GABA Synthesis and Cycling in Human Brain as Studied By ^{1}H and ^{13}C NMR Spectroscopy

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Introduction

GABA is the major inhibitory neurotransmitter in human cerebral cortex. Vigabatrin is an anti-epileptic medication that blocks the degradation of GABA by the enzyme GABA-transaminase. ^{1}H NMR studies have shown that vigabatrin (3 gram dose) acutely raises GABA from 1 to 3 mM within 90 minutes, after which GABA ceases to rise further [1]. It is unknown whether the new GABA steady-state is established due to inhibition of GABA synthesis or shunting of GABA to other metabolic pathways. Furthermore, due to the presence of two different isoforms of the enzyme which synthesizes GABA (GAD_{65} and GAD_{67}), and the differential sensitivity of these isoforms to increased levels of GABA, it is not known whether the entire GABA pool is metabolically active after elevation. In this study we used localized ^{13}C NMR spectroscopy in combination with an infusion of [1-^{13}C]-glucose to measure the turnover of GABA in the occipital/parietal cortex of epilepsy patients following an acute dose of vigabatrin.

Methods

Experiments were performed on a 2.1 T Oxford whole body magnet interfaced to a Bruker Avance spectrometer. ^{1}H NMR (MRI, GABA editing) and decoupling were done with two 13 cm ^{1}H surface coils driven in quadrature, while ^{13}C NMR was done with a 8.5 cm surface coil. ^{13}C NMR spectra were acquired from an 81-144 ml volume using a localized (3D ISIS/1D OVS) adiabatic polarization transfer sequence [2]. WALTZ-16 decoupling was applied during the entire acquisition period. Three patients (two women) with refractory complex partial seizures took 3 gram of vigabatrin tablets one hour before spectroscopy. Other medications included phenytoin or carbamazepine. The isotopic enrichment of the plasma glucose was rapidly increased by raising glucose levels to 8-10 mM with an infusion of 99 % [1-^{13}C]-glucose. Plasma glucose concentrations were monitored frequently throughout the study (3 hours) to insure a steady glucose concentration and isotopic enrichment. This study was approved by the Yale University Human Investigations Committee.

Results

Fig. 1 shows localized ^{13}C NMR spectra from a control and a vigabatrin-medicated patient (four hours after a 3 gram vigabatrin dose) after the infusion of [1-^{13}C]-glucose. Both spectra are averaged over 20 minutes at the end of the infusion period (60 and 180 minutes, respectively) when all resonances were at steady-state. The ^{13}C NMR spectra show a large number of resonances originating from metabolites which are labeled from [1-^{13}C]-glucose through oxidative metabolism, i.e. C2-C4 glutamate and glutamine. The C4-glutamate resonance was visible in all spectra after the onset of infusion and stabilized after ~45 minutes. Besides the single-labeled resonances, the C3 and C4 glutamate resonances show sidebands originating from double-labeled [3,4-$^{13}C_2$]-glutamate. Unlike the control spectrum, the vigabatrin spectrum shows a clear resonance from [2-^{13}C]-GABA at 35.3 ppm. [2-^{13}C]-GABA increased up to 2-3 hours after the onset of infusion. The increased absolute GABA concentration after vigabatrin medication was confirmed by edited ^{1}H MRS.

Discussion

The ^{13}C MRS data presented here confirms earlier ^{1}H MRS results that GABA is elevated ~2-3 times by acute dosing of vigabatrin. The high degree of [2-^{13}C]-GABA labeling indicates that the entire GABA pool is metabolically active. The maximum [2-^{13}C]-GABA intensity was not reached until 2-3 hours after the start of infusion. Compared with the acute rate of GABA increase measured by ^{1}H NMR (stabilization within 1.5 hours), the ^{13}C NMR results suggest that the GABA synthesis rate may be inhibited once the GABA concentration reaches steady-state after acute vigabatrin dosage.

References

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Acknowledgments

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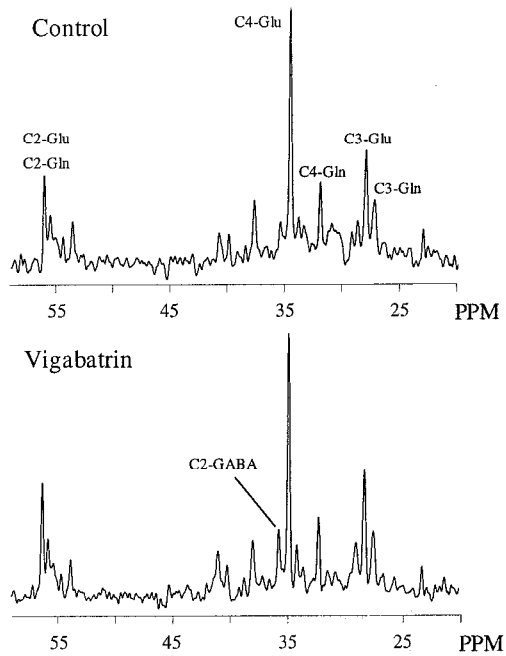

Figure 1 : Localized ^{13}C NMR spectra from a normal volunteer (top) and a vigabatrin-medicated epilepsy patient.