Published in final edited form as:

J Neural Transm. 2009 March ; 116(3): 291–300. doi:10.1007/s00702-009-0186-0.

Elevated Endogenous GABA Concentration Attenuates Glutamate-Glutamine Cycling between Neurons and Astroglia

Jehoon Yang^{1,2} and Jun Shen¹

¹ Molecular Imaging Branch, National Institute of Mental Health Intramural Research Program, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD, USA

² Samsung Biomedical Research Institute, 50 Ilwon-dong, Kangnam-Gu, Seoul, Korea

Abstract

In this study, the relationship between endogenous brain GABA concentration and glutamate-glutamine cycling flux (V_{cyc}) was investigated using in vivo 1H and $^1H\{^{13}C\}$ magnetic resonance spectroscopy techniques. Graded elevations of brain GABA levels were induced in rat brain after administration of the highly specific GABA-transaminase inhibitor vigabatrin (γ -vinyl-GABA). The glial-specific substrate $[2-^{13}C]acetate$ and $^1H\{^{13}C\}$ magnetic resonance spectroscopy were used to measure V_{cyc} at different GABA levels. Significantly reduced V_{cyc} was found in rats pretreated with vigabatrin. The reduction in group mean V_{cyc} over the range of GABA concentrations investigated in this study ($1.0 \pm 0.3 \sim 5.1 \pm 0.5 \mu\text{mol/g}$) was found to be nonlinear: $\Delta V_{cyc}/V_{cyc} = [GABA (\mu\text{mol/g})]^{-0.35} - 1.0$ ($r^2 = 0.98$). The results demonstrate that V_{cyc} is modulated by endogenous GABA levels, and that glutamatergic and GABAergic interactions can be studied in vivo using noninvasive magnetic resonance spectroscopy techniques.

Keywords

GABA; vigabatrin; glutamate; magnetic resonance spectroscopy

Introduction

Postmortem biochemical analyses suggest that, across the human brain, differences in endogenous γ -aminobutyric acid (GABA) concentrations can vary as much as 20-fold (Banay-Schwartz et al, 1993). The functional significance of this heterogeneous distribution of brain GABA has yet to be fully elucidated. GABA-elevating drugs such as vigabatrin, topiramate, and gabapentin have been shown to increase brain GABA concentrations and are used to treat epilepsy (Petroff et al, 1999; Kuzniecky et al, 2002; McDonald et al, 2005), and their anxiolytic and mood-stabilizing effects have also been recognized (Post et al, 1998; Kendell et al, 2005). A large amount of evidence exists noting increased GABA efflux after administration of GABA-elevating anticonvulsive drugs from studies using brain slices (Jackson et al, 2000), synaptosomes (Wood et al, 1988), and in vivo microdialysis (Qume et al, 1995; Smolders et al, 1997; Pierard et al, 1999). This increased GABA efflux has been attributed to a Ca^{2+} -independent nonvesicular mechanism mediated by the reversal of GABA transporters (Wu et al, 2003).

Functional brain activity is primarily composed of an interplay between excitation and inhibition. In any given brain region, output is based on a complex processing of incoming signals requiring both excitatory and inhibitory units. Glutamatergic neurons (e.g., cortical pyramidal neurons) receive a significant degree of GABA_A-mediated inhibition via interneurons. Characterization of the relationships between GABA concentration and synaptic release of glutamate under normal and diseased conditions is therefore important in understanding glutamatergic-GABAergic interactions and the mechanism of action of GABA-elevating drugs, especially for brain disorders that involve glutamate hyperactivity.

The neurotransmitter glutamate, when released by glutamatergic neurons, is primarily taken up by high-density [Na⁺-K⁺] glutamate transporters on astroglial end processes surrounding the presynaptic terminal and synaptic cleft (Danbolt et al., 1992), and then converted into glutamine by the glia-specific glutamine synthetase (EC 6.3.1.2). The released glutamate is replenished by astroglial release of glutamine, which is converted back into glutamate in neurons by phosphate-activated glutaminase (EC 3.5.1.2) and by de novo synthesis from glucose in astroglial cells (Hertz, 2004). Glutamate and glutamine reside predominantly in glutamatergic neurons and astroglial cells, respectively. The glutamate-glutamine cycling flux (V_{cyc}) between glutamatergic neurons and astroglia, which reflects synaptic glutamate release, can be determined in vivo using noninvasive ¹³C or ¹H{¹³C} magnetic resonance spectroscopy (MRS) techniques by measuring the kinetics of ¹³C label incorporation from ¹³C-labeled glucose into glutamate and glutamine (e.g., Sibson et al, Shen, 2006). Acetate is a glia-specific substrate (Waniewski and Martin, 1998). Exogenous [2-¹³C]acetate is initially metabolized by glial acetylCoA synthetase (acetate-CoA ligase; EC 6.2.1.1). Subsequently, the ¹³C label is incorporated into astroglial glutamine C4 (Bluml et al, 2003). The ¹³C label then enters neuronal compartments via V_{cyc} to label neuronal glutamate C4. Because of the high specificity of acetate metabolism, [2-¹³C]acetate has also been used in vivo to quantify V_{cyc} between glutamatergic neurons and astroglia (Lebon et al., 2002).

A previous study from our laboratory found that rats treated with the antidepressant/anti-panic drug phenelzine had elevated cortical GABA levels accompanied by reduced V_{cyc} rate (Yang and Shen, 2005), an observation that was in keeping with microdialysis studies that found that phenelzine attenuates glutamate efflux (Michael-Titus et al, 2000). In addition to raising brain GABA concentration, phenelzine also affects monoamine neurotransmitters by nonselectively inhibiting monoamine oxidase (EC 1.4.3.4, Parent et al, 2000). In comparison, vigabatrin is a highly specific inhibitor of GABA transaminase (EC 2.6.1.19), and it raises endogenous brain GABA concentrations in a dose-dependent manner. The aim of this study was to investigate the relationship between endogenous brain GABA concentration and V_{cyc} after vigabatrin treatment. In vivo Proton-Observed ¹³C-Edited (POCE, ¹H{¹³C}) spectroscopy and infusion of [2-¹³C]acetate were used to measure V_{cyc} in the rat brain at different endogenous GABA levels. We hypothesized that there would be a negative correlation between GABA concentration and V_{cyc} . A nonlinear, negative correlation between GABA concentration and V_{cyc} was found, indicating a significant glutamatergic effect for the GABA-elevating drug vigabatrin. Our results also suggest that glutamatergic-GABAergic interactions may be studied in the human brain using noninvasive in vivo MRS techniques.

Materials and Methods

Animal preparation

All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. Male Sprague-Dawley rats (Taconic, Germantown, NY; n = 29) were divided into four groups and studied according to procedures approved by the National Institute of Mental Health (NIMH) Intramural Research Program Animal Care and Use Committee. Animals in Group I (body weight: 154–191 g, n =

8) were not injected with vigabatrin and served as controls. Animals in Group II-IV received vigabatrin treatment 24 hours prior to MRS data acquisition (i.p., Sigma-Aldrich, St. Louis, MO; Group II: 250 mg/kg, body weight = 170–202 g, n = 7; Group III: 500 mg/kg, body weight = 171–194 g, n = 7; Group IV: 750 mg/kg, body weight = 171–204 g, n = 7). All rats were fasted for approximately 24 hours with free access to drinking water.

On the day of the in vivo MRS experiment, rats were orally intubated and mechanically ventilated with a mixture of ~70% N₂O, 30% O₂ and 1.5% isoflurane, which was discontinued after surgery. One femoral artery was cannulated for periodic sampling of arterial blood to monitor blood gases (pO₂, pCO₂), pH, and glucose concentration using a blood analyzer (Bayer Rapidlab 860, East Walpole, MA), as well as for monitoring arterial blood pressure levels. Two femoral veins (left and right) were also cannulated, one for intravenous infusion of α -chloralose (initial dose: 80 mg/kg supplemented with a constant infusion of 26.7 mg/kg/hr throughout the experiment) and the other for intravenous infusion of [2-¹³C]acetate (ISOTEC, Miamisburg, OH, 99% enrichment).

For POCE experiments that measured ¹³C enrichment of glutamate C4 and glutamine C4 following infusion of [2-¹³C]acetate, the intravenous acetate infusion protocol consisted of an initial bolus of 0.15 ml/100 g body weight/min of 0.9 M [2-¹³C]acetate for the first minute followed by a variable rate infusion of the same acetate solution, first at 1.6–3.0 ml/100 g body weight/hour for 30 minutes, and then at ~1.5–1.7 ml/100 g body weight/hour, with a total infusion period of three hours. The variable rate infusion protocol raised arterial blood acetate concentration to 76–118 mg/dL. The fractional enrichment of [2-¹³C]acetate reached 75.9% – 92.2% 10 minutes after the start of the [2-¹³C]acetate infusion, and was approximately constant ($76.3 \pm 6.1\% \sim 89.1 \pm 2.3\%$) over the course of in vivo POCE data acquisition. The fractional enrichments of [1-¹³C] and [6-¹³C]glucose in arterial blood steadily increased during the course of the [2-¹³C]acetate infusion, and reached $3.4 \pm 0.6\%$ and $3.8 \pm 0.5\%$ at the end of the 180 minute [2-¹³C]acetate infusion. Heart rate, arterial blood pO₂, pCO₂, mean blood pressure, and pH were maintained within the normal physiological range with few exceptions. End-tidal CO₂ and tidal pressure of ventilation were also monitored continuously. Rectal temperature was monitored and maintained at 37.5 ± 0.5 °C using an external pump for heat exchange by water circulation (BayVotex, Modesto, CA).

In vivo MRS methods

All Nuclear Magnetic Resonance (NMR) Spectroscopy experiments were performed on a Bruker microimaging spectrometer (Bruker Biospin, Billerica, MA) interfaced to an 11.7 Tesla 89-mm bore vertical magnet (Magnex Scientific, Abingdon, UK). ¹H and ¹H{¹³C} MRS experiments were performed to measure the total concentration and ¹³C labeling of brain metabolites. The spectroscopy voxel with a size of either $4.5 \times 2.5 \times 4.5$ mm³ or $4.0 \times 2.5 \times 4.0$ mm³ was centered along the brain midline in the rat neocortex using three-slice (coronal, horizontal, and sagittal) scout RARE imaging (field of view = 2.5 cm, slice thickness = 1 mm, recycle delay/echo time = 200/15 ms, rare factor = 8, 128 × 128 data matrix).

Short-TE ¹H MRS (Chen et al, 2004; echo-time = 18 ms, number of scans = 128) and a doubly selective homonuclear (¹H-¹H) polarization transfer GABA editing method (Shen et al., 2004; echo-time = 68 ms, number of scans = 256) were performed to measure the concentration of brain metabolites. For the ¹H{¹³C}MRS experiment, rats were infused with [2-¹³C]acetate with simultaneous, interleaved adiabatic POCE data acquisition (Yang and Shen, 2005; Yang et al, 2007). The adiabatic POCE method was slightly modified from a single-shot localization technique using three pairs of adiabatic refocusing pulses (Slotboom and Bovee, 1995). [¹³C] glutamate and [¹³C]glutamine C4 signals were edited by subtracting the even-numbered POCE subspectra from the corresponding odd-numbered ones. Re-shimming was performed after acquisition of each data block to maintain optimal B₀ homogeneity throughout the three hour

POCE data acquisition period. Quantification of the in vivo POCE spectra of rat brain was based on the phantom replacement method (Michaelis et al., 1993). Briefly, a water phantom containing glutamate, glutamine, and potassium chloride (pH = 7.0), which loaded approximately the same as a rat head, was used for quantification calibration. A two-dimensional stimulated echo acquisition mode (STEAM) method localizing a y column was used to place the first 180° null at the center of the selected spectroscopy voxel (Shen et al, 1999). The amplitude of the rectangular 180° pulse (the first pulse in the STEAM sequence) was used to measure small differences in coil loading between the water phantom and a rat head for absolute quantification of [^{13}C]glutamate and [^{13}C]glutamine C4 signals in the POCE spectra, as well as the total ([^{13}C] + [^{12}C]) concentration of glutamate and glutamine. In vivo MRS data were corrected for longitudinal relaxation saturation based on the metabolite null method to measure in vivo longitudinal relaxation time (Shen et al., 2004).

Analysis of plasma samples

Plasma samples were analyzed using gas chromatography/electron impact -mass spectrometry (GC/EI-MS) and in vitro $^1\text{H}\{^{13}\text{C}\}$ POCE spectroscopy. For the gas chromatography-mass spectrometry analysis of acetate, the protein complement of plasma was denatured and precipitated by adding methanol at a ratio of 1:4 (methanol). After centrifugation, 100 μl of the plasma extract supernatant was mixed with 10 μl of 1.0 M hydrochloric acid solution and analyzed by GC/EI-MS in single ion monitoring mode (SIM). The signal was recorded for molecular ions of acetic acid (60 m/z), 2- ^{13}C acetic acid (61 m/z), and propanoic acid (74 m/z). The ^{13}C fractional enrichment of [2- ^{13}C]acetate was determined by measuring the peak area ratio of acetic acid at 61 m/z to the sum of 60 m/z and 61 m/z. Total acetate concentration was determined using propanoic acid as the internal standard. For gas chromatography-mass spectrometry analysis of ^{13}C -labeled glucose generated via gluconeogenesis and other peripheral pathways (Schumann et al, 1991), after centrifugation and lyophilization, the residue was dissolved using 2% hydroxylamine in dry pyridine and incubated for one hour, followed by an additional 30 minute incubation with the addition of acetic anhydride. The resulting aldonitrile penta-acetate glucose derivative was analyzed using the signals of penta-O-acetyl-gluconitrile (314 m/z), penta-O-acetyl-(^{13}C)-gluconitrile (315 m/z), penta-O-acetyl-glucononitrile (187 m/z), and penta-O-acetyl-(^{13}C)-glucononitrile (188 m/z). For in vitro POCE spectroscopy analysis, plasma samples were extracted using perchloric acid (PCA, 12%), then centrifuged and lyophilized repeatedly as described previously (Yang and Shen, 2005). The POCE spectra of plasma PCA extracts dissolved in D_2O were recorded using a 2.5-mm diameter broadband inverse probe (Bruker Biospin, Billerica, MA) and a high-resolution version of the adiabatic POCE pulse sequence in which spatial localization, water, and outer volume suppression segments were deleted.

Metabolic modeling

^{13}C -labeling of glutamate and glutamine C4 was used to determine V_{cyc} between glutamatergic neurons and astroglia based on a previously described two-compartment metabolic model (Sibson et al, 1997; Shen et al, 1999; Lebon et al, 2002). In this model, intravenously infused glia-specific substrate [2- ^{13}C]acetate enters the astroglial cells first. In astroglia, the ^{13}C -label from [2- ^{13}C]acetate is incorporated into glutamine C4. Glutamine and glutamine synthetase are predominantly located in astroglia. Via the glutamate-glutamine cycle, the ^{13}C label is transferred to glutamate C4, which is predominantly located in glutamatergic neurons. The ^{13}C label on glutamate C4 has two metabolic fates: (i) displacement to glutamate C3 or C2 during the second turn of the neuronal tricarboxylic acid (TCA) cycle; or (ii) reincorporation into glutamine C4 via the glutamate-glutamine cycle. At isotopic steady state, the rate of ^{13}C label influx to neuronal glutamate C4 ($V_{\text{cyc}} * \text{fe}(\text{Gln C4})$) equals the corresponding outflux rate ($V_{\text{cyc}} * \text{fe}(\text{Glu C4}) + {}^nV_{\text{TCA}} * \text{fe}(\text{Glu C4})$) (Lebon et al, 2002):

$$\frac{V_{\text{cyc}}}{^nV_{\text{TCA}}} = \frac{\text{fe}(\text{Glu C4})}{[\text{fe}(\text{Gln C4}) - \text{fe}(\text{Glu C4})]}, \quad [1]$$

where fe represents ^{13}C fractional enrichment due to $[2-^{13}\text{C}]$ acetate metabolism in brain, and $^nV_{\text{TCA}}$ is the rate of the neuronal TCA cycle flux.

Some of the ^{13}C labels from $[2-^{13}\text{C}]$ acetate may also be incorporated into glucose by hepatic gluconeogenesis and other pathways, and gradually produce ^{13}C -labeled glucose (Schulmann et al, 1991). ^{13}C labels at glucose C1 and C6 are incorporated into the TCA cycle the same way as acetate C2. The labeling of glucose by $[2-^{13}\text{C}]$ acetate was found to be negligible in a ^{13}C MRS study of the human brain (Lebon et al, 2002). When the small amounts of $[1-^{13}\text{C}]$ and $[6-^{13}\text{C}]$ glucose become nonnegligible due to prolonged $[2-^{13}\text{C}]$ acetate infusion, they mainly contribute to glutamate C4 labeling, and their contribution can be corrected based on measured arterial input function and brain TCA cycle rate; the latter is known to be unaffected by vigabatrin treatment (Chapman et al., 1982; Manor et al, 1996). The same metabolic modeling approach used in $[1-^{13}\text{C}]$ or $[1,6-^{13}\text{C}_2]$ glucose infusion studies (Shen et al, 1999; Yang and Shen, 2005) was used for this correction. The contribution of $[1-^{13}\text{C}]$ and $[6-^{13}\text{C}]$ glucose to glutamine C4 labeling is much smaller due to the delayed appearance of ^{13}C labels on glutamine C4. In the present study Eq. [1] and contributions to glutamate and glutamine C4 from $[1-^{13}\text{C}]$ and $[6-^{13}\text{C}]$ glucose were fitted together (Boumezbeur et al, 2008) using the well-known Attractive Fixed Point (AFP) method.

Statistical analysis

Statistical differences were determined by one-way analysis of the variance (ANOVA) followed by Bonferroni post hoc analysis using the Statistical Package for the Social Sciences software (SPSS, version 12.0; SPSS Inc., Chicago, IL). The *F*-values and group and experimental degrees of freedom are included in the text as well as legends of the tables. The level of statistical significance was set at Bonferroni $p < 0.05$.

Results

Effect of vigabatrin treatment on brain metabolite concentrations

Figure 1 shows typical localized in vivo ^1H short-TE spectra from the neocortex of a Group I control rat (bottom trace) and from a Group III rat 24 hours after vigabatrin treatment (top trace, i.p., 500 mg/kg). Voxel size = $4.5 \times 2.5 \times 4.5 \text{ mm}^3$. Total number of scans = 128. The spectra were processed using Lorentz-Gauss transformation with $\text{lb} = -3$, $\text{gb} = 0.1$ prior to Fourier transformation. Only zero order phase correction was applied without using any baseline corrections. Marked elevation of the GABA β -methylene proton signal at 2.28 ppm and γ -methylene proton signal at 1.91 ppm in vigabatrin-treated rat is visually appreciable in Fig. 1. In comparison, the intensities of spectrally resolved glutamate γ -methylene proton signal at 2.35 ppm, glutamine γ -methylene proton signal at 2.46 ppm showed no change due to vigabatrin treatment. Similarly, the intensities of Glx (glutamate + glutamine) α -methine protons at 3.76–3.78 ppm and β -methylene protons at 2.11–2.14 ppm were not altered by administration of vigabatrin. Marked elevation in GABA due to vigabatrin blockade of the GABA shunt was also revealed in the edited spectra acquired using the one-dimensional homonuclear (^1H - ^1H) polarization transfer method. Cortical GABA concentrations in the control and vigabatrin-treated rats were determined using the one-dimensional homonuclear (^1H - ^1H) polarization transfer method to be 1.0 ± 0.3 (mean $\pm$ SD, $n = 8$, Group I), 2.3 ± 0.4 $\mu\text{mol/g}$ (mean $\pm$ SD, $n = 7$, Group II), 3.2 ± 0.5 (mean $\pm$ SD, $n = 7$, Group III), and 5.1 ± 0.5 (mean $\pm$ SD, $n = 7$, Group IV) $\mu\text{mol/g}$, respectively. One-way ANOVA analysis demonstrated

that vigabatrin treatment had a significant overall effect on endogenous brain GABA levels ($F(3, 25) = 102.8, p < 0.001$). A one-way ANOVA analysis with post hoc Bonferroni adjusted comparison found a significant difference in GABA concentration between each of the groups, with Bonferroni p values ranging from 0.004 to less than 0.001. The cortical GABA concentrations determined in vivo are in keeping with previously reported values determined using in vivo or ex vivo methods under conditions of minimal postmortem change (e.g., Miller et al., 1990; Manor et al., 1996; Patel et al., 2006; Yang et al., 2007). The short-TE ^1H spectra were analyzed using linear combination (LC) model (Provencher, 2001). All 11 metabolites used in LCModel fitting with a concentration greater than $1 \mu\text{mol/g}$ were listed in Table 1. Among them, GABA was the only metabolites which showed statistically significant changes in concentration 24 hours after vigabatrin treatment. The lack of significant change in other brain metabolites due to vigabatrin treatment observed in the current study is consistent with previous results reported in the literature (Manor et al., 1996; Preece and Cerdan, 1996; Patel et al., 2006).

^{13}C labeling of glutamate and glutamine detected using POCE spectroscopy

Figure 2 (bottom trace) shows the POCE difference spectrum accumulated over the 120–180 minute $[2-^{13}\text{C}]$ acetate infusion period from a Group I rat (voxel size = $4.5 \times 2.5 \times 4.5 \text{ mm}^3$; total number of scans = 768). The POCE spectra were processed using Lorentz-Gauss transformation with $\text{lb} = -4$, $\text{gb} = 0.2$ prior to Fourier transformation. Only zero order phase correction was applied without using any baseline corrections. Glutamate γ -methylene protons (2.35 ppm), glutamine γ -methylene protons (2.46 ppm), glutamate and glutamine β -methylene protons + N-acetylaspartate methyl protons (2.01–2.14 ppm), as well as the acetate methyl protons (1.92 ppm) were detected in the spectrum. The GABA α -methylene proton signal at 2.28 ppm was not significantly above the noise level. Figure 2 (top trace) shows the POCE spectrum accumulated over the 120–180 min $[2-^{13}\text{C}]$ acetate infusion period from a Group III rat that was pretreated with vigabatrin (500 mg/kg, i.p., 24 hours prior to data acquisition). All acquisition and data processing parameters were identical to those in the Figure 2 bottom trace. In the vigabatrin-treated rat, the GABA α -methylene proton signal at 2.28 ppm derived from $[2-^{13}\text{C}]$ acetate was appreciable. The most striking feature of Figure 2 is the reduced $[^{13}\text{C}]$ glutamate C4/ $[^{13}\text{C}]$ glutamine C4 intensity ratio due to vigabatrin treatment, indicating a reduced ^{13}C label flow from astroglial glutamine to neuronal glutamate.

The contribution of $[1-^{13}\text{C}]$ and $[6-^{13}\text{C}]$ glucose generated via hepatic gluconeogenesis and other peripheral pathways during intravenous $[2-^{13}\text{C}]$ acetate infusion to the fractional enrichments of $[^{13}\text{C}]$ glutamate C4 was found to be $\sim 1.3\%$ for rats in Groups I–IV. The corresponding contribution to $[^{13}\text{C}]$ glutamine C4 was $\sim 2.0\%$. Table 2 shows the direct contribution to the enrichment of $[^{13}\text{C}]$ glutamate C4 and $[^{13}\text{C}]$ glutamine C4 from infused $[2-^{13}\text{C}]$ acetate after correcting for the small contributions from $[1-^{13}\text{C}]$ and $[6-^{13}\text{C}]$ glucose and the 1.1% natural abundance. Reduction in ^{13}C label flow from astroglial glutamine C4 to neuronal glutamate C4 during $[2-^{13}\text{C}]$ acetate infusion was found in Group II – IV rats (Table 2). Because prolonged vigabatrin treatment does not affect the total concentration of glutamate ($[^{13}\text{C}]$ glutamate + $[^{12}\text{C}]$ glutamate) or glutamine (Manor et al., 1996; Preece and Cerdan, 1996; Patel et al., 2006), a mean glutamate concentration of $11.3 \mu\text{mol/g}$ and a mean glutamine concentration of $4.1 \mu\text{mol/g}$ were used to determine the fractional enrichment of $[^{13}\text{C}]$ glutamate C4 and $[^{13}\text{C}]$ glutamine C4.

Glutamate-glutamine cycling flux under elevated GABA concentration

The $V_{\text{cyc}}/V_{\text{TCA}}$ ratios and V_{cyc} values were determined using Eq. [1] according to the metabolic modeling analysis described above, and using previously determined neuronal TCA cycle rate in α -chloralose anesthetized young adult rat brain ($0.45 \mu\text{mol/g/min}$, Yang and Shen, 2005), as well as prior knowledge that vigabatrin pretreatment does not affect TCA cycle rate

(Chapman et al., 1982; Manor et al., 1996). One-way ANOVA analysis revealed a significant overall effect of elevated GABA concentration on V_{cyc} ($F(3, 25) = 15.3, p < 0.001$). Using post hoc Bonferroni adjusted comparison, the V_{cyc} of rats in Groups II- IV was found to significantly differ from that of rats in Group I (Bonferroni $p = 0.02$ for Group I vs. Group II and Bonferroni $p < 0.001$ for Group I vs. Groups III and IV, respectively). Polynomial contrast analysis showed that most of the between-group sum of squares was accounted for by a linear trend over dose of vigabatrin, and that this trend was statistically significant ($F(1,25) = 42.7, p < 0.001$). A two-tailed bivariate correlation test showed that the correlation between V_{cyc} and dose of vigabatrin was highly significant (Pearson's correlation coefficient $r = -0.78, p < 0.001$). Figure 3 shows attenuation of V_{cyc} as a function of GABA concentration. When a nonlinear power trendline was fitted to the group mean V_{cyc} vs. group mean GABA concentration,

$$\Delta V_{cyc}/V_{cyc} = [GABA (\mu\text{mol/g})]^{-0.35} - 1.0 \quad [2]$$

was obtained ($r^2 = 0.98$). Fitting the power trendline to ungrouped V_{cyc} vs. ungrouped [GABA], $\Delta V_{cyc}/V_{cyc} = [GABA (\mu\text{mol/g})]^{-0.34} - 1.0$ was obtained ($r^2 = 0.70$).

Discussion

Comparison to previous ^{13}C MRS study of vigabatrin treatment using $[1-^{13}\text{C}]\text{glucose}$ infusion

In a previous ^{13}C MRS study focusing on GABA synthesis that used $[1-^{13}\text{C}]\text{glucose}$ infusion, vigabatrin treatment (500 mg/kg, i.p., 24 hours prior to MRS data acquisition) was found to alter GABA levels and the rate of GABA turnover but not the rate of TCA cycle flux (Manor et al, 1996). In that study, high-resolution in vitro POCE MRS spectra of perchloric acid extracts of vigabatrin-treated and non-treated rat cortex were recorded after 30 minutes of $[1-^{13}\text{C}]\text{glucose}$ infusion. Figure 4 in Manor et al (Manor et al, 1996) showed that the $[^{13}\text{C}]\text{glutamine C4}/[^{13}\text{C}]\text{glutamate C4}$ intensity ratio measured from brain perchloric acid extracts was markedly reduced in vigabatrin-treated rats although the authors did not discuss its significance. Using $[1-^{13}\text{C}]\text{glucose}$ infusion, ^{13}C labels flow mostly from glucose C1 to pyruvate C3 and then to neuronal glutamate C4. Mainly via V_{cyc} , ^{13}C labels are subsequently incorporated into glutamine C4. As a consequence, neuronal glutamate C4 is the major metabolic precursor of astroglial glutamine C4 when $[1-^{13}\text{C}]$ or $[1,6-^{13}\text{C}_2]\text{glucose}$ infusion is employed. A reduced $[^{13}\text{C}]\text{glutamine C4}/[^{13}\text{C}]\text{glutamate C4}$ intensity ratio therefore indicates a reduced ^{13}C label flow from neuronal glutamate to astroglial glutamine during $[1-^{13}\text{C}]\text{glucose}$ infusion. The results from the Manor et al study is therefore in agreement with our current results obtained using $[2-^{13}\text{C}]\text{acetate}$ infusion. Quantitative information on V_{cyc} could not be extracted from the Manor et al study because no dynamic turnover time courses for glutamate and glutamine C4, which are required to extract V_{cyc} when $[1-^{13}\text{C}]\text{glucose}$ infusion is employed, were measured in that study.

Comparison to previous ^{13}C MRS study of phenelzine treatment using $[1,6-^{13}\text{C}_2]\text{glucose}$ infusion

We previously found that V_{cyc} was reduced by ~38% in rats pretreated with the antidepressant/anti-panic drug phenelzine (10 mg/kg, i.p.). The main function of phenelzine is inhibition of monoamine oxidase. As a result, concentrations of the amine neurotransmitters noradrenaline, dopamine, and serotonin are elevated after phenelzine treatment. Phenelzine also significantly increases brain GABA concentrations, presumably due to an unidentified metabolite of phenelzine that inhibits GABA transaminase (Parent et al, 2002). The reduced V_{cyc} seen after phenelzine treatment is consistent with results from microdialysis studies measuring glutamate

efflux (Michael-Titus et al, 2000). However, it was unclear whether altered GABA level alone would affect V_{cyc} .

In contrast to phenelzine, which affects monoaminergic neurotransmitter systems, vigabatrin is a well-known highly specific suicide inhibitor of GABA transaminase. Eq. [2] predicts that at $[GABA] = 2.0\text{--}2.6\ \mu\text{mol/g}$, a 22–28% reduction in V_{cyc} is expected. After i.p. injection of 10 mg/kg phenelzine, rat brain GABA levels reached $2.30 \pm 0.26\ \mu\text{mol/g}$ (Yang and Shen, 2005). The actual ~38% reduction in V_{cyc} due to phenelzine treatment is actually greater than that predicted by the V_{cyc} vs. $[GABA]$ relationship described by Eq. [2], indicating that elevated monoamines also contributed to the reduced V_{cyc} . Because serotonin, dopamine, and norepinephrine are known to inhibit Ca^{2+} -dependent, K^{+} -evoked glutamate efflux and veratrine-induced glutamate release (Maura et al, 1998; Crowder and Bradford, 1987), this finding is not surprising. Thus, based on our previous ^{13}C MRS study of phenelzine treatment using $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ infusion and the current study using $[2\text{-}^{13}\text{C}]\text{acetate}$ infusion and vigabatrin-treatment, both elevated GABA and monoamine neurotransmitters should have contributed to the reduced V_{cyc} in phenelzine-treated rats.

Relationship between GABA concentration and V_{cyc}

Although the glutamate-glutamine cycle has been recognized as a critical pathway linking glutamatergic neurons and astroglia, little information exists on the regulation of this flux. Previous work has shown that elevated GABA correlates with reduced ^{13}C label flow from neuronal glutamate C4 to astroglial glutamine C4 using $[1\text{-}^{13}\text{C}]$ or $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ infusion (Manor et al, 1996; Yang and Shen, 2005). In contrast to $[1\text{-}^{13}\text{C}]$ or $[1,6\text{-}^{13}\text{C}_2]\text{glucose}$ infusion, astroglial glutamine C4 is the primary metabolic precursor of neuronal glutamate C4 when exogenous $[2\text{-}^{13}\text{C}]\text{acetate}$ is infused. Therefore, the reduced $[^{13}\text{C}]\text{glutamine C4}/[^{13}\text{C}]\text{glutamate C4}$ intensity ratio seen in the previous work is consistent with our current findings, which demonstrated that using vigabatrin to raise GABA levels and block GABA catabolism reduced ^{13}C label flow from $[^{13}\text{C}]\text{glutamine C4}$ to $[^{13}\text{C}]\text{glutamate C4}$.

The results of the current study quantitatively establish the relationship between elevated GABA level and reduced V_{cyc} rate. The elevated GABA pool is predominantly localized in GABAergic neurons (Preece and Cerdan, 1996). The dependence of V_{cyc} on GABA concentration (see Figure 3) also indicates that, at the GABA level of $5.1 \pm 0.5\ \mu\text{mol/g}$, the inhibitory action of GABAergic interneurons on glutamatergic neurons approaches saturation. One-way ANOVA analysis with post hoc Bonferroni adjusted comparison showed that there was no statistically significant difference in V_{cyc} for Group II vs. Group III ($p = 0.3$) or for Group III vs. Group IV ($p = 1.0$). These results thus suggest that, although the effect of elevated GABA on V_{cyc} is quite significant at the lower end of the $1.0 \pm 0.3 \sim 5.1 \pm 0.5\ \mu\text{mol/g}$ concentration range, further increases in GABA concentration are less effective in reducing synaptic glutamate release. This fact was also reflected by the nonlinear feature of the $\Delta V_{cyc}/V_{cyc}$ vs. $[GABA]$ relationship (see Figure 3). The strong negative correlation between GABA levels and V_{cyc} indicates a strong interaction between GABAergic and glutamatergic systems and a large glutamatergic effect for GABA elevating drugs such as vigabatrin.

Implications for studying glutamate hyperactivity in human subjects

Converging evidence has indicated that glutamatergic and GABAergic systems play an important role in many psychiatric and neurological disorders (Bradford 1995; Kendall et al, 2005). For example, glutamate hyperactivity and decreased GABA level are associated with major depressive disorder (Sanacora et al, 1999; Kendell et al, 2005; Hasler et al, 2007), and GABA-elevating drugs such as vigabatrin, topiramate, and gabapentin effectively treat many brain disorders associated with glutamate hyperactivity (Bergin and Connolly, 2002; Post et al, 1998). The range of GABA concentrations investigated here is inclusive of that observed

in vivo in the human brain caused by GABA-elevating drugs (Petroff et al, 1999). The negative correlation between GABA concentration and V_{cyc} determined in rat brain in this study by the use of graded GABA levels therefore provides important insights into the interplay between GABA and glutamate in psychiatric and neurological diseases associated with GABAergic and glutamatergic dysfunction. It also gives a quantitative measure of the glutamatergic effects of GABA-elevating drugs.

Furthermore, in this study both GABA concentration and V_{cyc} were determined using noninvasive in vivo MRS techniques. Our results thus suggest that these methods can be used to study glutamatergic-GABAergic interactions, in order to assess the effects of GABA-elevating drugs on glutamatergic dysfunction in psychiatric and neurological patients.

In conclusion, a nonlinear negative correlation between GABA concentrations and V_{cyc} was found by elevating GABA concentrations using the highly specific GABA transaminase inhibitor vigabatrin. Our results provide evidence that V_{cyc} is modulated by endogenous brain GABA levels.

Acknowledgments

The authors are grateful to Mr. Christopher Johnson, Drs. Steve Li, Steve Fox and Su Xu for valuable help and Ms. Ioline Henter for editing the manuscript. This work was supported by the Intramural Research Program of the NIH, NIMH.

References

- Banay-Schwartz M, Palkovits M, Lajtha A. Heterogeneous distribution of functionally important amino acids in brain areas of adult and aging humans. *Neurochem Res* 1993;18:417–423. [PubMed: 8474566]
- Bergin AM, Connolly M. New antiepileptic drug therapies. *Neurol Clin* 2002;20:1163–82. [PubMed: 12616686]
- Blüml S, Moreno-Torres A, Shic F, Nguy CH, Ross BD. Tricarboxylic acid cycle of glia in the in vivo human brain. *NMR Biomed* 2002;15:1–5. [PubMed: 11840547]
- Boumezbeur, F.; Petersen, KF.; de Graaf, RA.; Cline, GW.; Behar, KL.; Shulman, GI.; Rothman, DL.; Mason, GF. *Proc Intl Soc Magn Reson Med*. Toronto: 2008. Combination of datasets from [2-¹³C] acetate and [1-¹³C] glucose experiments improve accuracy of metabolite rates determination in humans. Abstract # 196
- Bradford HF. Glutamate, GABA and epilepsy. *Prog Neurobiol* 1995;47:477–511. [PubMed: 8787032]
- Chapman AG, Riley K, Evens MC, Meldrum BS. Acute effects of sodium valproate and -vinyl GABA on regional amino acid metabolism in the rat brain: incorporation of 2-[¹⁴C]glucose into amino acids. *Neurochem Res* 1982;7:1089–1105. [PubMed: 6817155]
- Chen Z, Li SS, Yang J, Letizia D, Shen J. Measurement and automatic correction of high order B_0 inhomogeneity in the rat brain at 11.7 Tesla. *Magn Reson Imaging* 2004;22:835–842. [PubMed: 15234452]
- Crowder JM, Bradford HF. Inhibitory effects of noradrenaline and dopamine on calcium influx and neurotransmitter glutamate release in mammalian brain slices. *Eur J Pharmacol* 1987;143:343–52. [PubMed: 2891552]
- Danbolt NC, Storm-Mathisen J, Kanner BI. An [Na⁺ - K⁺]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. *Neuroscience* 1992;51:295–310. [PubMed: 1465194]
- Jackson MF, Esplin B, Capek R. Reversal of the activity-dependent suppression of GABA-mediated inhibition in hippocampal slices from gamma-vinyl GABA (vigabatrin)-pretreated rats. *Neuropharmacology* 2000;39:65–74. [PubMed: 10665820]
- Hasler G, van der Veen JW, Tumonis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 2007;64:193–200. [PubMed: 17283286]

- Hertz L. Intercellular metabolic compartmentation in the brain: past, present and future. *Neurochem Int* 2004;45:285–296. [PubMed: 15145544]
- Kendell SF, Krystal JH, Sanacora G. GABA and glutamate systems as therapeutic targets in depression and mood disorders. *Expert Opin Ther Targets* 2005;9:153–168. [PubMed: 15757488]
- Kuzniecky R, Ho S, Pan J, Martin R, Gilliam F, Faught E, Hetherington H. Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology* 2002;58:368–372. [PubMed: 11839834]
- Lebon V, Petersen KF, Cline GW, Shen J, Mason GF, Dufour S, Behar KL, Shulman GI, Rothman DL. Astroglial contribution to brain energy metabolism in humans revealed by ^{13}C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. *J Neurosci* 2002;22:1523–1531. [PubMed: 11880482]
- Manor D, Rothman DL, Mason GF, Hyder F, Petroff OA, Behar KL. The rate of turnover of cortical GABA from $[1-^{13}\text{C}]$ glucose is reduced in rats treated with the GABA-transaminase inhibitor vigabatrin (gamma-vinyl GABA). *Neurochem Res* 1996;21:1031–1041. [PubMed: 8897466]
- Maura G, Marcoli M, Tortarolo M, Andrioli GC, Raiteri M. Glutamate release in human cerebral cortex and its modulation by 5-hydroxytryptamine acting at h 5-HT_{1D} receptors. *Br J Pharmacol* 1998;123:45–50. [PubMed: 9484853]
- McDonald DG, Najam Y, Keegan MB, Whooley M, Madden D, McMenamin JB. The use of lamotrigine, vigabatrin and gabapentin as add-on therapy in intractable epilepsy of childhood. *Seizure* 2005;14:112–116. [PubMed: 15694564]
- Michaelis T, Merboldt KD, Bruhn H, Hancic W, Frahm J. Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. *Radiology* 1993;187:219–227. [PubMed: 8451417]
- Michael-Titus AT, Bains S, Jeetle J, Whelpton R. Imipramine and phenelzine decrease glutamate overflow in the prefrontal cortex—a possible mechanism of neuroprotection in major depression? *Neuroscience* 2000;100:681–684. [PubMed: 11036201]
- Miller JM, Jope RS, Ferraro TN, Hare TA. Brain amino acid concentrations in rats killed by decapitation and microwave irradiation. *J Neurosci Methods* 1990;31:187–192. [PubMed: 2329838]
- Parent MB, Habib MK, Baker GB. Time-dependent changes in brain monoamine oxidase activity and in brain levels of monoamines and amino acids following acute administration of the antidepressant/antipanic drug phenelzine. *Biochem Pharmacol* 2000;59:1253–63. [PubMed: 10736426]
- Parent MB, Master S, Kashlub S, Baker GB. Effects of the antidepressant/antipanic drug phenelzine and its putative metabolite phenylethylidenehydrazine on extracellular gamma-aminobutyric acid levels in the striatum. *Biochem Pharmacol* 2002;63:57–64. [PubMed: 11754874]
- Patel AB, de Graaf RA, Martin DL, Battaglioli G, Behar KL. Evidence that GAD65 mediates increased GABA synthesis during intense neuronal activity in vivo. *J Neurochem* 2006;97:385–396. [PubMed: 16539672]
- Petroff OA, Hyder F, Collins T, Mattson RH, Rothman DL. Acute effects of vigabatrin on brain GABA and homocarnosine in patients with complex partial seizures. *Epilepsia* 1999;40:958–64. [PubMed: 10403220]
- Piérard C, Pérès M, Satabin P, Guezennec CY, Lagarde D. Effects of GABA-transaminase inhibition on brain metabolism and amino-acid compartmentation: an in vivo study by 2D ^1H -NMR spectroscopy coupled with microdialysis. *Exp Brain Res* 1999;127:321–327. [PubMed: 10452220]
- Post RM, Denicoff KD, Frye MA, Dunn RT, Leverich GS, Osuch E, Speer A. A history of the use of anticonvulsants as mood stabilizers in the last two decades of the 20th century. *Neuropsychobiology* 1998;38:152–66. [PubMed: 9778604]
- Preece NE, Cerdan S. Metabolic precursors and compartmentation of cerebral GABA in vigabatrin-treated rats. *J Neurochem* 1996;67:1718–1725. [PubMed: 8858958]
- Provencher SW. Automatic quantification of localized in vivo ^1H spectra with LCModel. *NMR Biomed* 2001;14:260–264. [PubMed: 11410943]
- Qume M, Whitton PS, Fowler LJ. The effect of chronic treatment with the GABA transaminase inhibitors gamma-vinyl-GABA and ethanolamine-O-sulphate on the in vivo release of GABA from rat hippocampus. *J Neurochem* 1995;64:2256–2261. [PubMed: 7722510]

- Sanacora G, Mason GF, Rothman DL, Behar KL, Hyder F, Petroff OA, Berman RM, Charney DS, Krystal JH. Reduced cortical gamma-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* 1999;56:1043–7. [PubMed: 10565505]
- Schumann WC, Magnusson I, Chandramouli V, Kumaran K, Wahren J, Landau BR. Metabolism of [2-¹⁴C]acetate and its use in assessing hepatic Krebs cycle activity and gluconeogenesis. *J Biol Chem* 1991;266:6985–90. [PubMed: 2016310]
- Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo ¹³C NMR. *Proc Natl Acad Sci USA* 1999;96:8235–8240. [PubMed: 10393978]
- Shen J, Yang J, Choi I-Y, Li SS, Chen Z. A new strategy for in vivo spectral editing. Application to GABA editing using selective homonuclear polarization transfer spectroscopy. *J Magn Reson* 2004;170:290–298. [PubMed: 15388093]
- Shen J. ¹³C magnetic resonance spectroscopy studies of alterations in glutamate neurotransmission. *Biol Psychiatry* 2006;59:883–7. [PubMed: 16199016]
- Sibson NR, Dhankhar A, Mason GF, Behar KL, Rothman DL, Shulman RG. In vivo ¹³C NMR measurements of cerebral glutamine synthesis as evidence for glutamate-glutamine cycling. *Proc Natl Acad Sci U S A* 1997;94:2699–704. [PubMed: 9122259]
- Slotboom J, Bovee WMMJ. Adiabatic slice-selective rf pulses and a single-shot adiabatic localization pulse sequence,” *Concept Magn Reson*. 1995;7:193–217.
- Smolders I, Khan GM, Lindekens H, Prikken S, Marvin CA, Manil J, Ebinger G, Michotte Y. Effectiveness of vigabatrin against focally evoked pilocarpine-induced seizures and concomitant changes in extracellular hippocampal and cerebellar glutamate, gamma-aminobutyric acid and dopamine levels, a microdialysis-electrocorticography study in freely moving rats. *J Pharmacol Exp Ther* 1997;283:1239–1248. [PubMed: 9399999]
- Waniewski RA, Martin DL. Preferential utilization of acetate by astrocytes is attributable to transport. *J Neurosci* 1998;18:5225–5233. [PubMed: 9651205]
- Wood JD, Kurylo E, Lane R. Gamma-Aminobutyric acid release from synaptosomes prepared from rats treated with isonicotinic acid hydrazide and gabaculine. *J Neurochem* 1988;50:1839–43. [PubMed: 3373216]
- Wu Y, Wang W, Richerson GB. Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. *J Neurophysiol* 2003;89:2021–2034. [PubMed: 12612025]
- Yang J, Shen J. In vivo evidence for reduced cortical glutamate-glutamine cycling in rats treated with the antidepressant/antipanic drug phenelzine. *Neuroscience* 2005;135:927–37. [PubMed: 16154287]
- Yang J, Li SS, Bacher J, Shen J. Quantification of cortical GABA-glutamine cycling rate using in vivo magnetic resonance signal of [2-¹³C]GABA derived from glia-specific substrate [2-¹³C]acetate. *Neurochem Int* 2007;50:371–8. [PubMed: 17056156]

Figure 1.

Comparison of in vivo ^1H short-TE spectra from a control rat of Group I (bottom trace) and a Group III rat 24 hours after vigabatrin injection (top trace; 500 mg/kg, i.p., 24 hours prior to data acquisition). Voxel size = $4.5 \times 2.5 \times 4.5 \text{ mm}^3$. Total number of scans = 128. lb = -3, gb = 0.1. The spectra were phased using zero order phase only without any baseline corrections. In the ^1H short-TE spectrum of vigabatrin-treated rat, the elevation of GABA α -methylene proton signal at 2.28 ppm, the GABA β -methylene proton signal at 1.91 ppm due to vigabatrin treatment were clearly observed. No significant changes in the intensity of other metabolites were found in rats treated with vigabatrin. Cr: creatine, GABA: γ -aminobutyric acid, Gln: glutamine, GPC: glycerophosphocholine, Glu: glutamate, Glx: glutamate + glutamine, Lac:

lactate, M₁: macromolecule at 0.92 ppm, M₂: macromolecule at 1.21 ppm, M₃: macromolecule at 1.39 ppm, MI: *myo*-Inositol, NAA: N-acetylaspartate, PC: phosphocholine, PCr: phosphocreatine, Tau: taurine, VGB: vigabatrin.

Figure 2.

Comparison of in vivo POCE spectra accumulated over the 120–180 min [2-¹³C]acetate infusion period from a Group I rat (bottom trace; control) vs. a Group III rat (top trace; 500 mg/kg, i.p., 24 hours prior to data acquisition). Voxel size = $4.5 \times 2.5 \times 4.5 \text{ mm}^3$. Total number of scans = 768. lb = -4, gb = 0.2. The [¹³C]glutamate C4/[¹³C]glutamine C4 intensity ratio is reduced due to vigabatrin treatment, indicating a reduced ¹³C label flow from astroglial glutamine to neuronal glutamate. Ac: acetate; Gln: glutamine; Glx: glutamate + glutamine; Glu: glutamate; NAA: N-acetylaspartate; VGB: vigabatrin.

Figure 3.

V_{cyc} ($\mu\text{mol/g/min}$) vs. [GABA] ($\mu\text{mol/g}$). The error bar represents $\pm$ SD. A power trendline was fitted to group mean V_{cyc} vs. group mean [GABA,] which yields $\Delta V_{cyc}/V_{cyc} = [\text{GABA} (\mu\text{mol/g})]^{-0.35} - 1.0$ ($r^2 = 0.98$).

Table 1

Comparison of concentration of metabolites

Metabolites ^a	Group I	Group II ^b	Group III ^b	Group IV ^b
N-acetylaspartate	10.48 ± 0.28	10.39 ± 0.31	10.21 ± 0.44	10.23 ± 0.31
Aspartate	2.81 ± 0.19	2.59 ± 0.29	2.88 ± 0.17	2.71 ± 0.35
Creatine	3.43 ± 0.25	3.38 ± 0.13	3.59 ± 0.16	3.68 ± 0.22
GABA ^{c,d}	1.04 ± 0.28	2.28 ± 0.45	3.25 ± 0.54	5.14 ± 0.55
glutamate ^e	11.39 ± 0.29	11.43 ± 0.38	11.15 ± 0.47	11.08 ± 0.39
glutamine ^e	4.07 ± 0.33	4.06 ± 0.29	4.27 ± 0.48	4.21 ± 0.37
Lactate	0.41 ± 0.20	0.58 ± 0.12	0.62 ± 0.23	0.49 ± 0.18
myo-inositol	4.51 ± 0.32	4.62 ± 0.22	4.71 ± 0.48	4.32 ± 0.43
phosphocreatine	5.07 ± 0.25	5.11 ± 0.13	4.91 ± 0.16	4.82 ± 0.22
phosphorylethanolamine	2.37 ± 0.52	2.11 ± 0.55	2.43 ± 0.45	2.11 ± 0.39
Taurine	4.76 ± 0.21	4.82 ± 0.30	4.99 ± 0.38	4.79 ± 0.33

^a All concentrations were determined in vivo in the unit of $\mu\text{mol/g}$ and expressed as mean $\pm$ SD.

^b Measured 24 hours after vigabatrin treatment (Group II: 250 mg/kg, n = 7; Group III: 500 mg/kg, n = 7; Group IV: 750 mg/kg, n = 7).

^c One-way ANOVA analysis with post hoc Bonferroni adjusted comparison demonstrated significant difference in GABA concentration among each of the groups (Bonferroni $p = 0.004 \sim < 0.001$).

^d Determined using in vivo homonuclear (^1H - ^1H) polarization transfer spectral editing (Shen et al. 2004).

^e Referenced to concentrations determined using the phantom replacement method (Michaelis et al. 1993).

Table 2Glutamate and glutamine ^{13}C labeling and V_{cyc} determined from rat brain in vivo

^{13}C labeling and fluxes ^a	Group I	Group II ^b	Group III ^b	Group IV ^b
FE([4- ^{13}C]Glu) ^c (%)	9.0 ± 0.6	7.9 ± 0.6	8.8 ± 0.7	7.7 ± 0.6
FE([4- ^{13}C]Gln) ^c (%)	23.0 ± 3.4	23.4 ± 3.6	29.7 ± 3.0	28.1 ± 3.6
$V_{\text{cyc}}/{}^nV_{\text{TCA}}^d$	0.66 ± 0.11	0.52 ± 0.08	0.43 ± 0.08	0.38 ± 0.05
V_{cyc} (μmol/g/min) ^d	0.30 ± 0.05	0.23 ± 0.04	0.19 ± 0.04	0.17 ± 0.02

^a All values were expressed as mean ± SD.^b Measured 24 hours after vigabatrin treatment (Group II: 250 mg/kg, n = 7; Group III: 500 mg/kg, n = 7; Group IV: 750 mg/kg, n = 7).^c After correcting contributions from [1- ^{13}C] and [6- ^{13}C]glucose generated via hepatic gluconeogenesis and other peripheral pathways and the 1.1% natural abundance.^d One-way ANOVA analysis with post hoc Bonferroni adjusted comparison demonstrated significant difference among each of the groups (Bonferroni $p = 0.04 \sim < 0.001$) except for Group II vs. Group III (Bonferroni $p = 0.3$) and Group III vs. Group IV (Bonferroni $p = 1.0$). V_{cyc} of Group II- IV rats was found to be significantly different from that of Group I with Bonferroni $p = 0.02$ for Group I vs. Group II and Bonferroni $p < 0.001$ for Group I vs. Group III and IV, respectively. V_{cyc} was calculated using $V_{\text{cyc}}/{}^nV_{\text{TCA}}$ determined in this study and ${}^nV_{\text{TCA}} = 0.45 \mu\text{mol/g/min}$ from Yang and Shen, 2005.