Detection of GABA C1 turnover in rat brain in vivo

J. Yang[1,2], and J. Shen[3]

[1]NIMH, United States, [2]Samsung Biomedical Research Institute, Korea, Republic of, [3]NIMH, Bethesda, MD, United States

Introduction

GABA is the primary inhibitory neurotransmitter in mammalian brain. GABA turnover has been found to increase in the presence of high K^+ concentrations, electrical stimulation, or bicuculline-induced seizures ex vivo or in vivo (1,2). It is also regulated by changes in GABA receptor activity (3). For example, potentiation of postsynaptic GABAergic transmission by benzodiazepines or hypoglycemia down-regulates GABA turnover. ^{13}C NMR/MRS has been used to measure GABA turnover ex vivo and in vivo (e.g., 4-6). Essentially, all current ^{13}C NMR-based methods for studying GABA turnover use [1-^{13}C] or [1,6-$^{13}C_2$]glucose infusion and detection of ^{13}C-label incorporation into GABA C2. It was recently showed that ^{13}C label incorporation into glutamate and glutamine in the carboxylic/amide spectral region can be detected in vivo free from any lipid interference and using low RF power for proton decoupling (7). Here, we attempted to study GABA turnover and the effect of GABA transaminase inhibition in vivo in the rat brain at 11.7 Tesla. This study is the first to report detection of cerebral GABA C1 turnover in vivo.

Materials and Methods

All experiments were performed using a Bruker 11.7 Tesla spectrometer. Isoflurane-anesthetized male Sprague-Dawley rats (167–202 g, n = 5) were intravenously infusion with [2,5-$^{13}C_2$]glucose. Plasma glucose concentrations was rapidly raised to and maintained at ~19.8 mM. Rectal temperature was at ~37.5 °C. Normal arterial blood physiological parameters were maintained by small adjustments of respiration rate and volume (pH = 7.38 ± 0.03, pCO_2 = 41 ± 5 mmHg, pO_2 = 125 ± 22 mmHg, mean arterial blood pressure > 100 mmHg with few exceptions). Animals received gabaculine treatment (100 mg/kg, 0.6 cc, i.v.; BIOMOL Research Laboratories, Plymouth Meeting, PA) 2.5 hours after the start of the [2,5-^{13}C2]glucose infusion. Two hours after gabaculine administration, the infusate was switched to unlabeled glucose for a total of 4.4 hour isotope chase.

A 8.5 x 6 x 8.5 mm^3 spectroscopy voxel was placed at the gradient isocenter along the brain midline. A train of non-selective hard pulses with a nominal flip angle of 180^o spaced at 100 ms apart was used to generate broadband ^{1}H→^{13}C heteronuclear Overhauser enhancement. Direct three-dimensional spatial localization of ^{13}C spins in the carboxylic/amide region used a 0.75 ms adiabatic half-passage pulse, followed by three pairs of hyperbolic secant pulses (one pair per dimension, 2-ms per pulse, with phase factor = 5 and truncation level = 1%). The ^{13}C 180^o pulses also refocused the long-range heteronuclear ^{1}H-^{13}C couplings during TE. No additional outer volume suppression schemes were found to be necessary. Spectral width was set to 10 kHz with a data sampling time of 204.8 ms. During the data sampling time, ^{1}H decoupling was applied, which uses a pseudo noise decoupling scheme with constant γB_2 amplitude and randomly inverted phases (7) and a repetition unit of 0.2-0.4 ms. The pseudo noise decoupling scheme allowed effective broadband proton decoupling at 11.7 Tesla with a TR-averaged forward decoupling power at or above 5 mW. For each time point, 30 scans were acquired.

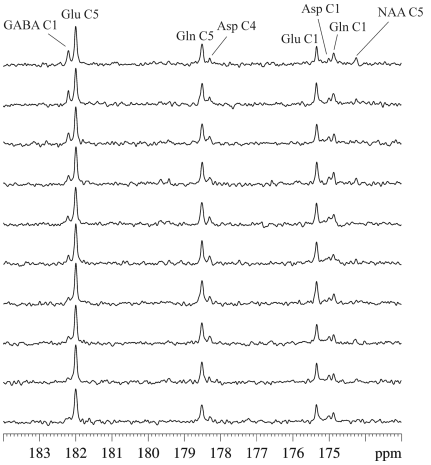

Fig. 1. ^{13}C spectra of GABA-transaminase inhibition during [2,5-$^{13}C_2$]glucose infusion. Total experimental time = ~2 hrs.

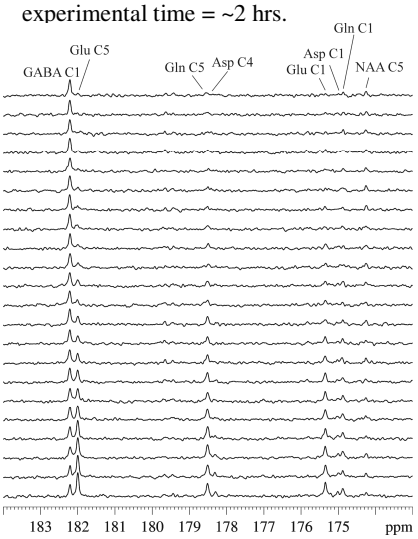

Fig. 2. ^{13}C spectra during isotope chase using unlabled glucose. Total experimental time = 4.4 hrs.

Results and Discussion

Typical in vivo ^{13}C spectra acquired during acute GABA-transaminase inhibition and continuous infusion of [2,5-$^{13}C_2$]glucose are shown in Figure 1. The narrowly confined lipid carboxylic signals at 172.5 ppm were completely suppressed by voxel localization, although the lipid signals do not present any spectral interference even without voxel localization. Upon administration of gabaculine, the most significant change in Figure 1 is the increase in GABA C1 at 182.3 ppm. The spectra in Figure 2 were acquired during isotope chase using unlabeled glucose. Signals of glutamate C5 and C1, glutamine C5 and C1, and aspartate C4 and C1 gradually lost their intensity during the ^{13}C isotope chase period due to the tricarboxylic acid cycles and the glutamate-glutamine cycle. The most striking observation from the isotope chase experiment was the slow turnover of GABA C1. After the isotope chase began, the continued increase in total GABA concentration was largely offset by the loss in ^{13}C labels from GABA C1. As a result, the variations in the signal intensity of GABA C1 during the 4.4 hour chase period were relatively small. At the end of the chase period, the plasma fractional enrichment of [2,5-$^{13}C_2$]glucose was determined to be 1.4 ± 1.6% (mean ± SD, n = 5). GABA C1 was the only major signal remaining at the end of the isotope chase. Similar to GABA, and in contrast to glutamate and glutamine, the N-acetylaspartate C5 signal at 174.3 ppm also showed fewer changes in intensity during the isotope chase. Based on high-resolution in vitro NMR analysis of brain perchloric acid extracts, the end point [^{13}C]GABA C1 concentration was 4.5 ± 0.3 μmol/g (n = 5, mean ± SD), corresponding to an isotopic fractional enrichment of 49.7 ± 2.6% (n = 5, mean ± SD).

This study also showed that low power broadband stochastic proton decoupling is feasible even at very high field strength, providing the impetus for developing carboxylic/amide ^{13}C MRS methods to study brain metabolism and neurotransmission in human subjects at high magnetic fields (e.g., 7 or 11.7 Tesla).

References 1. De Belleroche et al, J Neurochem 1972 19:585; 2. Chapman et al, J Neurochem 1983 41:886. 3. Lindgren S. J Neural Transm 1987 69:33-46; 4. Manor et al, Neurochem Res 1996 21:1031; 5. Yang et al, Magn Reson Med 2005 53:1258; 6. de Graaf et al, Neurochem Int 2006 48:508; 7. Li et al, Magn Reson Med 2007 57:265.