.023 ± 0.002; controls 0.032 ± 0.002; Wilcoxon two-sample test, $W = 16$ $p < 0.04$; Fig. 1C). By contrast, no statistically significant difference in Glx (a composite measure of glutamate and glutamine) was seen (patients Glx-to-NAA ratio 0.041 ± 0.002; controls 0.049 ± 0.004; $W = 22$, $p = 0.31$, Fig. 1D). There was no correlation between serum antibody titer and GABA level. There was no difference in the NAA concentrations between the groups (patients 2.04 ± 0.01; controls 2.05 ± 0.04; $W = 71$, $p = 0.57$).

Discussion

This study demonstrates lower cortical GABA levels in a group of four patients with epilepsy and high serum antibody titers to GAD than in a healthy age-matched control group. We wished to explore a region distant to the symptomatic lesion to avoid simply measuring the levels in an area of local damage. This finding, in cortex not directly affected by the disease process, contrasts with normal GABA levels outside the affected cortical areas in patients with SPS (Levy et al., 2005). One explanation for this observation could be that anti-GAD antibodies are directly pathogenic, in this case in epilepsy, producing a decrease in the activity of GAD within the GABAergic neurons and resulting in a low GABA level; this hypothesis is in line with the data from in vitro and animal studies (Vianello et al., 2008). It has been suggested previously that patients with lower occipital GABA content have slower rates of GABA synthesis (Petroff et al., 1996). It may be that the anti-GAD antibodies provide a causal explanation for this reduction in GABA. In this case immunosuppressive therapy would be expected to increase GABA levels in these patients.

An alternative explanation is that the antibodies are an epiphenomenon and secondary to damage to GABAergic neurons, although there is no direct evidence for such a process. The resulting release of the intracellular enzyme GAD into the cerebrospinal fluid and then into the circulation would lead to the formation of anti-GAD antibodies. In this case immunosuppressive therapy would not be expected to have a therapeutic effect. To reduce this confound we selected a region outside the epileptic focus (where the tissue is likely to be abnormal) to measure GABA levels. However, there is evidence that intravenous immunoglobulin treatment improves seizure control in SPS. It is possible that it is not the GAD antibodies per se that are pathogenic but additional unidentified antibodies that bind to the GAD-positive inhibitory neurons and interfere with their function (Dalakas, 2008).

In either case, the presence of serum autoantibodies in conjunction with a low GABA suggests that anti-GAD antibodies are, at the least, a marker for a disease process that is likely to be immune-mediated.

There are three possible confounds. First, cortical GABA is known to be decreased in poorly controlled patients with epilepsy outside the affected region (Petroff et al.,1996) but not in

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patients with well-controlled epilepsy in whom GABA levels are within the normal range outside the seizure focus (Petroff et al., 1996). Secondly, anticonvulsants and some antidepressants have the potential to *increase* cortical GABA (see Supporting Information). Finally the decrease in cortical GABA may reflect a specific loss of GABAergic interneurons. Although we cannot rule out this possibility, the normal NAA levels in these patients would make this possibility less likely.

However, these additional factors do not fully account for the results seen in our cohort. The two patients in our study not on anticonvulsants that increase GABA levels have well-controlled epilepsy and very low cortical GABA levels (01 and 02). This suggests that there is an additional mechanism reducing cortical GABA. The two patients with normal GABA levels (03 and 04) were taking pregabalin, and one additionally clobazam. However, their epilepsy remained poorly controlled, suggesting that low GABA levels may make patients more susceptible to having seizures, but are unlikely to be the only cause.

Although this study cannot prove causality, the coincidence of low GABA levels in patients with anti-GAD antibodies not on GABA-mimetic drugs does suggest a possible pathogenic role, in concordance with evidence from animal studies (Manto et al., 2007; Vianello et al., 2008). The lack of correlation between seizure control and GABA levels may reflect the interactions of the patient's medications, although other disease processes show poor "across patient" correlations between antibody titer and disease severity, demonstrating the complexity of the expression of the disease phenotype. We, therefore, believe that these findings may widen the potential treatment strategies to include immunomodulation therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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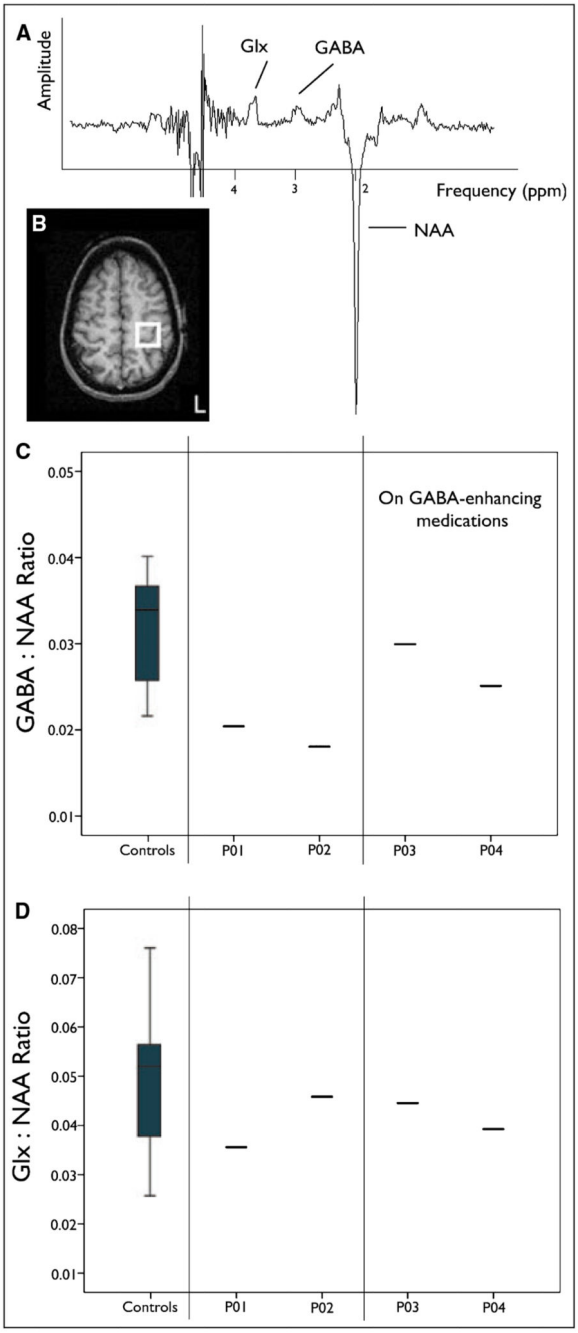

Figure 1.

(**A**) Typical GABA-optimized spectrum, showing GABA and Glx peaks (healthy control). (**B**) Typical placement of the 2 × 2 × 2 cm voxel of interest in the left sensorimotor cortex. (**C**) Differences in GABA-to-NAA ratios between patients and controls. Boxplot shows the median ± interquartile (IQ) range, with whiskers describing maxima and minima of the control group. A significantly lower GABA-to-NAA ratio can be seen in patients compared with controls. The two patients not on GABA-enhancing medications and with wellcontrolled epilepsy show persistently low GABA-to-NAA ratios. The two patients

taking GABA-modulatory medications with refractory epilepsy show higher levels than those whose epilepsy is well controlled, perhaps as a reflection of the different medication profiles. (**D**) Glx:-to-NAA ratios for patients and controls. No difference is seen in Glx, a composite measure of glutamate and glutamine, which cannot be separated at 3T.
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Table 1
Patient demographics and antibody titers

Patient	Age	Sex	Type of epilepsy	MRI lesions	Febrile seizures	EEG seizure focus	No. of seizures	Medications	Antibody titer (U/ml)
01	38	F	Tonic–clonic	None	No	Temporal lobe	Nonefor4 years	Levetiracetam Carbamazepine	6.2×10^4
02	34	F	Partial seizures Tonic–clonic	None	No	Temporal lobe	None for 5 years	Lamotrigine Topiramate Thyroxine Insulin	107
03	42	F	Localization-related	None	No	Temporal lobe	At least weekly	Phenytoin Pregabalin Thyroxine Sumatriptan Vitamin B12 Ferrous sulfate	2×10^4
04	46	F	Localization-related	None	No	Temporal lobe	At least nightly	Carbamazepine Meloxicam Clobazam Citalopram Pregabalin	110