Molecular Neurobiology

ISSN0893-7648/98/16(1): 97–121/$14.00

Measuring Human Brain GABA In Vivo

Effects of GABA-Transaminase Inhibition with Vigabatrin

Ognen A. C. Petroff* and Douglas L. Rothman

Departments of Neurology and Diagnostic Imaging, Yale University, New Haven, CT 06520

Abstract

Gamma-aminobutyric acid (GABA) plays a pivotal role in suppressing the origin and spread of seizure activity. Low occipital lobe GABA was associated with poor seizure control in patients with complex partial seizures. Vigabatrin irreversibly inhibits GABA-transaminase, raising brain and cerebrospinal fluid (CSF) GABA concentrations. The effect of vigabatrin on occipital lobe GABA concentrations was measured by in vivo nuclear magnetic-resonance spectroscopy. Using a single oral dose of vigabatrin, the rate of GABA synthesis in human brain was estimated at 17% of the Krebs cycle rate. As the daily dose of vigabatrin was increased to up to 3 g, the fractional elevation of brain GABA was similar to CSF increase. Doubling the daily dose from 3 to 6 g failed to increase brain GABA further. Increased GABA concentrations appear to reduce GABA synthesis in humans as it does in animals. With traditional antiepileptic drugs, remission of the seizure disorder was associated with normal GABA levels. With vigabatrin, elevated CSF and brain GABA was associated with improved seizure control. Vigabatrin enhances the vesicular and nonvesicular release of GABA. The release of GABA during seizures may be mediated in part by transporter reversal that may serve as an important protective mechanism. During a seizure, this mechanism may be critical in stopping the seizure or preventing its spread.

Index Entries: Human; brain; epilepsy; gamma-aminobutyric acid; vigabatrin; antiepileptic drugs; ^{1}H nuclear magnetic resonance spectroscopy; glutamic acid decarboxylase; GABA-transaminase; GABA-transporter.

Introduction

Gamma-aminobutyric acid has had a central role in neural control theory since it was first discovered in 1950 (Roberts and Frankel, 1950; Roberts, 1986, 1988). It is a major inhibitory neurotransmitter in mammalian brain and in human cortex (Roberts, 1986; Lloyd et al., 1986). GABA may serve as the primary inhibitory neurotransmitter at 25–50% of synapses in the mam-

*Author to whom all correspondence and reprint requests should be addressed.

malian brain (Lloyd et al., 1986; Kocsis and Mattson, 1996). Changes in GABA metabolism may play an important role in the origin and spread of seizure activity. 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 anti-epileptic drugs that target GABA metabolism (e.g., vigabatrin, gabapentin, valproate) hinges on the elevation of GABA concentration.

GABA is formed from the alpha-decarboxylation of glutamate by glutamic acid decarboxylase (GAD) and is metabolized to succinate by the sequential actions of GABA-transaminase (GABA-T) and succinic semi-aldehyde dehydrogenase (SSADH). There are several ways of increasing GABA activity in the brain. GABA agonists, e.g., progabide, diazepam, and phenobarbital, directly increase inhibitory chloride conductances or upregulate the effect of synaptically released GABA on the GABA-A receptor. GABA transporter blockers, e.g., tiagabine, prolong the action of GABA in the synaptic cleft by inhibiting uptake. Stimulating GABA synthesis and release, e.g., valproate, gabapentin, would increase synaptic GABA during neuronal activation. Slowing degradation of GABA, e.g., vigabatrin, valproate, by inhibiting GABA-T or SSADH increases intracellular and extracellular GABA concentrations. Recent studies in animal models and human patients show that multiple feedback mechanisms control both GABA concentration and inhibitory activity.

GABA Synthesis Is Regulated by Specific Modulators

The activity of GAD is believed to be primarily responsible for regulating the steady-state concentration of GABA in vivo through the pyridoxal-5'P-dependent interconversion of holo- (active) and apoenzyme (inactive) forms (Bernasconi et al., 1984; Martin, 1987). The activation of GAD (to holoenzyme) is stimulated by inorganic phosphate (Pi) and inhibited (increased level of apoenzyme) by ATP, GABA, glutamate, and aspartate (Martin, 1987). GAD is comprised of two major isoforms (65-kDa and 67-kD proteins), which are the products of two different genes (Martin and Rimvall, 1993; Erlander and Tobin, 1991) GAD_{65} also comprises the major pool of apoenzyme and may be involved in short-term changes in GABA synthesis flux and GABAergic function (Martin and Rimvall, 1993).

Cellular Compartmentation Is Important in GABA Metabolism

The metabolism of GABA associated with nerve terminals has been linked to a substrate cycle between neurons and astrocytes involving glutamate, GABA, and glutamine (Shank et al., 1993; Schousboe et al., 1992; Sonnewald et al., 1993). In this cycle, nerve-terminal GABA is synthesized from glutamate, enters the extracellular fluid by neurotransmitter release, from which it is either recycled into the nerve terminal or taken up by astrocytes (Fig. 1). In the astrocyte, GABA is broken down by GABA-T and resynthesized into glutamate through the tricarboxylic acid cycle (TCA cycle) of mitochondria. Astrocytic glutamate is converted into glutamine that is taken up by nerve terminals. In the nerve terminal, the released glutamine is hydrolyzed to glutamate by glutaminase (PAG). The flow of carbon between nerve terminal and glia, i.e., the glutamate-GABA-glutamine cycle, maintains nerve-terminal GABA transmitter stores (Schousboe et al., 1992; Sonnewald et al., 1993). Evidence based on selective lesioning, immunohistochemical localization of enzymes of GABA and glutamine metabolism, and isotopic labeling studies support the importance of this cycle (Shank et al., 1993, Schousboe et al., 1992; Sonnewald et al., 1993). GAD is highly enriched in nerve terminals. GABA-T, involved in the degradation of GABA, is enriched in nonsynaptic mitochondria associated with glia (Sonnewald et al., 1993; Sellstrom et al., 1975). Astrocytes serve as the site of glutamine synthesis because of the preferential localization of glutamine synthetase (GS) (Wiesinger, 1995; Ward et al., 1983;

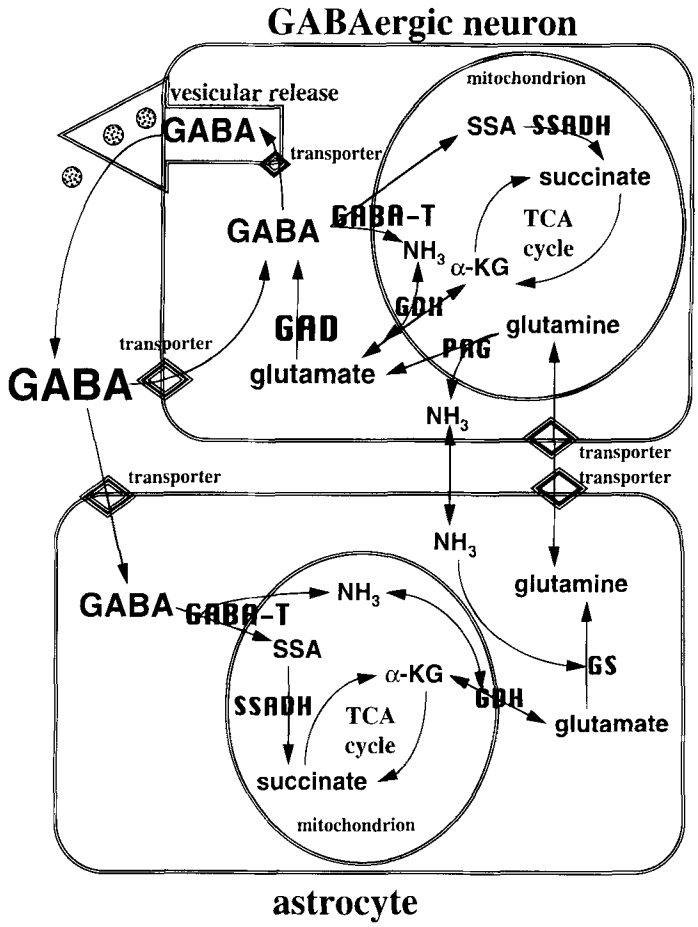

Fig. 1. Cartoon of GABA synapse: GABA is synthesized from glutamate by glutamic acid decarboxylase (GAD). A specific transporter system (diamond symbol) concentrates cytosolic GABA in synaptic vesicles for release. The synaptic actions of GABA are terminated by a high-affinity uptake system of neurons (approx 80%) and glia (approx 20%) (GABA transporter, diamond symbols). GABA taken up by the GABAergic neuron can be repackaged in vesicles. Alternatively, cytosolic GABA is metabolized to succinate by the sequential actions of GABA-transaminase (GABA-T) and succinic semi-aldehyde dehydrogenase and enters the Krebs cycle as succinate. This metabolic route can replenish intermediates in mitochondria (anaplerosis). Glutamate dehydrogenase (GDH) catalyzes the fast equilibrium between alpha-ketoglutarate (α-KG) and glutamate.

GABA-T, involved in the degradation of GABA, is enriched in nonsynaptic mitochondria associated with glia. Astrocytes serve as the site of glutamine synthesis because of the preferential localization of glutamine synthetase (GS). Glutamine produced from exogenous glutamate is readily released in cultured astrocytes. Glutamine is deaminated to glutamate by phosphate-activated glutaminase (PAG). GABA synthesis is stimulated by glutamine in synaptosomes, where it serves as a major precursor of the releasable pool of glutamate and GABA.

Peng et al., 1993). Glutamine produced from exogenous glutamate is readily released in cultured astrocytes and GABA synthesis is stimulated by glutamine in synaptosomes, where it serves as a major precursor of the releasable pool of glutamate and GABA (Wiesinger, 1995; Ward et al., 1983; Peng et al., 1993).

The synaptic actions of GABA are terminated by a high-affinity uptake system of neurons and glia (Wood and Sidhu, 1986). GABA taken up by the GABAergic neuron can be repackaged in vesicles for neurotransmission (Fyske and Fonnum, 1988). If catabolized by GABA-T and SSADH, it can replenish interme-

diates in the mitochondrial TCA cycle (anaplerosis). If GABA is taken up by non-GABAergic neurons, glia, or lost to the circulation, the GABAergic neuron is obligated to replace the four carbon skeleton of GABA from glutamine or TCA cycle intermediates lost when glutamate leaves the mitochondria (Wiesinger, 1995; Peng et al., 1993; Schousboe et al., 1993; Ottersen et al., 1992). Neuronal expression of these critical anaplerotic enzymes appears to be low or absent (Wiesinger, 1995). Neurons lack pyruvate carboxylase or malic enzyme, which are necessary to synthesize TCA cycle intermediates. Malate, alpha-ketoglutarate, or citrate synthesized by glial anaplerotic enzymes could supply needed TCA cycle intermediates (Peng et al., 1993; Schousboe et al., 1993; Leo et al., 1993). Glutamine, synthesized by glia, appears to be the main route for replenishment of glutamate, and thus GABA, in GABAergic neurons.

Measuring GABA with Nuclear Magnetic Resonance Spectroscopy

GABA was first detected in vivo in 1984 with ^{1}H nuclear magnetic-resonance spectroscopy (NMRS) in rat brain (Rothman et al., 1984) using, then newly developed, spectroscopic-editing techniques. This technology has advanced greatly since then because of improvements in water suppression, localization, and hardware.

Human GABA measurements were made of 14 cm^3 volume centered in the midline of the occipital lobe using an 8-cm diameter surface coil and a 2.1 Tesla spectrometer. Localization techniques included: 3D-ISIS (three-dimensional image selected in vivo spectroscopy), outer volume suppression, selective excitation, and a surface spoiler coil. Homonuclear editing of the 3.0 ppm C4 GABA resonance was performed using the spin-spin editing pulse sequence described previously (Rothman et al., 1993; Behar et al., 1994; Petroff et al., 1995). 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 (Fig. 2). Spectral editing of the GABA C4 resonance at 3.0 ppm was achieved by applying a DANTE (delays alternating with nutations for tailored excitations) pulse train to selectively invert the 1.9 ppm C3 resonance. Two primary spectra were obtained with and without the DANTE. They were subtracted to obtain a difference spectrum revealing the GABA signal.

Quantitation of GABA Concentration

After correction for relaxation properties, the integrated resonance areas in NMRS are proportional to concentration. The coefficients for a system of equations needed to estimate metabolite concentrations were determined from localized spectra of model solutions (phantoms) containing GABA, creatine, and other brain metabolites and macromolecules (Rothman et al., 1993, 1992; Petroff et al., 1995). Our experience with human and animal macromolecules is particularly helpful (Rothman et al., 1993; Behar et al., 1994; Petroff et al., 1995, 1989; Behar and Ogino, 1991, 1993). Using these input functions, the in vivo spectrum is modeled as a linear sum of these signals. The creatine signal was assigned a concentration of 9 mmol/kg, the value in human cortex (Petroff et al., 1995, 1989).

Human Brain GABA in Control Subjects

Ten men and nine women with no history of seizures served as the control group (Petroff et al., 1996a,b,c). The mean age of the nonepileptic subjects was 35 yr (SD 7, range 26–58). Mean brain GABA concentration was (1.18 mmol/kg, 95% CI 1.13–1.24, n = 19). Five subjects were studied serially over 2 yr to assess the precision of the brain GABA measurements. 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.

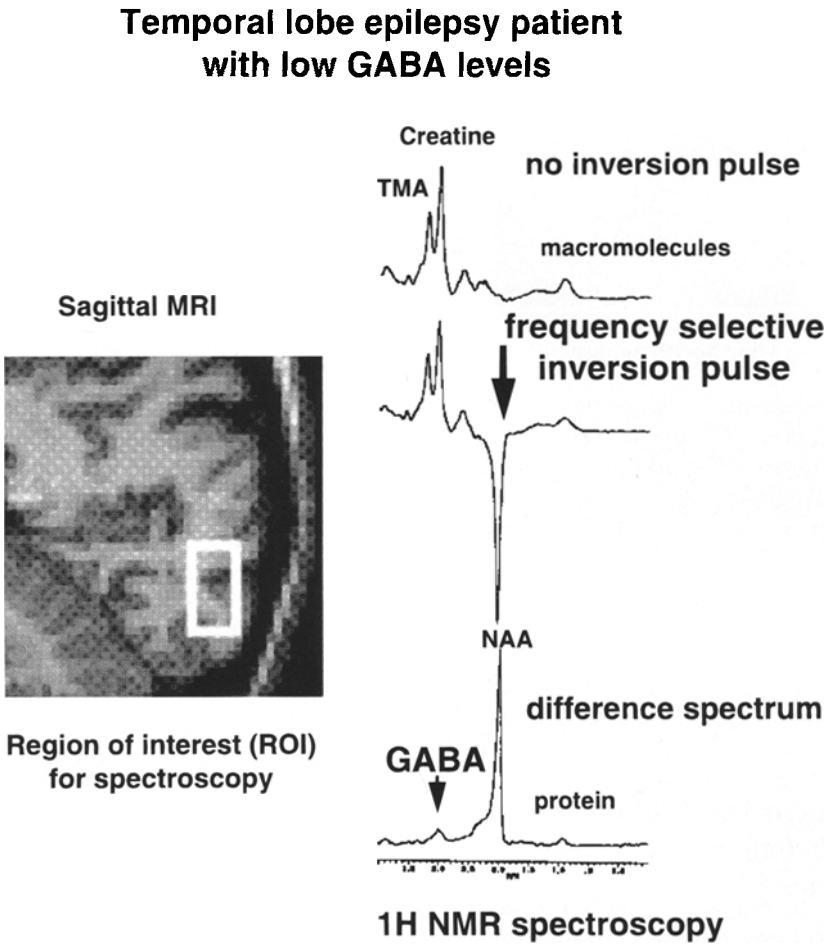

Fig. 2. The midline sagittal image on the left shows the location of the 14-cm^3 region of interest used for the NMRS measurement of GABA. On the right, ^1H spectra showing the spin–spin homonuclear editing process for GABA. In the top spectrum the J-modulation of the GABA C4 methylene (3.0 ppm) was inhibited by the semiselective refocusing pulse null at 1.91 ppm (the GABA C3 methylene resonance). Large noncoupled resonances from creatine (3.0 ppm) and trimethylamine groups (3.2 ppm) obscure the GABA resonance. In the middle spectrum, a frequency-selective inversion pulse was applied to invert the GABA C3 resonance and cause the outer sidebands of the GABA C4 triplet to invert by J-modulation. Subtraction of the middle from the top spectrum yields the bottom difference spectrum. All the signals at 3.0 ppm are J-coupled to resonances at 1.9 ppm. *N*-acetylated groups (2.0 ppm), mainly signals from *N*-acetylaspartate, are labeled NAA. The signals at 3.2 ppm, mainly trimethylamines such as choline, are labeled TMA. All spectra were plotted on the sample amplitude and frequency scales. Spectra were made of a patient with daily complex partial seizures treated with carbamazepine. The patient has below normal brain GABA (0.7 mmol/kg brain).

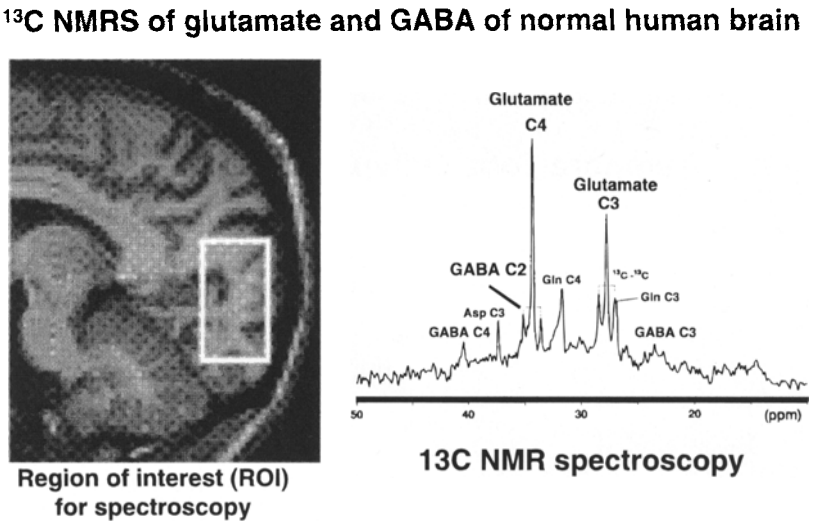

Fig. 3. broadband decoupled ^{13}C spectrum of the human occipital lobe obtained between 120–180 min after the start of an iv infusion of 1-^{13}C-glucose. The sagittal image on the left shows the location of the 144-cm^3 region of interest used. Label from 1-^{13}C-glucose enters the tricarboxylic acid (TCA) cycle as 3-^{13}C-pyruvate (28.5 ppm) and exits initially as 4-^{13}C-glutamate (34.4 ppm). Glutamate is in fast exchange with α-ketoglutarate. From 4-^{13}C-glutamate, label appears as 2-^{13}C-GABA (35.3 ppm) and 4-^{13}C-glutamine (31.3 ppm). Label that continues to circulate in the TCA cycle appears as 2-^{13}C-aspartate (53.1 ppm), 3-^{13}C-aspartate (37 5 ppm), 2-^{13}C-glutamate (55.5 ppm), and 3-^{13}C-glutamate (27.9 ppm). The latter forms 2-^{13}C-glutamine (55.0 ppm), 3-^{13}C-glutamine (27.0 ppm), 3-^{13}C-GABA (24.5 ppm), and 4-^{13}C-GABA (40.4 ppm). The resonances have been relabeled from the original to emphasize that the majority of intensity in the human brain ^{13}C spectrum at 35.3 ppm is from GABA C2 (Gruetter et al., 1994).

^{13}C NMRS of Glutamate, Glutamine, and GABA Labeling from Glucose in Human Cortex

Cerebral metabolism of [1-^{13}C] D-glucose was studied with localized ^{13}C NMRS during iv infusion of enriched [1-^{13}C] glucose in healthy subjects (Gruetter et al., 1994). A volume of 144 cm^3 was measured within the occipital-parietal region using a double surface coil consisting of a 7 cm diameter ^{13}C coil and a concentric 14 cm ^{1}H coil (Fig. 3). Compared with an external standard, the [4-^{13}C]-glutamate concentration was 2.4 m*M* (95% Cl 2.1–2.7). When combined with the isotopomer data, the calculated metabolically active glutamate concentration was 9.1 m*M* (95% Cl 6.9–11). Similarly, the [4-^{13}C]-GABA concentration was 0.4 m*M* (95% Cl 0.1–0.7) and the metabolically active brain GABA pool was 1.2 m*M* (95% Cl 0.2–2.2).

Glutamate and GABA Turnover Is Virtually Complete Within 2 h

The human brain glutamate and GABA pools incorporated label at close to the theoretical value expected from the plasma glucose fractional enrichment within 2 h (Gruetter et al., 1994; Mason et al., 1995). [4-^{13}C]-glutamate labeled rapidly, reaching close to isotopic steady state at 60 min. Isotopomer analysis of the spectra at 2 h yielded a ^{13}C isotopic fraction of C4 glutamate of 27% (95% Cl 24–30, n = 4), which was slightly less than half the enrichment of [1-^{13}C] glucose in plasma (63%, 95% Cl 60–66). A metabolic model was used to calculate of the flux into [4-^{13}C]-glutamate from plasma glucose (0.72 μmol/g/min, 95% Cl 0.56–0.88). This suggests that almost the entire pools of these amino acids are derived from glucose in the normal human brain.

GABA and Human Epilepsy

Seizures are a common neurological problem. Significant reductions in CSF GABA concentration are seen in patients with various epileptic syndromes (Wood et al., 1979). Other studies failed to find significant differences (Tunnicliff and Raess, 1991). In contrast, cortical biopsies from some patients with epilepsy show increased GABA in spiking cortex (Lloyd et al., 1986; Petroff et al., 1995; Perry et al., 1981; Perry, 1982; Sherwin and van Gelder, 1986; Sherwin et al., 1988; Hamberger et al., 1991; Peeling and Sutherland, 1993). Autopsy and biopsy measurements of GABA are difficult to interpret because GABA synthesis continues during hypoxia, whereas GABA catabolism ceases (Lloyd et al., 1986; Petroff et al., 1989; Perry et al., 1981). The hypoxia-induced reduction in GABA catabolism may be ameliorated by freezing the brain *in situ* before resection. Animal models of epilepsy indicate that GABA synaptic function decreases in many seizure states (Lloyd et al., 1986; Sherwin and van Gelder, 1986; Löscher and Schmidt, 1994; Kaura et al., 1995; Horton, 1991). GABA levels are low in some models of focal epilepsy.

GABA Release Is Reduced in the Human Seizure Focus

Microdialysis-based GABA measurements during spontaneous, complex-partial seizures of patients undergoing electrophysiological monitoring using hippocampal depth electrodes show that seizure-induced increases in extracellular GABA were diminished in epileptogenic hippocampi (During and Spencer, 1993; During, 1994). Potassium-induced GABA release was increased in the seizure focus compared with the contralateral hippocampus (During et al., 1995). Glutamate-induced release of GABA was decreased markedly in epileptogenic hippocampus, to a similar degree as seen during spontaneous seizures (During et al., 1995). Glutamate-induced release of GABA may be mediated in part by transporter reversal. Transporter function, although critical for terminating normal synaptic transmission, may be important for the maintenance of normal inhibition. During intense glutamate-induced excitation, i.e., a seizure, this mechanism may be critical in rapidly terminating the seizure or preventing its spread beyond the focus (During and Spencer, 1993; During et al., 1995). An increase in cytosolic GABA would be expected to augment this response.

Alterations in GABA Metabolism in an Amygdala-Kindled Rat Seizure Model

In the seizure focus, potassium-induced release of GABA was increased and glutamate-induced release was decreased in the amygdala-kindled rat seizure model (During et al., 1995). This pattern of GABA release was similar to the one seen in patients with temporal lobe seizures. A decrease in glutamate-induced GABA release was associated with a decrease in the number of GABA transporters in the amygdala of kindled rats (During et al., 1995). The loss of GABA transporters would account for the diminished GABA release during spontaneous seizures in human epileptogenic hipocampi. The nonvesicular release of GABA would be an important protective mechanism suppressing the evolution and spread of seizure activity. Loss of this mechanism could contribute to epileptogenicity.

Metabolic Abnormalities Are Widespread in Temporal-Lobe Epilepsy

Temporal-lobe epilepsy is characterized by wide-spread interictal hypometabolism affecting much of the temporal lobe and can involve much of the hemisphere (Ackermann et al., 1986; Spencer et al., 1995; Lu et al., 1997). Studies of patients undergoing electroconvulsive therapy show diffuse ictal hypermetabolism followed by diffuse, bilateral hypometabolism (Ackerman et al., 1986). These studies suggest that focal seizures have widespread metabolic effects involving cortex, remote from the seizure focus. Medically

refractory epilepsy is too often multifocal or global in nature (Fountain and Lothman, 1995). Resection of the temporal lobe achieved long-term remission of seizure in only 75% of patients with mesial temporal sclerosis (Spencer, 1996). The presence of secondarily generalized seizures increased the risk of recurrence. Mechanisms that prevent spread of seizure activity beyond the seizure focus are important for remission of epilepsy.

Nuclear Magnetic-Resonance Studies of Human Epilepsy

Several recent studies examined changes in pH, phosphate, lactate, and *N*-acetylaspartate within the epileptic focus of patients using ^{1}H and ^{31}P NMRS (Kuzniecky and Jackson, 1995; Laxer, 1996). Acidosis was observed in the temporal lobes of patients with intractable complex-partial seizures by one group of investigators, but not by another (Laxer, 1996; Hugg et al., 1992; Kuzniecky et al., 1992; Garcia et al., 1994; Chu et al., 1996). *N*-acetylaspartate and the *N*-acetylaspartate to creatine ratio was decreased in the epileptogenic temporal lobe, suggesting loss of neurons or altered mitochondrial metabolism. *N*-Acetylaspartate is synthesized by neuronal mitochondria and serves a marker for neuronal cytosol (Petroff et al., 1995; Behar and Ogino, 1991). Creatine is found in neurons and glia. Decreased *N*-acetylaspartate levels are widespread, involving much of the temporal lobe in patients with hippocampal sclerosis (Matthews et al., 1990; Hugg et al., 1993; Cendes et al., 1994; Jackson et al., 1994; Connelly et al., 1994; Gadian, 1995; Hetherington et al., 1995). Lesser decreases in *N*-acetylaspartate are seen in the contralateral temporal lobe. With bilateral disease, *N*-acetylaspartate levels improve in the remaining temporal lobe following successful resection of the epileptogenic temporal lobe (Hugg et al., 1996). As might be anticipated, lactate levels are elevated in the epileptogenic temporal lobe during and after complex partial seizures (Cendes et al., 1997). With good seizure control, lactate levels return to normal. Similar results have been reported with frontal-lobe seizures. Recent developments involving ^{1}H-NMRS-editing techniques allowed noninvasive measurements of human brain GABA, glutamate, and glutamine (Rothman et al., 1984, 1992; Petroff et al., 1995, 1996a,b,c).

Low Brain GABA Level is Associated with Poor Seizure Control

Low GABA levels outside of the epileptic focus may facilitate spread of epileptiform discharges beyond the focus. Patients with complex-partial seizures had lower mean GABA levels (1.02 mmol/kg brain, 95% CI 0.93–1.11, $n = 28$, $p < 0.02$) than subjects without epilepsy (1.18 mmol/kg, 95% CI 1.13–1.24, $n = 19$; Petroff et al., 1996c). There was no significant difference in mean GABA levels among patients treated with traditional antiepileptic medications (AEDs). Brain GABA was more than 2 SD below normal in 40% of patients treated with no AEDs, carbamazepine, phenytoin, valproate, and barbiturates alone and in combinations. There was no correlation between age and brain GABA for patients or controls. There was no difference in mean GABA between men and women for patients or controls.

Low GABA levels predispose but may in themselves not be sufficient for seizures to become clinically manifest. There was a significant association between low GABA levels and recent seizures (correlation coefficient 0.548, $p < 0.01$, degrees of freedom 32, Petroff et al., 1996c). Better seizure control was associated with normal occipital lobe GABA concentrations. Patients with seizures within a day of the measurement had lower GABA levels (0.9 mmol/kg, 95% CI 0.8–1.1, $n = 7$) than patients who were seizure free for five or more years (1.3, 95% CI 1.1–1.5, n=4). Poor seizure control was associated with low brain GABA.

NMRS measurements of GABA may complement electroencephalography (EEG) in the assessment of the risk of seizure recurrence. Half of patients with epileptiform features had occipital GABA levels more than 2 SD below normal (Petroff et al., 1996c). One third of patients with normal interictal EEG had low GABA levels.

Spectroscopic measurements of occipital-lobe GABA may better define the risk of seizure recurrence as part of the evaluation following a first seizure. Abnormalities on EEG may be associated with recent or frequent seizures. The risk of recurrent seizures in adult patients after a first seizure is increased when such abnormalities are present (van Donselaar et al., 1992). Patients with below-normal GABA levels may have a greater chance of having another seizure.

GABA-Transaminase Inhibitors

Vigabatrin Is a Selective GABA-Transaminase Inhibitor

Vigabatrin was synthesized as a rational approach to antiepileptic drug therapy (Schechter, 1989; Jung and Palfreyman, 1995). It was designed to increase brain GABA concentration by inhibiting GABA-transaminase. 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. Neuronal GABA transaminase appear to be more sensitive to vigabatrin than astrocytic GABA transaminase. A high-affinity, active-uptake system demonstrated in cultured neurons, but not astrocytes, accounts for part of the difference (Schousboe et al., 1986). The time course of seizure protection following acute administration of vigabatrin is closely related to the increase in synaptosomal GABA concentration (Jung and Palfreyman, 1995; Schousboe et al., 1986; Löscher and Frey, 1987).

Following the acute administration of vigabatrin, rat brain GABA levels rise proportionate to the dose. A vigabatrin dose of 1000 mg/kg raises rat brain GABA levels from 2 to 8 m*M*/kg (Böhlen et al., 1979). Peak serum (4.5 mmol) and brain (0.2 m*M*) vigabatrin concentrations were achieved in less than 2 h following a single 1500 mg intraperitoneal dose (Bolton et al., 1989). GABA-T inhibition was 30% at 4 h and reached a maximum of 65% inhibition at 48 h. A single dose of 50 mg/kg resulted in a maximal GABA-T inhibition of 20%.

Brain GABA Increased Rapidly After GABA-Transaminase Inhibition

Using rat brain as a model system, the rate of GABA turnover in vivo following GABA-transaminase inhibition was measured using ^{1}H NMRS (Behar and Boehm, 1994). Gabaculine (3-amino-2,3-dihydrobenzoic acid), which is similar in action to vigabatrin, was administered (100 mg/kg, iv) to inhibit GABA-T. Gabaculine is a more potent GABA-T inhibitor (IC_{50} 1.8 μ*M*) than vigabatrin (IC_{50} 350 μ*M*) and the dose given is known to rapidly achieve 50–100% inhibition. Brain GABA levels increased nearly linearly from 1.9 (pre-gabaculine) to 6.7 μmol/g over 4 h, at a rate of 2 μmole/g/h. An analysis of the extracted brain tissue by high resolution, analytical ^{1}H NMRS confirmed that the increased peak intensities measured in vivo corresponded to GABA and no other metabolite. GABA is closely linked to glutamate and TCA cycle metabolism. Interruption of GABA metabolism could have effects on energy metabolism. The acute effects of GABA-transamination inhibition on the rate of GABA accumulation, high-energy phosphates (ATP, phosphocreatine), and intracerebral pH was measured in vivo using ^{31}P NMRS interleaved with ^{1}H NMRS. High-energy phosphates, inorganic phosphates (P_i) and intracellular pH (pH_i) were unchanged compared to control (pre-gabaculine) values. GABA-T inhibitors such as gabaculine and vigabatrin do not affect high energy phosphate levels in vivo. GAD is the key rate-controlling enzyme of GABA synthesis. These results show that an acute increase in GABA concentration does not perturb key modulators of GAD over this short time frame.

The Rate of GABA Synthesis Is Decreased in Epileptogenic Brain

The GABA-T inhibitor, vigabatrin, reduces seizure severity in proportion to the rise in tis-

sue GABA in an amygdala-kindling rat model (Löscher et al., 1989). The rate of rise of brain GABA after vigabatrin was slower in the kindled amygdala, suggesting that rate of GABA synthesis may be decreased in epileptogenic brain. Slower rates of GABA synthesis could explain low GABA concentrations associated with frequent seizures.

Estimates of GABA Synthesis in Human Brain

Pharmacokinetics of Vigabatrin in Humans

Vigabatrin has been shown to be a safe and effective anti-epileptic medication in several human studies (Schechter et al., 1984; Halonen et al., 1988; Reikkinen et al., 1989a,b; Ben-Menachem et al., 1991, 1989; Ben-Menachem, 1989; Kälviäinen et al., 1993; Chadwick et al., 1996; Pitkänen et al., 1993; French et al., 1996). The drug is supplied as 500-mg tablets and is well absorbed with a bioavailability of 60–80%. Single 3-g doses result in >0.5 m*M* plasma vigabatrin levels in 1–2 h, regardless of food intake (Schechter, 1989). There is virtually no binding to serum proteins. Vigabatrin has a serum half-life ($T_{1/2}$) of 5–7 h and is 70% excreted by the kidney with no active metabolites (Gram, 1996).

The Rise in GABA Following a Single Oral Dose of Vigabatrin Provides an Estimate of the Minimum Rate of GABA Synthesis

In patients with complex partial seizures, a single oral 50 mg/kg dose of vigabatrin achieved peak serum levels in less than 1 h (0.6 m*M*) and CSF level (0.012 m*M*) in less than 6 h (Ben-Menachem et al., 1988). Seven patients (5 women) with complex partial epilepsy were given a single dose of 50 mg/kg vigabatrin. Serial GABA measurements were made before and after vigabatrin (Fig. 4). Brain GABA increased rapidly within 2 h of a single oral dose and remained the same for 24 h. The patients reported no side effects and were calm, but not drowsy (Petroff et al., 1996d).

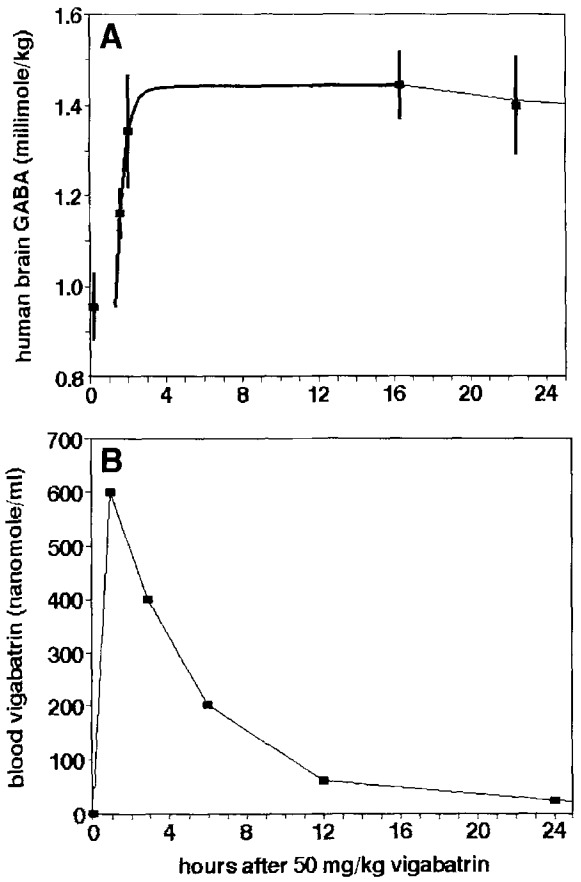

Fig. 4. Serial mean brain GABA concentrations with standard error (bar) are plotted as a function of the time after ingestion of 50 mg/kg vigabatrin. Below are serum vigabatrin concentrations reported after a single 50 mg/kg oral dose (Ben-Menachem et al., 1988). Metabolic modeling suggests brain GABA was synthesized at a minimum rate of 1.1 μmol/g/h. Brain GABA begins to rise approx 1.3 h after ingestion with a time constant of 0.43 h. Assuming that a single dose of 50 mg/kg inhibited GABA-T by 15%, the rate of GABA synthesis in human brain may be as high as 0.12 μmol/g/min.

The rate of GABA synthesis may be estimated by measuring the rise in brain GABA following administration of GABA-transami-

nase inhibitors (Behar and Boehm, 1994). The rise in GABA was modeled using a single exponential, i.e.,

$$C_t = (C_{final} - C_{initial})(1 - e^k(t - d)) + C_{initial}.$$

Brain GABA begins to rise (d) approx 1.3 h after ingestion with a rate constant (k) of 2.3 inverse hours. Brain GABA was synthesized at a minimum rate of 1.1 mmol/kg/h. This value underestimates the rate of synthesis. GABA-T was not inhibited completely. Studies in a rat model suggest GABA-T is 15% inhibited by a single dose of 50 mg/kg (Bolton et al., 1989). Assuming this value, the rate of GABA synthesis (Vgad) in human brain may be as high as 0.12 μmol/g/min. Comparing this value with the previously discussed rate of glutamate synthesis via the TCA cycle (0.72 μmol/g/min), suggests that 17% of the TCA cycle flux is used to synthesize GABA in human brain.

A Single Dose of Vigabatrin Raises GABA for Approx 1 Wk

Vigabatrin concentrations in human CSF following a single oral 50 mg/kg dose peaked within 6 h and decreased with an apparent half-life ($T_{1/2}$) of 12 h (Ben-Menachem et al., 1988). Increases in human CSF GABA were obvious within 6 h of drug administration (Fig. 5). Total CSF GABA levels increased steadily during the first days (pre-vigabatrin 4.7 nmol/mL, 6.2 at 24 h). At 1 wk, the concentrations were almost the same (5.9 nmol/mL). The prolonged elevation of GABA was not unexpected since vigabatrin irreversibly inhibits GABA-T. The inhibited enzyme must be degraded and new enzyme synthesized before GABA-T activity is restored.

In a similar set of experiments, a single 50 mg/kg oral dose of vigabatrin increased brain GABA within 2 h from 1.0 mmol/kg (95% CI 0.8–1.1, n 7) to 1.3 (95% CI 1.0–1.7) (Petroff et al., 1996c). By the next day, brain GABA increased further to 1.4 mmol/kg (95% CI 1.3–1.6). Levels declined gradually to 1.2 (95%

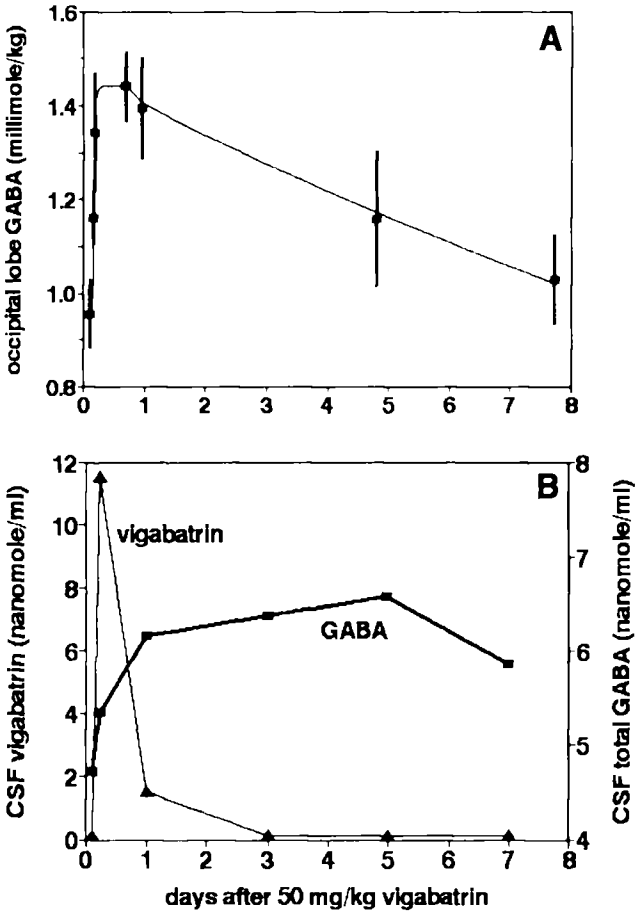

Fig. 5. The top graph shows serial mean brain GABA concentrations with standard error (bar) as a function of the time after ingestion of a single 50 mg/kg dose of vigabatrin. The line models the decrease in brain GABA using a single exponential. Below are CSF total GABA and vigabatrin concentrations reported after a single 50 mg/kg oral dose (Ben-Menachem et al., 1988). Brain GABA levels remain the same for over 24 h as CSF GABA levels continue to rise for several days. CSF vigabatrin concentrations have a half life of 12 h. Once-a-day dosing with vigabatrin should be sufficient to ensure elevated brain GABA.

CI 0.8–1.5) by day five and 1.0 (95% CI 0.8–1.3) at day eight. The patients reported no side effects. The prolonged effect of a single dose indicates once-a-day dosing should be more than sufficient to maintain brain GABA at an elevated level, and presumably therapeutic ef-

fect. Even the inadvertent (but known) missed dose could be taken later without significant loss of effect or probability of side effect. Most clinical studies used a twice-a-day dosing schedule.

The rapid rise of brain GABA levels without adverse effects suggests oral loading with vigabatrin may provide a method to quickly begin antiepileptic drug therapy. Vigabatrin promptly elevates brain GABA and presumably offers partial protection against further seizures within hours of the first oral dose. None of our patients appeared to have a worsening of seizure control during the first 2 d after a single 3-g dose of vigabatrin. Six of seven patients were seizure free. Linear regression showed that for every 0.1 mmol/kg increase in peak GABA over the pre-vigabatrin levels, patients were seizure free for 0.5 d (95% CI 0.4–0.6, correlation coefficient 0.97, $n = 7$, $p < 0.001$). Daily doses raise brain GABA more than a single dose can with further improvement in seizure control.

Metabolic Adaption to Prolonged Elevation of GABA

Cerebrospinal Fluid Concentrations of GABA and Vigabatrin in Humans

Serial human studies of vigabatrin were limited to blood and cerebrospinal fluid (CSF) (Schechter et al, 1984; Halonen et al., 1988; Reikkinen et al., 1989a,b; Ben-Menachem et al., 1989, 1991, 1988; Ben-Menachem, 1989; Kälviäinen et al., 1993; Chadwick et al., 1996; Grove et al., 1981). They showed that CSF vigabatrin increased linearly as the dose of vigabatrin increased to 6 g daily (1.35 nmol/mL/g daily dose, 95% CI 1.26–1.44, degrees of freedom 14, correlation coefficient 0.976). Short-term therapy, lasting 3–14 d, increased CSF GABA in proportion to dose (0.5, 1, 2, and 6 g/d). Three to 6 mo of therapy with doses of 1–3.5 g/d showed CSF total GABA increased in a dose-dependent manner. Human CSF total GABA levels increased by

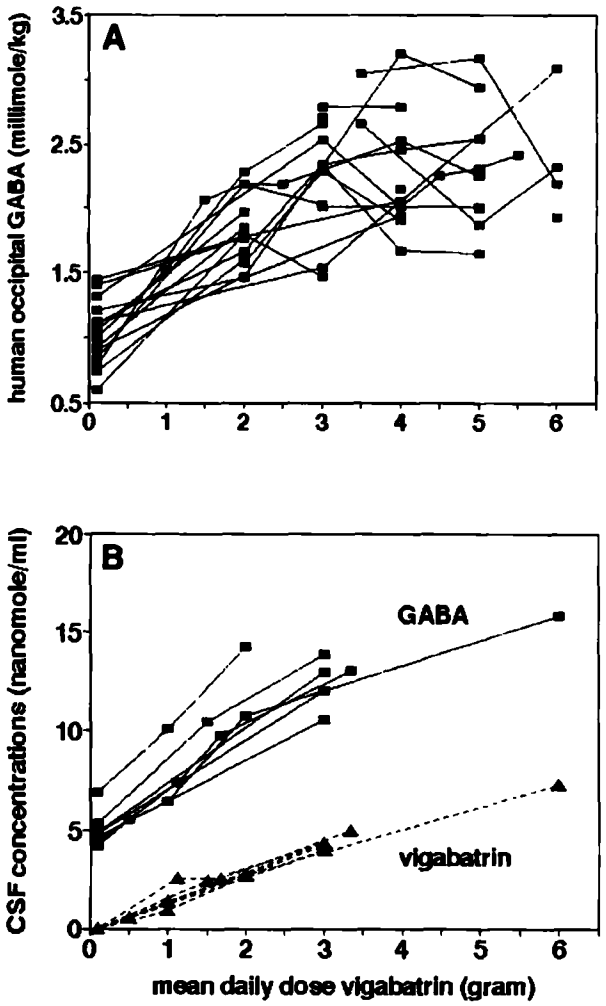

Fig. 6. The top graph shows serial brain GABA concentrations as the daily dose of vigabatrin is increased. The lines connect serial measurements of individual patients. When more than one measurement was made at the same daily dose, the average was used. Occipital-lobe GABA increased with daily dose to 3 g. Increasing the daily dose to 6 g failed to increase GABA in most patients. There is no difference in the mean GABA concentration with daily doses of 3, 4, 5, or 6 g. Below are mean CSF total GABA and vigabatrin concentrations as daily vigabatrin dose increased adapted from several sources (Halonen et al., 1988; Ben-Menachem et al., 1989, 1991; Grove et al., 1981. Schechter and Sjoerdsma, 1990). CSF vigabatrin concentrations increased in proportion to daily dose (1.35 nmols/mL/g daily dose, standard error 0.04, degrees of freedom 14, correlation coefficient 0.976).

58% at 1 g, 125% at 2 g, 151% at 3 g, and 214% at 6 g daily.

One patient underwent temporal-lobe resection while on vigabatrin (3.5 g daily for 3 yr; Ben-Menachem et al., 1993). Cortical and CSF GABA concentrations were reported as 3 μmol/g and 10.5 nmol/mL, respectively. Cortical vigabatrin concentrations were not measured.

Using serial, in vivo NMRS, we studied 25 patients undergoing treatment for intractable epilepsy with the GABA-T inhibitor, vigabatrin, as the daily dose was increased (Petroff et al., 1996b,e). Our data suggest that brain GABA levels increased to 178% (95% CI 163–193, n = 16) of pretreatment values with low dose (27 mg/kg daily) and to 226% (95% CI 209–243, n = 25) with standard dose (48 mg/kg daily) vigabatrin (Fig. 6). There was no further increase at high-dose vigabatrin (228%, 95% CI 202–254, n = 14, at 72 mg/kg daily). The fractional elevation of brain GABA with vigabatrin dose is similar to CSF increases suggesting a proportional relationship between cellular and extracellular GABA concentration. The increase in brain GABA with the addition of vigabatrin was the same for all AEDs tested. The response when vigabatrin was added to carbamazepine or phenytoin was the same as with valproate and barbiturates.

Metabolic adaptation occurs in human brain with prolonged elevation of GABA. Increasing the amount of GABA-transaminase inhibition by doubling the daily dose of vigabatrin from 3 to 6 g/d failed to raise GABA further. This suggests that increased GABA concentration reduces GABA synthesis. Experiments in animals show only partial inhibition of brain GABA-transaminase on doses of vigabatrin 10 times larger than those used in humans (Jung and Palfreyman, 1995; Bolton et al., 1989). Following the acute administration of vigabatrin, rat brain GABA levels rise proportionate to the dose. Doubling the dose of vigabatrin from 3 to 6 g/d was expected to increase the inhibition of human-brain GABA transaminase and increase GABA, but this did not occur.

Increased GABA Inhibits Synaptic GABA Synthesis

GAD has several isoforms localized to synaptic terminals, neuronal perikarya, and glia. Until recently, feedback inhibition by GABA was considered not to be important because of the high concentration of GABA required for inactivation of GAD (approx 16 m*M*) relative to the concentration of GABA (1–2 m*M*) in cerebral cortex. Adaptation to the acute effects of vigabatrin occurs in gerbil, mouse, and rat models (Jung and Palfreyman, 1995; Löscher and Frey, 1987; Rimvall and Martin, 1994; Jung et al., 1977; Neal and Shah, 1990). Recent studies have found that GAD activity is decreased when GABA concentration is elevated chronically by vigabatrin roughly in proportion to GABA concentration. Comparison of whole-tissue and synaptosome GABA concentration following chronic GABA elevation suggested that this inhibition is considerably greater in nerve terminals. The level of GAD_{67} protein is sensitive to GABA concentration and decreases as GABA levels rise (Rimvall and Martin, 1994), which has been proposed as a mechanism for the drop in GAD activity. Adaptation to the acute effects of vigabatrin was shown in rat, mouse, and gerbil models. After 10 d of vigabatrin treatment (120 mg/kg), whole brain levels increase 200%, yet synaptosomal GABA levels increase only 80–90%. Increasing the dose to 150 mg/kg fails to increase whole-brain or synaptosomal GABA further. The increased neuronal concentrations of GABA decreases GAD_{67} to 20% of normal. Whole-brain GAD enzymatic activity decreases by 25%. The role of prolonged elevations of GABA in controlling GAD activity is not yet known, since enzyme level and activity assayed in vitro does not always reflect activity in vivo. The importance of this mechanism is supported by studies we performed in rats indicating a 70% reduction in GABA-synthesis rates 24 h after GABA was elevated using vigabatrin (Manor et al., 1996).

Turnover of GABA is Decreased in Rat Cortex When GABA Concentrations Were Increased to Above Normal Levels

Recently it has been shown that increased GABA levels reduces GAD_{67} protein, one of two major isoforms of GAD. Treatment with vigabatrin (45 mg/kg) for 5 d resulted in a 50% increase in rat whole-brain GABA, a 35% decrease in the GAD_{67} isoform, and a 5–10% drop in GAD activity measured in vitro (Rimvall and Martin, 1994). The effect of GABA elevation on GABA synthesis were assessed in rat brain in vivo using ^{1}H and ^{13}C-edited NMRS at 24 h following a single dose of vigabatrin (500 mg/kg ip; Manor et al., 1996).

GABA concentration increased twofold at 24 h (from 1.3 μmol/g, 95% Cl 1.1–1.5, n = 18, to 2.7, 95% Cl 2.2–3.2, n = 21) and GABA-T activity was inhibited by 60%. Tricarboxylic acid-cycle flux was not affected by vigabatrin treatment (0.47 μM/g/min, 95% Cl0. 27–0.67, n=6), compared to untreated rats (0.52 μM/ g/min, 95% Cl 0.33–0.71, n=6). GABA-C2 isotopic enrichment measured in acid extracts rose more slowly in vigabatrin-treated compared to untreated rats, reaching >90% of the glutamate-C4 isotopic enrichment after 3 h (Fig. 7). In contrast, GABA isotopic enrichment was greater than glutamate isotopic enrichment in untreated rats. A metabolic model consisting of a single glutamate pool failed to account for the fast labeling of GABA from glutamate. Metabolic modeling analysis based on two (noncommunicating) glutamate pools revealed an approx 70% decrease in the rate of GABA synthesis following vigabatrin treatment, from 0.14 (untreated) to 0.04 μmol/g/ min vigabatrin-treated). These findings, in conjunction with the previously reported differential effect of elevated GABA on the GAD isoforms, suggests that GAD_{67} may account for a major fraction of cortical GABA synthesis in alpha-chloralose anesthetized rat brain in vivo.

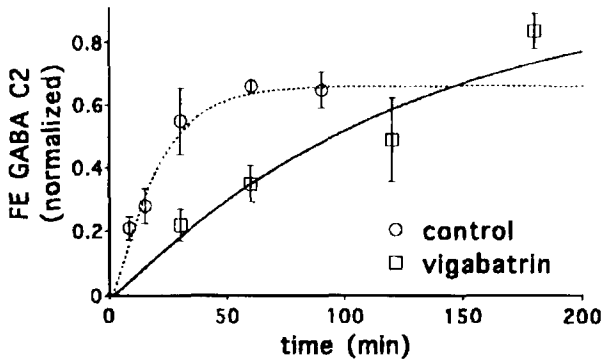

Fig. 7. The rate of GABA turnover was decreased markedly within 24 h of a single dose of vigabatrin. GABA-C2 fractional enrichment (FE) rose more slowly in vigabatrin-treated compared to untreated rats, reaching >90% of the glutamate FE after 3 h. In contrast, GABA FE ≥ glutamate FE in untreated rats. Tricarboxylic acid-cycle flux, with alpha chloralose anesthesia, was not affected by vigabatrin treatment (untreated 0.47 μmol/g/min, vigabatrin-treated 0.52). GABA concentration increased twofold at 24 h. Ex vivo measurements showed that GABA-T activity was inhibited by 60% (untreated 10.8 mM/h, vigabatrin treated 4.1). Metabolic-modeling analysis based on two (noncommunicating) glutamate pools revealed an approx 70% decrease in the rate of GABA synthesis (untreated 0.14 μmol/g/min, vigabatrin-treated 0.04).

On a Constant Dose of Vigabatrin, GABA Declined in Some Patients, But Remained the Same in Most

Vigabatrin remains an effective antiepileptic medication over 3–5 yr of use (Ben-Menachem, 1991; Pitkänen et al., 1993; French et al., 1996). Serial CSF measurements in 10 patients treated with 50 mg/kg vigabatrin administered once a day showed a 300% increase in GABA over pretreatment levels at 6 mo (Ben-Menachem et al., 1989, 1991). Human CSF GABA remained elevated (260%) at 12 and 32 mo. In our study, serial GABA measurements were obtained of six patient taking a constant dose, four on a high dose, and two on a standard dose (Petroff et al, 1996b,e). Brain GABA levels decreased over a period of 4–14 mo in half the patients. Levels remained the same after 12 mo (Fig. 8).

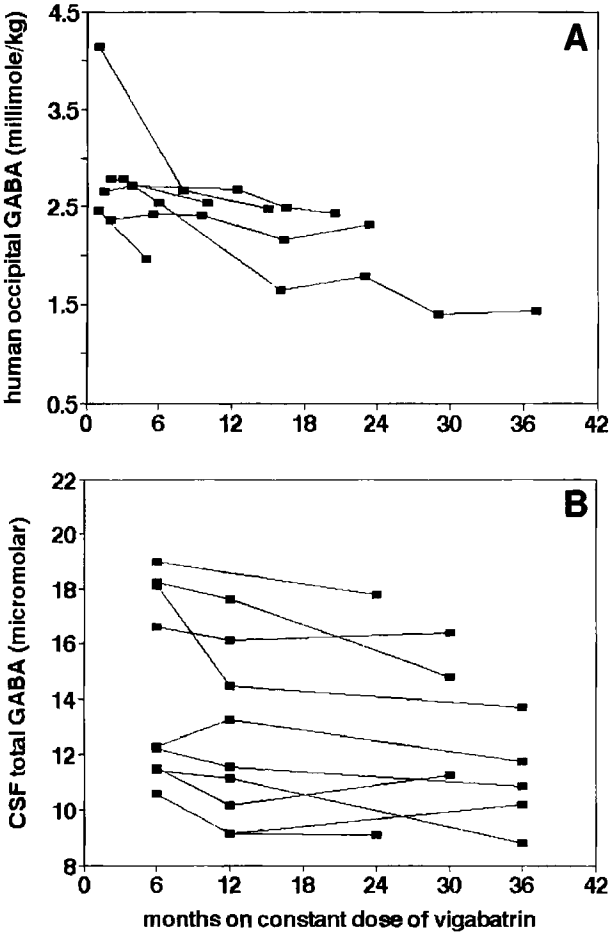

Fig. 8. Serial GABA measurements with a constant daily dose of vigabatrin. The top graph shows occipital-lobe GABA levels of individual patients on a constant dose of vigabatrin. The graph below shows CSF total GABA of a different group of patients on a constant dose of vigabatrin, adapted from Ben-Menachem et al. (1991). Brain and CSF GABA remained above normal, although a decline was observed in some patients.

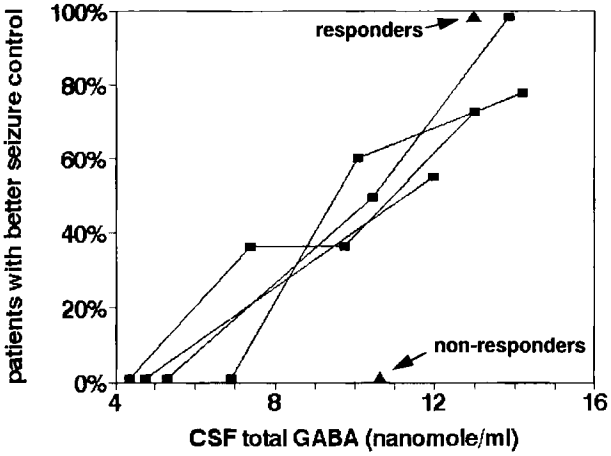

Fig. 9. Seizure control improved as CSF total GABA increased. Total GABA levels above 12 n*M*/mL were associated with significantly improved seizure control (seizure frequency reduced by more than 50%) (Schechter et al, 1984; Ben-Menachem et al., 1989; Reikkinen et al., 1989b). Significantly lower CSF GABA levels were seen in patients whose seizure control did not improve significantly. Reducing vigabatrin from 3 to 1.5 g daily, decreased CSF total GABA from 13.9 to 10.5 nmol/mL and seizure control deteriorated by 50% in those patients who had responded to the higher dose (Reikkinen et al., 1989b).

Effect of GABA on Seizure Control

In animal models, the time course of seizure protection following acute administration of vigabatrin is closely related to the increase in synaptosomal GABA concentration (Jung and Palfreyman, 1995). Improved seizure control correlated with increased CSF total-GABA concentrations (Schechter et al., 1984; Ben-Menachem et al., 1989; Reikkinen et al., 1989b). Total GABA levels above 12 nmol/mL were associated with significantly improved seizure control in 50–70% of patients (Fig. 9). CSF GABA levels were higher in patients whose seizure control improved with the addition of vigabatrin compared to those patients who failed to improve (Schechter et al., 1984; Halonen et al., 1988; Ben-Menachem et al., 1989; Reikkinen et al., 1989b). Reducing vigabatrin from 3 to 1.5 g daily decreased CSF total GABA from 13.9 to 10.5 nmol/mL and seizure control deteriorated by 50% (Reikkinen et al., 1989b).

Our study confirmed and extended these observations. Starting vigabatrin reduced seizure frequency by over 50%, from six to seven per month to two to three (Petroff et al., 1996b,e). Improved seizure control was not associated with further increases of vigabatrin dose. The improve-

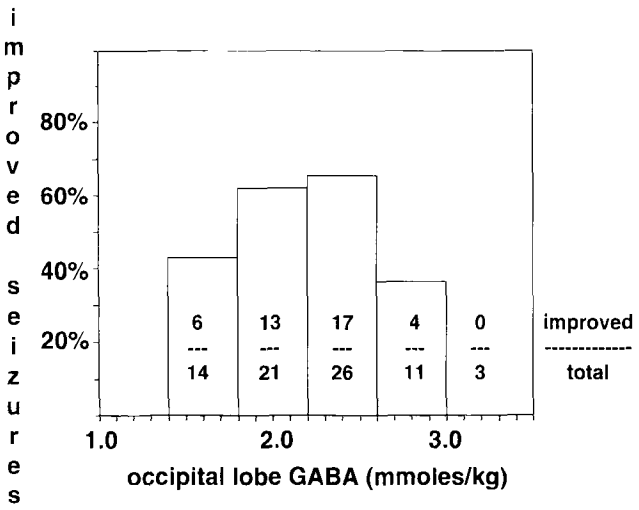

Fig. 10. Seizure control as occipital-lobe GABA concentrations increased. Seizure frequency reduced by more than 50% when GABA levels rose above 1.8 mmol/kg and was best at GABA levels between 1.8 and 2.5 mM/kg. Some patients' seizure control did not improve. Further increases in human brain GABA above 2.5 mmol/kg fail to provide additional protection.

ment in seizure control correlated with increased brain-GABA concentration, not with vigabatrin dose (Fig. 10). Starting vigabatrin improved seizure control twofold when GABA levels increased above 1.8 mmol/kg. Maximal improvement in seizure control was seen at GABA levels between 2.2 and 2.6 mmol/kg. Two thirds of patients whose brain GABA was in this range had more than a 50% improvement in seizure control.

In the human epileptic focus, GABA release in response to a seizure is blunted and abbreviated. Remote from the focus, patients with complex-partial seizures had lower mean GABA levels than subjects without epilepsy. Low GABA levels predispose but, in themselves, may not be sufficient for seizures to become clinically manifest. Below-normal GABA levels were associated with poor seizure control. Low GABA levels outside of the epileptic focus may facilitate spread of epileptiform discharges beyond the focus. On traditional AEDs, remission of the seizure disorder was associated with normal GABA levels. With vigabatrin, elevated CSF and brain GABA was associated with improved seizure control.

Reversal of the GABA Transporter Can Contribute to Seizure Protection

The action of GABA in the synapse is terminated by a sodium-dependent, chloride-dependent high-affinity uptake system on neurons and glia (Schousboe and Westergaard, 1995; Levi and Gallo, 1995). Neurons have a higher capacity for GABA uptake than astrocytes. Estimates of 80% neuronal and 20% glial uptake have been proposed (Schousboe and Westergaard, 1995). In the adult cortex, glia contain little GABA and metabolize it rapidly under normal circumstances (Martin, 1995). GABA uptake by glia is functionally important in epilepsy. GABA analogs, e.g., nipecotic acid, which preferentially inhibit glial GABA uptake act as anticonvulsants (Kocsis and Mattson, 1996; Schousboe and Westergaard, 1995).

Glutamate-induced, calcium-independent release of GABA was decreased markedly in the human, epileptogenic hippocampus (During et al., 1995). The release of GABA during seizures may be mediated in part by transporter reversal and may serve as an important protective mechanism. During a seizure, this mechanism may be critical in rapidly terminating the seizure or preventing its spread beyond the focus (During and Spencer, 1993; During et al., 1995). A decrease in the density of GABA transporters in the epileptic focus was associated with a decrease in glutamate-induced GABA release (During et al., 1995). The loss of GABA transporters would account for the diminished GABA release during spontaneous seizures in human epileptogenic hippocampi. The nonvesicular release of GABA is an important protective mechanism suppressing the evolution and spread of seizure activity.

Gabapentin Enhances Nonvesicular Release of GABA

Gabapentin has come into clinical use as adjunctive therapy in the treatment of epilepsy

(Kocsis and Mattson, 1996; Petroff et al., 1996a). It promotes the release of GABA from glia and perhaps neurons by the reversal of the sodium-dependent GABA transporter (Taylor, 1994; Kocsis and Honmou, 1994; Honmou et al., 1995). This action would tend to increase extracellular GABA not only within the seizure focus, but also the surrounding volume of brain. Gabapentin significantly increased human occipital lobe GABA (Petroff et al., 1996a). Patients who responded to gabapentin had above-normal GABA and the best seizure control (Petroff et al., 1997). Increased cytosolic GABA would enhance the nonvesicular release of GABA when the GABA transporters operate in reverse. This mechanism would not operate under normal conditions in which vesicular release of GABA dominates. Drugs that increase inhibition primarily during seizures would be expected to have a very favorable side-effect profile. Both gabapentin and vigabatrin have such favorable profiles (Kocsis and Mattson, 1996; Chadwick et al., 1996).

Glutamate Can Reverse Glial GABA-Transporter Function

Glial cells express non-NMDA subtypes of glutamate receptors. Micromolar concentrations of kainate, AMPA, and quisqualate stimulate the calcium-independent, nonvesicular release of GABA from astrocytes (Gallo et al., 1989, 1991; Martin, 1992). The reuptake of GABA is coupled to the influx of sodium. Under conditions of depolarization and elevated intracellular sodium concentrations, the GABA transporters operate in reverse. Nonvesicular release of GABA can occur by the reverse operation of the GABA transporter under conditions associated with seizure activity (Kocsis and Mattson, 1996; Levi and Gallo, 1995).

Vigabatrin, like other GABA-transaminase inhibitors, increases GABA concentrations in neurons and glia (Rimvall and Martin, 1994; Martin, 1995; Neal et al., 1989; Storm-Mathisen et al, 1986). Synaptic GABA is increased too (Gram et al., 1989). Increased GABA concentrations in the astroglial cytosol would augment GABA release when GABA transporters reverse (Fig. 11). Vigabatrin enhances the nonvesicular release of GABA by glia (Kocsis and Mattson, 1996). Vigabatrin potentially could compensate for the decrease in glutamate-induced, calcium-independent release of GABA observed in the human epileptic focus (During et al., 1995). Enhanced nonvesicular release of GABA by astrocytes during a seizure would be expected to increase GABA-mediated inhibition both in the focus and the surrounding cortex serviced by activated glia.

Limitations on the Effectiveness of Increased GABA

The United States cooperative study of vigabatrin compared seizure control using vigabatrin doses of 1, 3, and 6 g/d (French et al., 1996; Penry et al, 1993). The same percentage of patients responded to 3 or 6 g/d therapy. Our study showed no further improvement in seizure control as dose increased from 3 to 6 g/d using a twice-a-day dosing schedule (Petroff et al., 1996b,e). Brain-GABA levels also failed to increase further. Downregulation of GABA synthesis and residual GABA catabolism unaffected by vigabatrin could limit the effectiveness of high-dose drug.

Seizure control improved when GABA increased above 1.8 μmol/kg. Further increases in human-brain GABA above 2.5 m*M*/kg fail to provide additional protection. Seizure control with GABA levels above 3.0 μmol/kg was the same as before vigabatrin. Elevated brain GABA levels failed to improve seizure control in some patients (Petroff et al., 1996b,e).

Clinical studies show that 20–30% of patients who initially benefitted from vigabatrin therapy suffered a worsening of seizure control with prolonged treatment (Jung and Palfreyman, 1995; Ben-Menachem et al., 1991; Kälviäinen et al., 1995). Whether the lessening of seizure control was associated with declining levels of brain GABA is unclear from our small study (Petroff et al., 1996b,e).

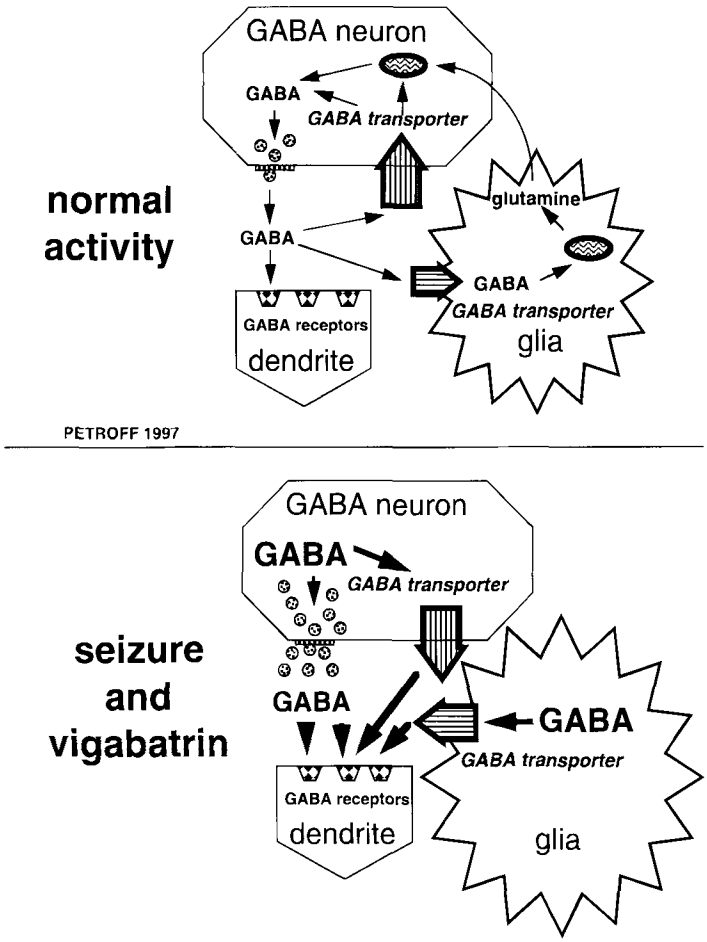

Fig. 11. Cartoons of GABAergic synapses during normal activity and the start of seizure activity after vigabatrin therapy: During normal activity, extracellular GABA concentrations are controlled by the rate of synaptic release and limited by transporter-mediated reuptake Vigabatrin increases cytosolic GABA in neurons and glia. With neuronal depolarization, synaptic release of GABA increases with vigabatrin. During intense neuronal depolarization with increased intracellular sodium, nonvesicular release of GABA increases. GABA-transporter reverses and the increased cytosolic GABA floods the extracellular space surrounding depolarized portions of GABAergic neurons. Augmented nonvesicular release of GABA by glia was demonstrated in rat models treated with vigabatrin. Augmented nonvesicular GABA release by glia, triggered by high extracellular glutamate during seizure activity, would flood the extracellular space surrounding glia with GABA.

Several possible mechanisms could explain our observations. Desensitization of the $GABA_A$ receptor/chloride-channel complex during prolonged exposure to GABA was proposed as contributing to epileptogenesis (Tasker and Dudek, 1991). Increased GABA concentrations in the postsynaptic neuron might downregulate $GABA_A$-receptor functioning (Wood and Davies, 1991; Maloteaux et al., 1987). This effect was seen following 5 d vigabatrin treatment (150 mg/kg) in a rat model. Alternatively, $GABA_A$-receptor density may be downregulated by elevated GABA levels (Mhartre and Ticku, 1994; Kang and Miller, 1991). Prolonged exposure to GABA might have the paradoxical effect of decreasing GABA-mediated inhibition at times of normal synaptic release.

The potency of diazepam in displacing [^{11}C]-flumazenil from brain benzodiazepine receptors was enhanced in vigabatrin-treated baboons, contrasting with the reduced anticonvulsant effects of diazepam in these animals (Schmid et al., 1996). No effect was observed on the displacement of [^{11}C]-flumazenil by the inverse agonist, methyl-beta-carboline-3-carboxylate. Vigabatrin reduced the proconvulsant effect of the inverse agonist at all doses.

High-dose vigabatrin could increase extracellular GABA significantly by increasing quantal release of GABA during normal neurotransmission and by diminishing reuptake by GABA transporters. Vigabatrin worsens seizures that respond to GABA-B receptor blockade, e.g., petit mal (Fisher and Kerrigan, 1995; Lortie et al., 1993; Macdonald and Meldrum, 1995). This mechanism could lower seizure protection with high concentrations of GABA. Recently, a mechanism for biphasic, inhibitory and excitatory, dendritic responses to GABA was proposed (Staley et al., 1995). Activity-dependent GABA-mediated excitation may contribute to epileptogenesis.

Summary

GABA is the major inhibitory neurotransmitter in human cortex. Extensive studies in animals have shown that GABA plays a pivotal role in suppressing the origin and spread of seizure activity. 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 (e.g., vigabatrin, gabapentin, tiagabine) hinges on the elevation of GABA concentration. Serial occipital-lobe GABA measurements were made using in vivo nuclear magnetic-resonance spectroscopy. Below-normal occipital-lobe GABA, remote from the seizure focus, was associated with poorly controlled seizures. Using a single dose of vigabatrin to abruptly increase GABA-transaminase inhibition, the rate of GABA synthesis in human brain was estimated at 17% of the TCA cycle rate. As the daily dose of vigabatrin is increased, the fractional elevation of brain GABA is similar to cerebrospinal fluid increases. Therefore, a proportional relationship exists between cellular and extracellular GABA concentration. GABA levels initially increased linearly with vigabatrin dose, but cease to increase above a dose of 3 g/d. This suggests that increased GABA concentration reduces GABA synthesis. Vigabatrin administered 24 h prior to the measurements reduced the rate of GABA synthesis by 70% in a rate model. Improvement in seizure control correlated with increased brain GABA concentration, not with vigabatrin dose. The significance of understanding the regulation of GABA concentration in humans is enhanced by our recent finding that improved seizure control correlated with the elevation of GABA to above normal levels in patients receiving vigabatrin and gabapentin. Nonvesicular release of GABA during seizure, enhanced by gabapentin and vigabatrin, may serve as an important protective mechanism terminating the spread of seizure activity.

Acknowledgments

Supported by NIH-NINDS grants: RO1-NS32518, PO1-NS06208, R29-NS032126. Vigabatrin was provided by Hoechst Marion Roussel. We thank Richard H. Mattson for inviting us to participate in his studies using vigabatrin, Kevin Behar for useful discussions about GABA metabolism, and Terry Nixon for modifications and maintenance of the spectrometer.

References

Ackermann R. F., Engel J., and Phelps M. E. (1986) Identification of seizure-mediating brain structures with the deoxyglucose method: studies of

human epilepsy with positron emission tomography, and animal seizure models with contact autoradiography. *Adv. Neurol.* **44,** 921–934.

Böhlen P., Huot S., and Palfreyman M. G. (1979) The relationship between GABA concentrations in brain and cerebrospinal fluid. *Brain Res.* **167,** 297–305.

Behar K. and Boehm D. (1994) Measurement of GABA following GABA-transaminase inhibition by gabaculine: a ^{1}H and ^{31}P NMR spectroscopic study of rat brain in vivo. *Magn. Reson. Med.* **31,** 660–667.

Behar K. L. and Ogino T. (1991) Assignment of resonances in the ^{1}H spectrum of rat brain by two-dimensional shift correlated and J-resolved NMR spectroscopy. *Magn. Reson. Med.* **17,** 285–304.

Behar K. L. and Ogino T. (1993) Characterization of macromolecule resonances in the ^{1}H NMR spectrum of rat brain. *Magn. Reson. Med.* **30,** 38–44.

Behar K. L., Rothman D. L., Spencer D. D., and Petroff O. A. C. (1994) Analysis of macromolecule resonances in ^{1}H NMR spectra of human brain. *Magn. Reson. Med.* **32,** 294–302.

Ben-Menachem E. (1989) Pharmacokinetic effects of vigabatrin on cerebrospinal fluid amino acids in humans. *Epilepsia* **30(Suppl. 3),** S12–S14.

Ben-Menachem E., Perrson L. I., and Schechter P. J. (1988) Effects of single doses of vigabatrin on CSF concentrations of GABA, homocarnosine, homovanillic acid and 5-hydroxyindoleacetic acid in patients with complex partial epilepsy. *Epilepsy Res.* **2,** 96–101.

Ben-Menachem E., Persson L. I., and Schechter P. J. (1989). The effect of different vigabatrin treatment regimens on CSF biochemistry and seizure control in epileptic patients. *Br. J. Clin. Pharmacy* **27(Suppl. 1),** 79S–85S.

Ben-Menachem E., Persson Mumford J. P., Haegele K. D., and Huebert N. (1991) The effect of long-term vigabatrin therapy on selected neurotransmitter concentrations in cerebrospinal fluid. *J. Child. Neurol.* **6(Suppl. 2),** 2S11–2S16.

Ben-Menachem E., Hamberger A., and Mumford J. (1993) Effect of long-term vigabatrin therapy on GABA and other amino acid concentrations in the central nervous system a case study. *Epilepsy Res.* **16,** 241–243.

Bernasconi R., Bittiger H., Schmutz M., Martin P., and Klein M. (1984) Is the estimation of the GABA turnover rate in vivo a tool to differentiate between various types of drugs interfering with the GABA/benzodiazapine/ionophore receptor complex? *Neuropharmacology* **23,** 815–816.

Bolton J. B., Rimmer E., Williams J., and Richens A. (1989) The effect of vigabatrin on brain and platelet GABA-transaminase activities. *Br. J. Clin. Pharmacy* **27(Suppl. 1),** 35S–42S.

Cendes F., Andermann F., Preul M. C., and Arnold D. L. (1994) Lateralization of temporal lobe epilepsy based on regional metabolic abnormalities in proton magnetic resonance spectroscopic images. *Ann. Neurol.* **35,** 211–216.

Cendes F., Stanley J. A., Dubeau F., Andermann F., and Arnold D. L. (1997) Proton magnetic resonance spectroscopic imaging for discrimination of absence and complex partial seizures. *Ann. Neurol.* **41,** 74–81.

Chadwick D. W., Marson T., and Kadir Z. (1996) Clinical administration of new antiepileptic drugs: an overview of safety and efficacy. *Epilepsia* **37(Suppl. 6),** S17–S22.

Chu W. J., Hetherington H. P., Kuzniecky R. I., Vaughan, U. T., Twie D. B., Faught R. E., Gilliam F. G., and Elgavish G. A. (1996) Is the intracellular pH different from normal in the focus of patients with temporal lobe epilepsy? A ^{31}P NMR study. *Neurology* **47,** 756–760.

Connelly A., Jackson G. D., Duncan J. S., King M. D., and Gadian D. G. (1994) Magnetic resonance spectroscopy in temporal lobe epilepsy. *Neurology* **44,** 1411–1417.

During M. J. (1994) Dynamic neurochemical alterations in human temporal lobe epilepsy. *Clin. Neuroscience* **2,** 53–63.

During M. J. and Spencer D. D. (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* **341,** 1607–1613.

During M. J., Ryder K. M., and Spencer D. D. (1995) Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature* **376,** 174–177.

Erlander M. G. and Tobin A. J. (1991) The structural and functional heterogeneity of glutamic acid decarboxylase: a review. *Neurochem. Res.* **16,** 215–226.

Fisher R. S. and Kerrigan J. F. (1995) Vigabatrin: toxicity, in *Antiepileptic Drugs,* 4th ed. (Levy R. H., Mattson R. H., and Meldrum B. S., eds.) Raven, New York, pp. 931–939.

Fountain N. B. and Lothman E. W. (1995) Pathophysiology of status epilepticus. *J. Clin. Neurophysiol.* **12,** 326–342.

French J. A., Mosier M., Walker S., Sommerville K., Sussman N., and the Vigabatrin Protocol 024 Investigative Cohort. (1996) A double-blind, placebo-controlled study of vigabatrin three g/day in patients with uncontrolled complex partial seizures. *Neurology* **46,** 54–61.

Fyske E. M. and Fonnum F. (1988) Uptake of gamma-aminobutyric acid by a synaptic vesicle fraction isolated from rat brain. *J. Neurochem.* **50,** 1237–1242.

Gadian D. G. (1995) N-acetylaspartate and epilepsy. *Magn. Reson. Imag.* **13,** 1193–1195.

Gallo V., Giovannini C., Suergiu R., and Levi G. (1989) Expression of excitatory amino acid receptors by cerebellar cells of the type-2 astrocyte lineage. *J. Neurochem.* **52,** 1–9.

Gallo V., Patrizio M., and Levi G. (1991) GABA release triggered by the activation of neuronlike non-NMDA receptors in cultured type-2 astrocytes is carrier-mediated. *Glia* **4,** 245–255.

Garcia P. A., Laxer K. D., van der Grond J., Hugg J. W., Matson G. B., and Weiner M. W. (1994) Phosphorus magnetic resonance spectroscopic imaging in patients with frontal lobe epilepsy. *Ann. Neurol.* **35,** 217–221.

Gram L. (1996) Pharmacokinetics of new antiepileptic drugs. *Epilepsia* **37(Suppl. 6),** S12–S16.

Gram L., Larsson O. M., Johnsen A., and Schousboe A. (1989) Experimental studies of the influence of vigabatrin on the GABA system. *Br. J. Clin. Pharmacy* **27(Suppl. 1),** 13S–17S.

Grove J., Schechter P. J., Tell G., Koch-Weser J., Sjoerdsma A., Warter J. M., Marescaux C., and Rumbach L. (1981) Increased gamma-aminobutyric acid (GABA), homocarnosine, and beta-alanine in cerebrospinal fluid of patients treated with gamma-vinyl GABA (4-amino-hex-5-enoic acid). *Life Sci.* **28,** 2431–2439.

Gruetter R., Novotny E. J., Boulware S. D., Mason G. F., Rothman D. L., Prichard J. W., and Shulman R. G. (1994) Localized ^{13}C NMR spectroscopy in the human brain ofamino acid labeling from ^{13}C glucose. *J. Neurochem.* **63,** 1377–1385.

Halonen T., Lehtinen M., Pitkänen A., Ylinen A., and Riekkinen P. J. (1988) Inhibitory and excitatory amino acids in CSF of patients suffering with complex partial seizures during chronic treatment with gamma-vinyl GABA (vigabatrin). *Epilepsy Res.* **2,** 246–252.

Hamberger A., Nystrom B., Larsson S., Silvenius H., and Nordborg C. (1991) Amino acids in the neuronal microenvironment of focal human epileptic lesions. *Epilepsy Res.* **9,** 32–43.

Hetherington H., Kuzniecky R., Pan J., Mason G., Morawetz R., Harris C., Faught E., Vaughan T., and Pohost G. (1995) Proton nuclear magnetic resonance spectroscopic imaging of human temporal lobe epilepsy at 4.1 T. *Ann. Neurol.* **38,** 396–404.

Honmou O., Kocsis J. D., and Richerson G. B. (1995) Gabapentin potentiates the conductance increase induced by nipecotic acid in CA1 pyramidal neurons in vitro. *Epilepsy Res.* **20,** 193–202.

Horton R. W. (1991) GABA dysfunction in animal models of epilepsy, in *GABA Mechanisms in Epilepsy* (Tunnicliff G. and Raess B. U., eds.), Wiley-Liss, New York, pp. 121–147.

Hugg J. W., Laxer K. D., Matson G. B., Maudsley A. A., Husted C. A., and Weiner M. W. (1992) Lateralization of human focal epilepsy by ^{31}P magnetic resonance spectroscopic imaging. *Neurology* **42,** 2011–2018.

Hugg J. W., Laxer K. D., Matson G. B., Maudsley A. A., and Weiner M. W. (1993) Neuron loss localizes human temporal lobe epilepsy by in vivo proton magnetic resonance spectroscopic imaging. *Ann. Neurol.* **34,** 788–794.

Hugg J. W., Kuzniecky R. I., Gilliam F. G., Morawetz R. B., Faught R. E., and Hetherington H. P. (1996) Normalization of contralateral metabolic function following temporal lobectomy demonstrated by ^{1}H magnetic resonance spectroscopic imaging. *Ann. Neurol.* **40,** 236–239.

Jackson G. D., Connelly A., Cross J. H., Gordon I., and Gadian D. G. (1994) Functional magnetic resonance imaging of focal seizures. *Neurology* **44,** 850–856.

Jung M. J., Lippert B., Metcalf B. W., Böhlen P., and Schechter P. J. (1977) Gamma-vinyl-GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: effects on brain metabolism in mice. *J. Neurochem.* **29,** 797–802.

Jung M. J. and Palfreyman M. G. (1995) Vigabatrin: mechanisms of action, in *Antiepileptic Drugs,* 4th ed. (Levy R. H., Mattson R. H., and Meldrum B. S., eds.) Raven, New York, pp. 903–913.

Kälviäinen R., Halonen T., Pitkanen A., and Reikkinen P. J. (1993) Amino acid levels in the cerebrospinal fluid of newly diagnosed epileptic patients: effect of vigabatrin and carbamazepine monotherapies. *J. Neurochem.* **60,** 1244–1250.

Kälviäinen R., Merbaala E., Sivenius J., and Reikkinen P. J. (1995) Vigabatrin clinical use, in *Antiepileptic Drugs*, 4th ed. (Levy R. H., Mattson R. H., and Meldrum B. S., eds.) Raven, New York, pp. 925–930.

Kang I. and Miller L. G. (1991) Decreased $GABA_A$ receptor subunit mRNA concentrations following chronic lorazepam administration. *Br. J. Pharmacol.* **103,** 1285–1287.

Kaura S., Bradford H. F., Young A. M. J., Croucher M. J., and Hughes P. D. (1995) Effect of amygdaloid kindling on the content and release of amino acids from the amygdaloid complex: in vivo and in vitro studies. *J. Neurochem.* **65,** 1240–1249.

Kocsis J. D. and Honmou O. (1994) Gabapentin increases GABA-induced depolarization in rat neonatal optic nerve. *Neurosci. Lett.* **169,** 181–184.

Kocsis J. D. and Mattson R. H. (1996) GABA levels in the brain: a target for new antiepileptic drugs. *Neuroscientist* **6,** 326–334.

Kuzniecky R. I. and Jackson G. D. (1995) *Magnetic Resonance in Epilepsy*. Raven, New York.

Kuzniecky R., Elgavish G. A., Hetherington H. P., Evanochko W. T., and Pohost G. M. (1992) In vivo ^{31}P nuclear magnetic resonance spectroscopy of human temporal lobe epilepsy. *Neurology* **42,** 1586–1590.

Laxer, K. D. (1996) ^{31}P magnetic resonance spectroscopy of epilepsy, in *Neuroimaging in Epilepsy* (Cascino G. D. and Jack C. R., eds.) Butterworth-Heinemann, Boston, pp. 145–150.

Leo G. C., Driscoll B. F., Shank R. P., and Kaufman E. (1993) Analysis of [1-^{13}C]d-glucose metabolism in cultured astrocytes and neurons using nuclear magnetic resonance spectroscopy. *Dev. Neurosci.* **15,** 282–288.

Levi G. and Gallo V. (1995) Release of neuroactive amino acids from glia, in *Neuroglia* (Kettenmann H. and Ransom B. R., eds.) Oxford University Press, New York, pp. 815–826.

Lloyd K. G., Bossi L., Morselli P. L., Munari C., Rougier M., and Loiseau H. (1986) Altered GABA-mediated synaptic transmission in human epilepsy. *Adv. Neurol.* **44,** 1033–1044.

Lortie A., Chiron C., Mumford J., and Dulac O. (1993) The potential for increasing seizure frequency, relapse, and appearance of new seizure types with vigabatrin. *Neurology* **43,** S24–S27.

Löscher W. and Frey H. H. (1987) One to three day dose intervals during subchronic treatment of epileptic gerbils with gamma-vinyl GABA: anticonvulsant efficacy and alterations in regional brain GABA levels. *Eur. J. Pharmacol.* **143,** 335–342.

Löscher W. and Schmidt D. (1994) Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? *Epilepsy Res.* **17,** 95–134.

Löscher W., Jäckel R., and Müller F. (1989) Anticonvulsant and proconvulsant effects of inhibitors of GABA degradation in the amygdala-kindling model. *Eur. J. Pharmacol.* **163,** 1–14.

Lu D., Margouleff C., Rubib E., Labar D., Schaul N., Ishikawa T., Kazumata K., Antonini A., Dhawan V., Hyman R. A., and Eidelberg D. (1997) Temporal lobe epilepsy: correlation of proton magnetic resonance spectroscopy and ^{18}F Fluorodeoxyglucose positron emission tomography. *Magn. Reson. Med.* **37,** 18–23.

Macdonald R. L. and Meldrum B. S. (1995) Principles of antiepileptic drug action, in *Antiepileptic Drugs*, 4th ed. (Levy R. H., Mattson R. H., and Meldrum B. S., eds.) Raven, New York, pp. 61–77.

Maloteaux J. M, Octave K. N., Gossuin A., Laterre C., and Trouet A. (1987) GABA induces down-regulation in the benzodiazepine-GABA receptor complex in rat cultured neurons. *Eur. J. Pharmacol.* **144,** 173–183.

Manor D., Rothman D. L., Mason G. F., Hyder F., Petroff O. A. C., and Behar K. L. (1996) The rate of turnover of cortical GABA from [1-13C] glucose is reduced in rats treated with the GABA-transaminase inhibitor vigabatrin (gamma-vinyl GABA). *Neurochem. Res.* **21,** 1037–1047.

Martin D. L. (1987) Regulatory properties of brain glutamate decarboxylase. *Cell Mol. Neurobiol.* **7,** 237–253.

Martin D. L. (1992) Synthesis and release of neuroactive substances by glial cells. *Glia* **5,** 81–94.

Martin D. L. (1995) The role of glia in the inactivation of neurotransmitters, in *Neuroglia* (Kettenmann H. and Ransom B. R., eds.) Oxford University Press, New York, pp. 732–745.

Martin D. L. and Rimvall K. (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J. Neurochem.* **60,** 395–407.

Mason G. F., Gruetter R., Rothman D. L., Behar K. L., Shulman R. G., and Novotny E. J. (1995) Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J. Cereb. Blood Flow Metab.* **18,** 12–25.

Matthews P. M., Andermann F., and Arnold D. L. (1990) A proton magnetic resonance spectroscopic study of focal epilepsy in humans. *Neurology* **40,** 985–989.

Mhartre M. C. and Ticku M. K. (1994) Chronic GABA treatment downregulates the $GABA_A$ receptor $alpha_2$ and $alpha_3$ subunit mRNAs as well as polypeptide expression in primary cultured cerebral cortical neurons. *Brain Res. Mol. Brain Res.* **24,** 159–165.

Neal M. J., Cunningham J. R., Shah M. A., and Yazulla S. (1989) Immunocytochemical evidence that vigabatrin in rats causes GABA accumulation in glial cells of the retina. *Neurosci. Lett.* **98,** 29–32.

Neal M. J. and Shah M. A. (1990) Development of tolerance to the effects of vigabatrin (gamma-vinyl-GABA) on GABA release from the rat cerebral cortex, spinal cord and retina. *Br. J. Pharmacol.* **100,** 324–328.

Ottersen O. P., Zhang N., and Walberg F. (1992) Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. *Neuroscience* **46,** 519–534.

Peeling J. and Sutherland G. (1993) ^1H magnetic resonance spectroscopy of extracts of human epileptic neocortex and hippocampus. *Neurology* **43,** 589–594.

Peng L., Hertz L., Huang R., Sonnewald U., Petersen S. B., Wetergaard N., Larsson O., and Schousboe A. (1993) Utilization of glutamine and TCA cycle constituents as precursors for transmitter glutamate and GABA. *Dev. Neurosci.* **15,** 367–377.

Penry J. K., Wilder B. J., and Sachdeo R. C. (1993) Multicenter dose-response study of vigabatrin in adults with focal (partial) epilepsy (abstract). *Epilepsia* **34(Suppl. 6),** 67.

Perry T. (1982) Cerebral amino acid pools, in *Handbook of Neurochemistry*, 2nd ed., vol. 1, (Lajtha A., ed.), Plenum Press, New York, pp. 151–180.

Perry T. L., Hansen S., and Gandham S. S. (1981) Postmortem changes of amino compounds in human and rat brain. *J. Neurochem.* **36,** 406–412.

Petroff O. A. C., Behar K. L., and Rothman D. L. (1997) Measuring brain GABA in patients with complex partial seizures, in *Jasper's Basic Mechanisms of the Epilepsies* (Delgado-Escueda A. V., Wilson W., Olsen R. W., and Porter R. J., ed.) Lippincott-Raven, Philadelphia (in press).

Petroff O. A. C., Rothman D. L., Behar K. L., Lamoureux D., and Mattson R. H. (1996a) The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. *Ann. Neurol.* **39,** 95–99.

Petroff O. A. C., Rothman D. L., Behar K. L., and Mattson R. H. (1996b) Human brain GABA levels rise following initiation of vigabatrin therapy, but fail to rise further with increasing dose. *Neurology* **46,** 1459–1463.

Petroff O. A. C., Rothman D. L., Behar K. L., and Mattson R. H. (1996c) Low brain GABA level is associated with poor seizure control. *Ann. Neurol.* **40,** 908–911.

Petroff O. A. C., Rothman D. L., Behar K. L., Collins T. L., and Mattson R. H. (1996d) Human brain GABA levels rise rapidly following initiation of vigabatrin therapy. *Neurology* **47,** 1567–1571.

Petroff O. A. C, Rothman D. L., Behar K. L., and Mattson R. H. (1996e) Human brain gamma-aminobutyric acid (GABA) levels and seizure control following initiation of vigabatrin therapy. *J. Neurochem.* **67,** 2399–2404.

Petroff O. A. C., Rothman D. L., Behar K. L., and Mattson R. H. (1995a) Initial observations on the effect of vigabatrin on the in vivo ^1H spectroscopic measurements of GABA, glutamate, and glutamine in human brain. *Epilepsia* **36,** 457–464.

Petroff O. A. C., Pleban L. A., and Spencer D. D. (1995b) Symbiosis between *in vivo* and *in vitro* NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of epileptic human brain. *Magn. Reson. Imag.* **13,** 1197–1211.

Petroff O. A. C., Spencer D., Alger J. R., and Prichard J. W. (1989) High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. *Neurology* **39,** 1197–1202.

Pitkänen A., Ylinen A., and Matilainen R. (1993) Long-term antiepileptic efficacy of vigabatrin in drug-refractory epilepsy in mentally retarded patients: a 5 year follow-up study. *Arch. Neurol.* **50,** 24–29.

Reikkinen P. J., Pitkänen A., Ylinen A., Sivenius J., and Halonen T. (1989a) Specificity of vigabatrin for the GABAergic system in human epilepsy. *Epilepsia* **30(Suppl. 3),** S18–S22.

Reikkinen P. J., Ylinen A., Halonen T., Sivenius J., and Pitkänen A. (1989b) Cerebrospinal fluid GABA and seizure control with vigabatrin. *Br. J. Clin. Pharmacy* **27(Suppl. 1),** 87S–94S.

Rimvall K. and Martin D. L. (1994) The level of GAD67 protein is highly sensitive to small in-

creases in intraneuronal gamma-aminobutyric acid levels. *J. Neurochem.* **62,** 1375–1381.

Roberts E. (1986) Failure of GABAergic inhibition: a key to local and global seizures. *Adv. Neurol.* **44,** 319–341.

Roberts E. (1988) The establishment of GABA as a neurotransmitter, in *GABA and Benzodiazepines Receptors*, vol. 1. (Squires R. F., ed.) CRC Press, Boca Raton, FL., pp. 1–21.

Roberts E. and Frankel S. (1950) Gamma-aminobutyric acid in brain: its formation from glutamic acid. *J. Biol. Chem.* **187,** 55–63.

Rothman D. L., Behar K. L. , Hetherington H. P., and Shulman R. G. (1984) Homonuclear ^{1}H-double resonance difference spectroscopy of the rat brain in vivo. *Proc. Natl. Acad. Sci. USA* **81,** 6330–6334.

Rothman D. L., Hanstock C. C., Petroff O. A. C., Novotny E. J., Prichard J. W., and Shulman R. G. (1992) Localized ^{1}H NMR measurements of glutamate in the human brain. *Magn. Reson. Med.* **25,** 94–106.

Rothman D. L., Petroff O. A. C., Behar K. L., and Mattson R. H. (1993) Localized ^{1}H NMR measurements of GABA levels in human brain in vivo. *Proc. Natl. Acad. Sci. USA* **90,** 5662–5666.

Schechter P. J. (1989) Clinical pharmacology of vigabatrin. *Br. J. Clin. Pharmacy* **27(Suppl. 1),** 19S–22S.

Schechter P. J. and Sjoerdsma A. (1990) Clinical relevance of measuring GABA concentrations in cerebrospinal fluid. *Neurochem. Res.* **15,** 419–423.

Schechter P. J., Hanke N. F. J., Grove J., Huebert N., and Sjoerdsma A. (1984) Biochemical and clinical effects of gamma-vinyl-GABA in patients with epilepsy. *Neurology* **34,** 182–186.

Schmid L., Bottlaender M., Brouillet E., Fuseau C., and Mazière M. (1996) Vigabatrin modulates benzodiazepine receptor activity in vivo: a positron emission tomography study in baboon. *J. Pharmacol. Exptl. Ther.* **276,** 977–983.

Schousboe A., Larsson O. M., and Seiler N. (1986) Stereoselective uptake of the GABA-transaminase inhibitors gamma-vinyl GABA and gamma-acetylenic GABA into neurons and astrocytes. *Neurochem. Res.* **11,** 1497–1505.

Schousboe A., Westergaard N., Sonnewald U., Petersen S. B., Yu A. C. H., and Hertz L. (1992) Regulatory role of astrocytes for neuronal biosynthesis and homeostasis of glutamate and GABA. *Prog. Brain Res.* **94,** 199–211.

Schousboe A., Westergaard N., Sonnewald U., Petersen S. B., Huang R., Peng L., and Hertz L. (1993) Glutamate and glutamine metabolism and compartmentation in astrocytes. *Dev. Neurosci.* **15,** 359–366.

Schousboe A. and Westergaard N. (1995) Transport of neuroactive amino acids in astrocytes, in *Neuroglia* (Kettenmann H. and Ransom B. R., eds.) Oxford University Press, New York, pp. 246–258.

Sellstrom A., Sjoberg L. B., and Hamberger A. (1975) Neuronal and glial systems for gamma-aminobutyric acid metabolism. *J. Neurochem.* **25,** 393–398.

Shank R. P., Leo G. C., and Zielke H. R. (1993) Cerebral metabolic compartmentation as revealed by nuclear magnetic resonance analysis of D-[1-13C]glucose metabolism. *J. Neurochem.* **61,** 315–323.

Sherwin A. L. and van Gelder N. M. (1986) Amino acid and catecholamine markers of metabolic abnormalities in human focal epilepsy. *Adv. Neurol.* **44,** 1011–1032.

Sherwin A., Robitaille Y., Quesney F., Olivier A., Villemure J., Leblanc R., Feindel W., Andermann E, Gotman J., Andermann F., Ethier R., and Kish S. (1988) Excitatory aminoacids are elevated in human epileptic cerebral cortex. *Neurology* **38,** 920–923.

Sonnewald U., Westergaard N., Schousboe A., Svendsen J. S., Unsgard G., and Petersen S. B. (1993) Direct demonstration by [^{13}C]NMR spectroscopy that glutamine from astrocytes is a precursor for GABA synthesis in neurons. *Neurochem. Int.* **22,** 19–29.

Spencer S. S., Theodore W. H., and Berkovic S. F. (1995) Clinical applications: MRI, SPECT, and PET. *Magn. Reson. Imag.* **13,** 1119–1124.

Spencer S. S. (1996) Long-term outcome after epilepsy surgery. *Epilepsia* **37,** 807–813.

Staley K. J., Soldo B. L., and Proctor W. R. (1995) Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science* **269,** 977–981.

Storm-Mathisen J., Ottersen O. P., Fu-Long T., Gunderson V., Laake J. H., and Nordbo G. (1986) Metabolism and transport of amino acids studied by immunocytochemistry. *Med. Biol.* **64,** 127–132.

Tasker J. G. and Dudek F. E. (1991) Electrophysiology of GABA-mediated synaptic transmission and possible roles in epilepsy. *Neurochem. Res.* **16,** 251–262.

Taylor C. P. (1994) Emerging perspectives on the mechanism of action of gabapentin. *Neurology* **44(Suppl. 5),** S10–S16.

Tunnicliff G. and Raess B. U. (1991) GABA neurotransmitter activity in human epileptogenic brain, in *GABA Mechanisms in Epilepsy* (Tunnicliff G. and Raess B. U., eds.) Wiley-Liss, New York, pp. 105–119.

van Donselaar C. A., Schimsheimer R., Geerts A. T., and Declerck A. C. (1992) Value of the electroencephalogram in adult patients with untreated first seizure. *Arch. Neurol.* **49,** 231–237.

Vaughan T., and Pohost G. (1995) Proton nuclear magnetic resonance spectroscopic imaging of human temporal lobe epilepsy at 4.1 T. *Ann. Neurol.* **38,** 396–404.

Ward H. K., Thanki C. M., and Bradford H. F. (1983) Glutamine and glucose as precursors of transmitter amino acids: ex vivo studies. *J. Neurochem.* **40,** 855–860.

Wiesinger H. (1995) Glia-specific enzyme systems, in *Neuroglia,* (Kettenmann H. and Ransom B. R., eds.) Oxford University Press, New York, pp. 488–499.

Wood J. D. and Davies M. (1991) Regulation of the $GABA_A$ receptor/ion channel complex by intracellular GABA levels. *Neurochem. Res.* **16,** 375–379.

Wood J. D. and Sidhu H. S. (1986) Uptake of gamma-aminobutyric acid by brain tissue preparations: a re-evaluation. *J. Neurochem.* **46,** 739–744.

Wood J. H., Hare T. A., Glaeser B. S., Ballenger J. C., and Post R. M. (1979) Low cerebrospinal fluid gamma-aminobutyric acid content in seizure patients. *Neurology* **29,** 1203–1208.