Further evidence for a correlation between increased endogenous GABA concentration and decreased glutamate-glutamine cycling flux

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Introduction

There is experimental evidence in the literature that elevated endogenous GABA concentration in brain is accompanied by a reduced glutamate efflux [e.g., 1,2]. It has been shown recently that administration of the antidepressant/antipanic drug phenelzine increases endogenous GABA concentration and simultaneously decreases the glutamate-glutamine cycling flux between neurons and glia, the latter is manifested by a reduced ^{13}C-label incorporation from [1,6-$^{13}C_2$]glucose into [4-^{13}C]glutamine [3]. Interestingly, increased endogenous GABA concentration has also been shown to correlate with an attenuation in BOLD response to functional stimulation [4]. We hypothesize that increased endogenous GABA concentration reduces the glutamate-glutamine cycling flux between neurons and glia. In this study, we test this hypothesis by measuring the ^{13}C-label incorporation into the predominantly neuronal [4-^{13}C]glutamate from the glia-specific substrate [2-^{13}C]acetate 24 hours after administration of the GABA-elevating, antiepileptic drug vigabatrin.

Methods

Male Sprague-Dawley rats were studied using an 11.7 Tesla 89-mm bore vertical magnet (Magnex Scientific, Abingdon, UK). The control group (n = 4) and the vigabatrin-treated group (n = 4, 500 mg/kg, i.p., 24 hrs prior to MRS study) were infused with [2-^{13}C]acetate for a period of 180 minutes. All rats were orally intubated and mechanically ventilated with a mixture of ~70% N_2O, 30% O_2 and 1.5% isoflurane, which was discontinued after surgery with anesthesia maintained using alpha-chloralose instead (initial dose: 80 mg/kg supplemented with a constant infusion of 26.7 mg/kg/hr throughout the experiment). Prior to intravenous acetate infusion, GABA editing spectra were acquired from a voxel of 4.5 x 2.5 x 4.5 mm^3 centered along the brain midline in the neocortex. The GABA editing method uses doubly selective homonuclear polarization transfer, which detects the GABA-4 resonance at 3.0 ppm while preserving the NAA resonance at 2.0 ppm. The time-resolved turnover kinetics of [4-^{13}C]glutamate and [4-^{13}C]glutamine from the same spectroscopy voxel was measured using the Proton-Observed, Carbon-13 Edited (POCE) method.

Results and Discussion

Fig. 1 shows a comparison of the edited GABA spectra between a control rat and one treated with vigabatrin. NS = 256 with 5 Hz exponential line-broadening. Only zero order phase correction was applied without using any baseline corrections. In agreement with previous results, the brain GABA concentration was markedly elevated 24 hrs after vigabatrin treatment (500 mg/kg, i.p.). Quantitatively, [GABA] was determined to be 2.8 ± 0.5 µmol/g ww (mean ± SD, n = 4) in the vigabatrin-treated group as compared to 1.0 ± 0.3 µmol/g ww (mean ± SD, n = 4) determined from the control group. A comparison of the adiabatic POCE spectra accumulated over the 120-180 min period was shown in Fig. 2. NS = 768, lb = -4, gb = 0.2. Only zero order phase correction was applied to the POCE difference spectra without using any baseline corrections. In the POCE spectra shown in Fig. 2, ^{13}C-label incorporation into [4-^{13}C]glutamine, [4-^{13}C]glutamate, and [3-^{13}C]glx was detected. In the vigabatrin-treated rat (upper trace, Fig. 2), ^{13}C-label incorporation into [2-^{13}C]GABA was also observed. The most striking observation of the POCE data is the reduced [4-^{13}C]glutamate/[4-^{13}C]glutamine ratio in the vigabatrin-treated group. Since [2-^{13}C]acetate is a glia-specific substrate, the labeling of [4-^{13}C]glutamate from [2-^{13}C]acetate, which is predominantly located in neurons, has been used as a measure of the glutamate-glutamine cycling flux in the human brain [5]. In this study, we found a significant reduction (30%, $p < 0.05$, unpaired t test) of the [4-^{13}C]glutamate/[4-^{13}C]glutamine ratio in the vigabatrin-treated group (0.78 ± 0.22, mean ± SD, n = 4), as compared to the controls (1.11 ± 0.08, mean ± SD, n = 4), indicating a significantly reduced glutamate-glutamine cycling flux between neurons and glia accompanying an elevated endogenous GABA concentration due to the irreversible inhibition of GABA-transaminase by vigabatrin. The results presented here, therefore, are in agreement with previous studies using microdialysis [1,2] and MRS with ^{13}C-labeled glucose infusion [3], providing further evidence to support our hypothesis that increased endogenous GABA concentration reduces the glutamate-glutamine cycling flux in brain.

Fig. 1

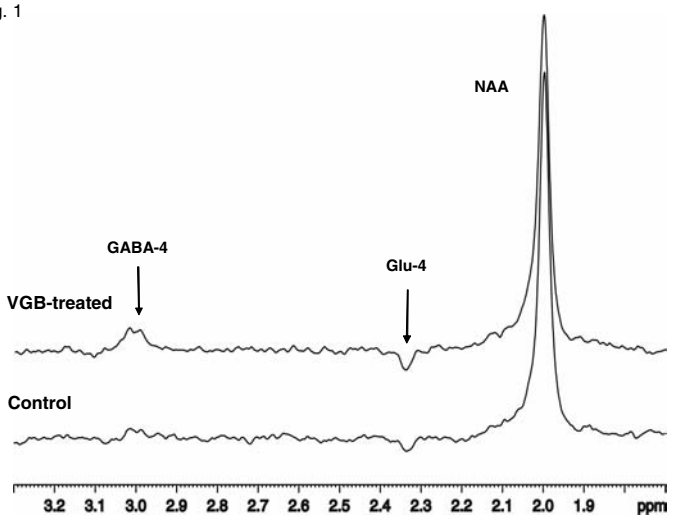
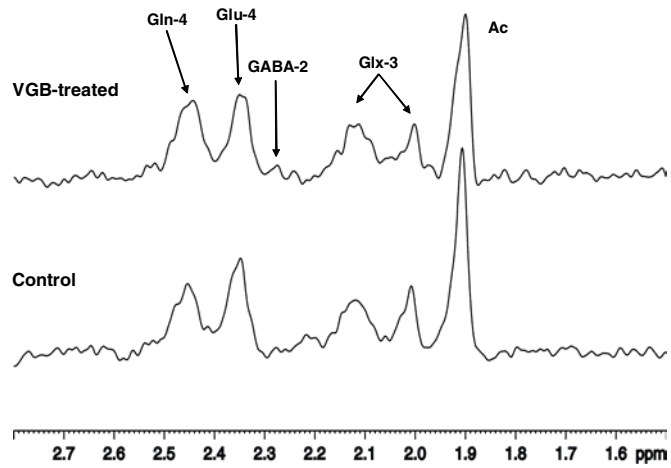


Fig. 2



References

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