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Effects of valproate and other antiepileptic drugs on brain glutamate, glutamine, and GABA in patients with refractory complex partial seizures

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Preclinical studies suggested valproate increased brain γ-aminobutyric acid (GABA) with no major effects on brain glutamate or glutamine. Valproate increased human cerebrospinal fluid GABA and glutamine in some studies; others reported no effect. *In vivo* measurements of glutamate, glutamine, and GABA were made of a 14 cm^3 volume in the occipital cortex using a ^1H spectroscopy with a 2.1 Tesla magnetic resonance spectrometer and an 8 cm surface coil. Ten control subjects and 14 patients with refractory complex partial seizures were examined. Brain glutamine concentrations were above normal in three of five patients taking valproate and two of nine taking carbamazepine or phenytoin. Mean glutamine levels of patients taking valproate were higher than control subjects and patients taking carbamazepine or phenytoin. Brain glutamate concentrations were above normal in four of nine patients taking phenytoin or carbamazepine and two of five taking valproate. Brain GABA levels were below normal in four of nine patients taking carbamazepine or phenytoin and one of five taking valproate. Above normal glutamate or below normal GABA was present in nine of 14 patients and may contribute to their refractory epilepsy. Increased brain glutamine associated with valproate therapy may reflect mild hyperammonemia.

Key words: glutamine; glutamate; human; epilepsy; valproate; ^1H nuclear magnetic resonance spectroscopy.

INTRODUCTION

Carbamazepine, phenytoin, and valproate all treat complex partial seizures effectively[1,2]. Carbamazepine and phenytoin are not thought to have a direct GABAergic mechanism of action[3,4]. These antiepileptic drugs (AED) do not increase CSF or brain GABA significantly in adult patients with epilepsy[5–7].

The initial preclinical studies in rodents of the neurochemical effects of valproate suggested a GABAergic anticonvulsant mechanism[8–11]. Large doses (400 mg/kg) of valproate increased rat whole brain GABA by 46% without significant changes in aspartate, glutamate, glutamine, or glycine[8]. Studies of epileptic children and adults treated with valproate reported increased GABA concentrations in cerebrospinal fluid (CSF)[12,13]. No significant alterations in CSF GABA levels were seen in adult patients with epilepsy, Parkinsonism, or schizophrenia treated with valproate[14–16].

The effect of valproate on glutamate and glutamine concentrations appears dependent on the species and dose studied[9,10,17]. Brain glutamate concentrations were reported to be increased, decreased, and unchanged in rodents treated with valproate[9,10,17–19]. Increased concentrations of glutamine were reported in mouse brain, rat cortex, and baboon CSF[17–20]. Valproate was reported to increase CSF glutamine in children but not in adults[14,21].

In vivo measurements of brain glutamate, glutamine, and GABA in patients with epilepsy were made possible by advances in nuclear magnetic resonance (NMR) spectroscopy[22–25]. We describe our initial experience in making combined measurements of glutamate, glutamine, and GABA in patients taking valproate, phenytoin, or carbamazepine.

MATERIALS AND METHODS

Five men and five women volunteered as medication free, control subjects. The mean age of the nonepileptic subjects was 36 years (SD 9, range 26–58). Glutamine, glutamate, and GABA levels were

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measured in 14 patients (9 women) with complex partial seizures (Table 1). The mean age of the patients was 36 years (SD 8, range 25–53). Antiepileptic drugs used included carbamazepine monotherapy (4 patients), phenytoin monotherapy (4), phenytoin with carbamazepine (1), valproate monotherapy (2), and valproate with carbamazepine (3). All patients were evaluated extensively by the Yale Epilepsy Program and suffered from refractory complex partial seizures. None showed an occipital focus on electroencephalogram. Patients kept a seizure diary. Prior to each spectroscopic measurement, patients were interviewed concerning seizure frequency and side effects of the medications. All subjects gave informed consent for the study which was approved by the Yale Human Investigations Committee.

Studies were performed at Yale Medical School with a 2.1 Tesla (89.43 MHz for ^{1}H) Oxford Magnet Technologies 1 meter bore magnet equipped with a modified Biospec spectrometer (Bruker Instruments, Billerica, MA, USA) and OMT shielded gradients and power supplies (Oxford Magnetic Technology, Oxford, UK). Each subject's head was positioned in an adjustable holder (designed for minimal motion and maximal comfort) such that a flat surface spoiler and an 8 cm distributed capacitance ^{1}H transceiver radio-frequency (RF) coil lay just below the occipital cortex. Prior to the ^{1}H spectroscopy measurement a gradient echo image of the subject's brain was obtained. From the image, a $3.0 \times 1.5 \times 3.0$ cm (14 cm^3) volume in the occipital cortex was chosen for spectroscopic measurements. An automated shimming routine was used to optimize the static (B_0) field homogeneity in the sensitive volume[26].

Glutamine and glutamate measurements were made using macromolecule suppressed, short echo time (TE) spectra[24,27]. Localization was achieved by a 3D-ISIS sequence, outer volume suppression, selective excitation, and a surface spoiler coil. An inversion recovery pulse and a semiselective refocusing pulse were used for water suppression. The TE time was 17 ms with the semiselective refocusing pulse optimum at 2.30 ppm and 7.16 ppm, repetition time of 4.1 s, and an inversion recovery delay of 1.03 s. 'Metabolite-nulled' spectra were obtained by a broadband inversion recovery sequence which suppresses all metabolites with a long T_1. Subtraction of the 'metabolite-nulled' (short T_1 macromolecule resonances only) spectrum from the full short-TE spectrum results in a flat spectral baseline (Fig. 1). Other spectral acquisition conditions included a sweep width of 2500 Hz and acquisition time of 410 ms.

Glutamine and glutamate concentrations were measured as previously described[22,24]. *In vivo* time domain data were zero filled to 32 768 points. Prior to Fourier transformation, time domain spectra were filtered with a -2 Hz exponential function followed by a 7.5 Hz Gaussian window. The processing added approximately 1 Hz to the natural linewidth. After Fourier transformation, a zero-order phase correction was performed. After subtraction of the metabolite nulled spectrum, a zero- and first-order baseline correction from 4.5 to -0.5 ppm and 10 to 5.5 ppm was performed. Spectra were scaled by the amplitude of the creatine resonance. The ^{1}H spectral region between 1.6 and 3.1 ppm was deconvoluted using proprietary software written in MATLAB. The coefficients for a system of equations needed to estimate metabolite concentrations were determined from localized spectra of model solutions (phantoms) containing glutamate, glutamine, GABA, creatine, aspartate, *N*-acetylaspartate, succinate, and other brain metabolites[22,24]. Using these input functions, the *in vivo* spectrum was modeled as a linear sum of these signals. A Gaussian function with the same linewidth as creatine in the subject's spectrum was used to apodize the spectra obtained from the

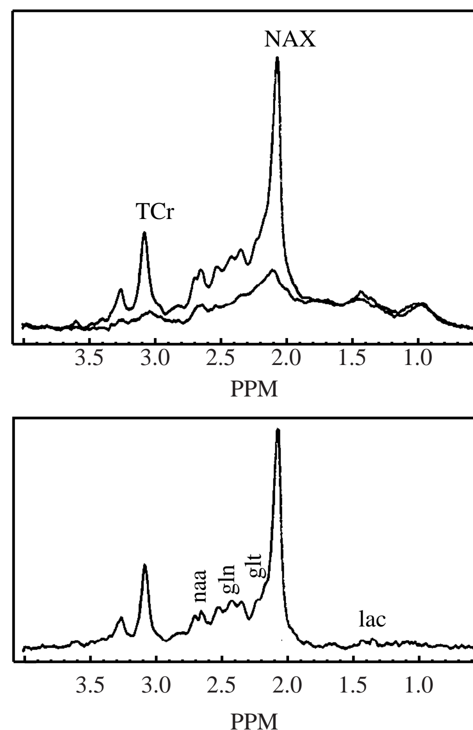

Fig. 1: A short-TE spectrum with and without the 'metabolite-nulled' inversion recovery sequence was obtained in the same volume as for the GABA measurement (top). Subtracting the short T_1 weighted spectrum from the unweighted spectrum yields a spectrum (bottom) containing little or no signals originating from macromolecule resonances. The remaining signals are all from small molecules, metabolites, which have long T_1 relaxation times. The flat baseline, free of signals from macromolecules with short T_1 relaxation times, allows the metabolite signals to be analyzed quantitatively. Abbreviations used: *N*-acetylaspartate and other signals at 2.0 ppm (NAX), creatine, phosphocreatine, and other signals at 3.0 ppm (TCr), *N*-acetylaspartate (naa), glutamine (gln), glutamate (glt), and lactate (lac).

Table 1:

ID	Age yr	Onset epilepsy yr	Gender	Frequency of seizures in past month	Daily dose of antiepileptic drugs	Blood levels of drugs mg/l
a.	34	20	Female	30	Carbamazepine 1000 mg	11
b.	38	28	Female	30	Carbamazepine 1200 mg	6
c.	36	19	Male	0	Carbamazepine 1200 mg	9
d.	29	2	Female	30	Carbamazepine 500 mg	13
e.	52	18	Male	8	Carbamazepine 2000 mg	11
					Phenytoin 500 mg	11
f.	29	23	Male	30	Phenytoin 700 mg	16
g.	38	32	Male	0	Phenytoin 400 mg	19
h.	53	6	Female	6	Phenytoin 300 mg	9
i.	39	14	Female	0	Phenytoin 300 mg	8
j.	34	5	Female	1	Carbamazepine 1200 mg	11
					Valproate 1250 mg	51
k.	36	12	Female	2	Carbamazepine 1600 mg	9
					Valproate 1500 mg	52
l.	25	12	Male	3	Carbamazepine 800 mg	10
					Valproate 1500 mg	103
m.	25	16	Female	4	Valproate 3000 mg	78
n.	34	21	Female	15	Valproate 1500 mg	63

pure compounds. This method is similar to that used by Provencher[28] except that the fit is in the frequency domain and macromolecules are suppressed by the pulse sequence. The mean value of all control subjects was assigned the concentrations of glutamine (4.2 mM), glutamate (9.1 mM), and GABA (1.2 mM) for the occipital lobe measured using ^{13}C spectroscopy and an external concentration reference[29].

Homonuclear editing of the 3.0 ppm C4 GABA resonance was performed using the spin–spin (J) editing pulse sequence described previously[23, 24]. The localization techniques were 3D-ISIS sequence, outer volume suppression, selective excitation, and surface spoiler coil. An inversion recovery pulse and a semiselective refocusing pulse was used for water suppression. Spectral editing was used to separate the GABA C4 resonance at 3.0 ppm from overlapping resonances by applying a 26.5 ms DANTE pulse applied symmetrically in time about the center of the sequence to improve editing selectivity to the 1.9 ppm C3 resonance. Spectral acquisition conditions were TR 3.39 s, TE 68 ms, sweep width 2500 Hz, and acquisition time 410 ms.

In the edited spectra, GABA was measured as previously described[23, 25]. *In vivo* time domain data were zero filled to 32 768 points and apodized with a 3 Hz exponential function prior to Fourier transformation. The GABA resonance at 3.0 ppm in the ^{1}H NMR edited spectrum were integrated over a limited bandwidth and compared with the limited bandwidth integrated resonance of creatine obtained in the same measurement set. Coefficients needed to convert the GABA/creatine ratios to concentrations were measured in model solutions[23, 30].

Student's t-test two-tailed probability distribution tables were used to calculate a 95% confidence interval (95% CI) of the group mean difference. Tables based on the binomial distribution were used to obtain the 95% CI for population medians[31]. When multiple measurements for an individual were available, the mean value was used.

RESULTS

There were no significant gender or age related differences in brain glutamine levels of control subjects. Brain glutamine concentrations were above normal in three of five patients taking valproate (Fig. 2). Mean glutamine levels of patients taking valproate (5.7 mM, 95% CI 4.4–6.9, $n = 5$) were higher than control subjects (4.2 mM, 95% CI 4.0–4.4, $n = 10$, $P < 0.01$) and patients taking carbamazepine or phenytoin (4.2 mM, 95% CI 3.9–4.6, $n = 9$, $P < 0.01$).

There were no significant gender or age related differences in glutamate levels among control subjects. Brain glutamate concentrations were above normal in 43% of patients including two patients taking valproate (Fig. 3). Mean glutamate was higher in patients taking carbamazepine or phenytoin (9.9 mM, 95% CI 9.3–10.5, $n = 9$, $P < 0.05$) than in control subjects (9.2 mM, 95% CI 9.0–9.5, $n = 10$). One patient taking valproate had below normal glutamate.

There were no gender or age related differences in occipital lobe GABA levels of our control subjects. Brain GABA levels were below normal in 36% of patients with epilepsy (Fig. 4). Mean GABA was lower in patients taking carbamazepine or phenytoin (1.0 mM, 95% CI 0.8–1.2, $n = 9$) than in control subjects (1.2 mM, 95% CI 1.1–1.3, $n = 10$, $P < 0.05$). There were no significant differences in mean or median GABA concentrations between patients treated with valproate and carbamazepine or phenytoin.

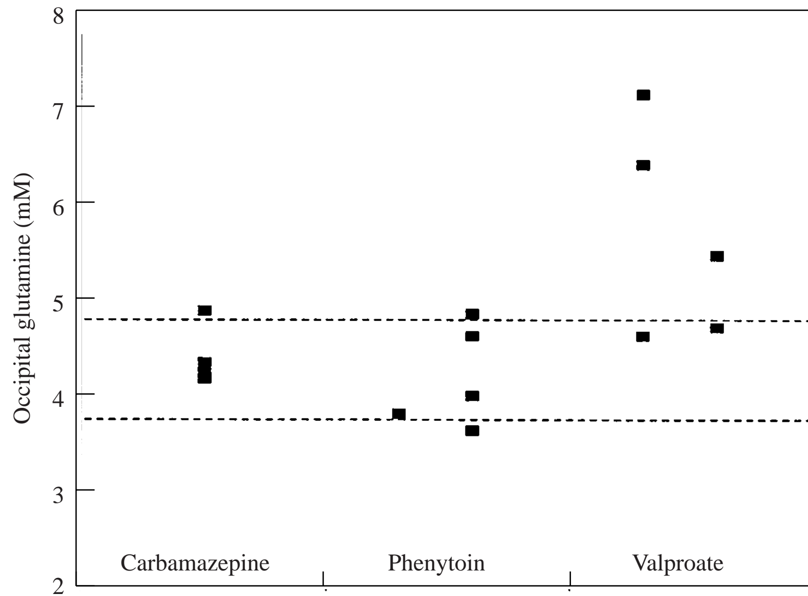

Fig. 2: Valproate increased human occipital lobe glutamine. Brain glutamine was above normal in 60% of patients taking valproate, 20% taking phenytoin, and 25% taking carbamazepine. The dashed lines indicate two standard deviations (± 2 SD) above and below the mean of 10 normal subjects. Four patients on carbamazepine monotherapy, four on phenytoin monotherapy (to the right in the phenytoin column) plus one on phenytoin and carbamazepine (to the left), and two patients on valproate monotherapy (to the right in the valproate column) plus three on combined therapy with carbamazepine (to the left) were examined.

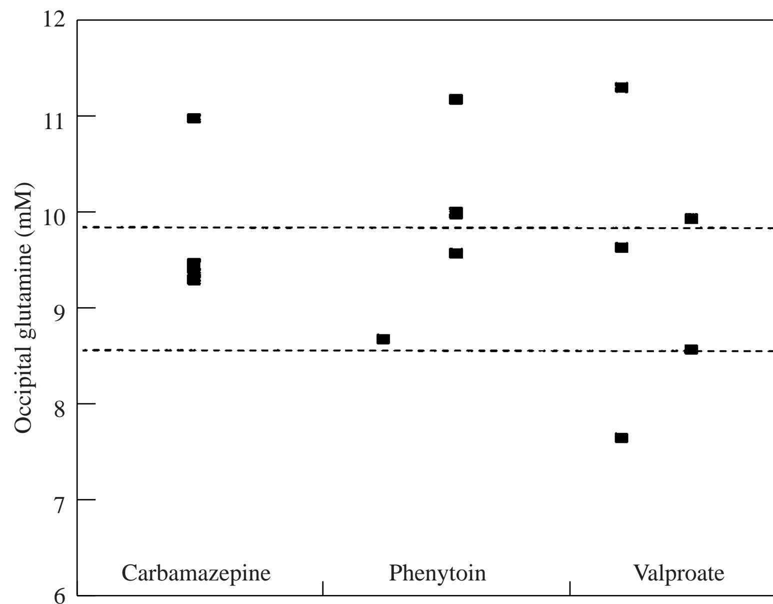

Fig. 3: Brain glutamate was above normal in 44% of patients taking carbamazepine or phenytoin and 40% of those taking valproate or valproate and carbamazepine. The dashed lines indicate two standard deviations (± 2 SD) above and below the mean of 10 normal subjects.

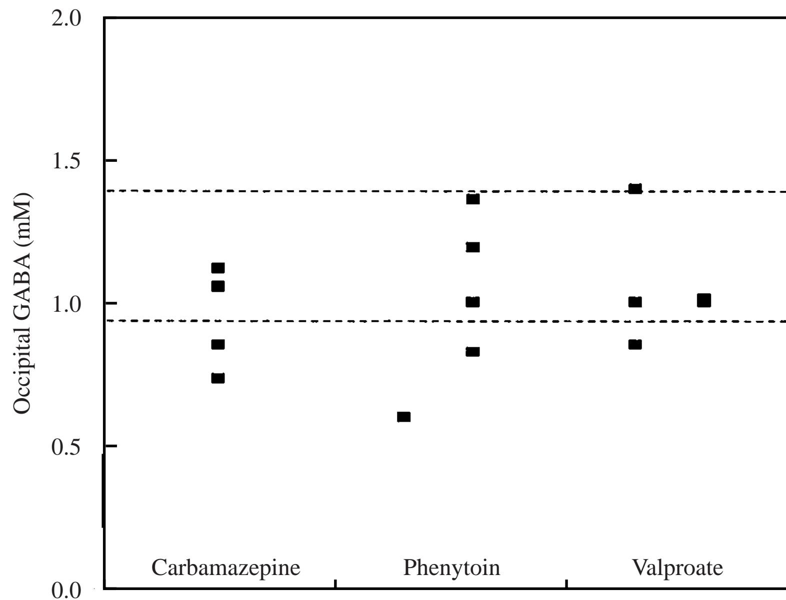

Fig. 4: Human occipital lobe GABA concentrations are decreased in 36% of patients with complex partial seizures. Brain GABA was below normal in 50% of patients taking carbamazepine, 40% taking phenytoin, and 20% taking valproate. The dashed lines indicate two standard deviations (± 2 SD) above and below the mean of 10 normal subjects.

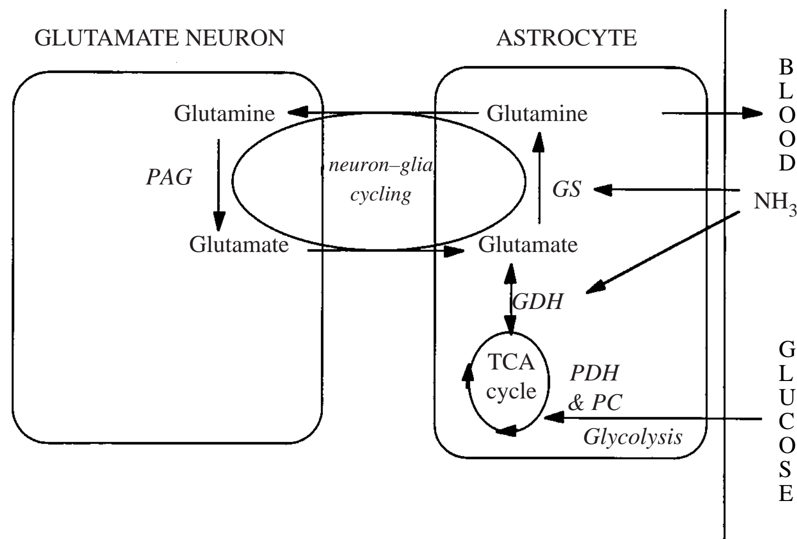

Fig. 5: Schematic of glutamate–glutamine cycling between neurons and glia. Neuronal glutamate is lost during glutamate transmitter release. In glutamatergic neurons, the continuing loss of glutamate would deplete mitochondria of critical TCA cycle intermediates and lead to neuronal energy failure, possible mitochondrial damage, and potential neuronal apoptosis[52–54]. The metabolic pathway through phosphate activated glutaminase (PAG), primarily localized in neurons, supplies the needed carbon to maintain the TCA cycle intermediates[37, 52, 55]. The flow through PAG is proportional to neuron–glia cycling and controls neuronal glutamate concentrations[50, 52, 55]. The action of glutamate in the synaptic cleft is terminated by glial uptake mechanisms. Glutamine synthetase (GS), exclusively found in glia, is critical in recycling the carbon skeletons lost from neurons through neurotransmitter release. The rate of glutamine synthesis is proportional to the rate of ammonia (NH_3) detoxification and neuron–glia cycling[37, 52]. Aspartate aminotransferase and glutamate dehydrogenase (GDH) catalyze the rapid equilibrium between glutamate and α-ketoglutarate. GDH is enriched in glia. The glycolytic and pyruvate dehydrogenase (PDH) pathways from glucose feed substrate into the tricarboxylic acid (TCA) cycle providing energy. However, this metabolic pathway cannot replenish TCA cycle intermediates (anaplerosis). The anaplerotic pathway from glucose through pyruvate carboxylase (PC) to glutamate is active in glia exclusively and replenishes TCA cycle intermediates lost when glutamine is exported to neurons and blood.

The median monthly seizure frequency at the time of the spectroscopy was eight (95% CI 0.3–30, $n = 9$) for the group taking carbamazepine or phenytoin and three (range 1–15, $n = 5$) for the group taking valproate. Patients taking carbamazepine or phenytoin had the best and the worst seizure control. Three of four patients with daily seizures had below normal GABA. Mean GABA was lower in patients with daily seizures (0.8 mM, 95% CI 0.7–1.1, $n = 4$) than patients with fewer than one seizure a month (1.2 mM, 95% CI 0.8–1.5, $n = 3$, $P < 0.05$). Two of four patients with daily seizures had above normal brain glutamate. However, mean glutamate was not significantly higher with daily seizures (10.3 mM, 95% CI 9.0–11.6, $n = 4$) than with better control (9.7 mM, 95% CI 8.9–10.4, $n = 3$). Mean brain glutamine was the same for both groups (daily seizures 4.5 mM, 95% CI 4.1–4.9, $n = 4$; best control 4.2, 95% CI 2.9–5.5, $n = 3$).

DISCUSSION

Valproate did not increase significantly GABA concentrations in the occipital lobe of adult patients with complex partial seizures. There was no difference in brain GABA between patients taking carbamazepine or phenytoin and patients taking valproate. Brain GABA in many animal models increases at higher doses of valproate[9,10,17]. The median daily dose taken by our patients (22 mg/kg, range 18–33, $n = 5$) was low compared to the doses used in animal models. Of the newer AEDs, gabapentin, topiramate, and vigabatrin clearly increase brain GABA in epilepsy patients with doses used clinically[25,32–35]. Topiramate, gabapentin, and lamotrigine increase brain GABA in healthy subjects[36]. Curiously, only vigabatrin significantly raises brain GABA in rodent models.

Valproate increased brain glutamine in 60% of patients. The rate of glutamine synthesis is limited primarily by its substrates, glutamate and ammonia[37–39]. Raised ammonia levels increase glutamine synthesis and decrease catabolism, thereby raising glutamine concentrations. Hyperammonemia is a common consequence of valproate administration[40]. Valproate increases production of ammonia in human kidney and increases glutamine removal[41]. Valproate also inhibits the activity of the urea cycle and can unmask partial defects in various enzymes[42,43]. Whether our patients had asymptomatic defects in urea cycle enzymes unmasked by valproate or merely asymptomatic mild hyperammonemia is unknown. None had clinical signs of hepatic or renal disease and had normal findings on routine blood work. Blood ammonia was not tested. Elevated CSF glutamine was reported in children treated with valproate or barbiturates and adults treated with phenobarbital[6,21,44]. Adult patients treated with vigabatrin also had above normal brain glutamine[24]. Whether the metabolic changes associated with increased glutamine contribute to improved seizure control or deleterious side-effects remains to be investigated.

Glutamate concentrations are higher in epileptic human cortex[45–47]. A third of our patients had above normal glutamate in cortex remote from the seizure focus. Two thirds, nine of 14, had either above normal glutamate or below normal GABA. Low brain GABA levels are associated with frequent seizures[7]. Either condition could contribute to increased cerebral excitability[3,4,35,48].

Elevated serum ammonia may have an impact on seizure control through its effects on cerebral glutamate homeostasis. Brain glutamate concentrations are influenced primarily by glutamate concentrations within glutamatergic neurons[49,50]. Significant changes in the glial glutamate concentrations would be difficult to detect. Glutamate levels in astrocytes (<1 mM) are far lower than those found in glutamatergic neurons. It is within the range of the Michaelis–Menten constant of glutamine synthetase which would allow the rate of glutamine synthesis to respond to an increase in glial glutamate concentration[37–39]. Glia efficiently remove glutamate released by neurons and terminate the action of released glutamate (Fig. 5). Inhibition of glutamine synthesis produces seizures by raising both glial and extracellular glutamate concentrations[45]. Increased blood ammonia is thought to lower glial glutamate concentrations significantly while raising glutamine concentrations[38,39,51]. Decreased glial glutamate would facilitate glutamate uptake, potentially making the brain less excitable.

CONCLUSIONS

Valproate does not increase significantly brain GABA in patients with epilepsy. Increased glutamine may reflect mild hyperammonemia associated with valproate therapy. Below normal GABA or above normal glutamate was present in 64% of patients with refractory complex partial seizures and may contribute to poor seizure control.

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