Increased cortical TCA cycle rate during functional stimulation determined at 11.7 Tesla

J. Yang[1], D. Li[1], J. Shen[1]
[1]NIMH, Bethesda, MD, United States

Introduction

Recent technical improvement in localized spectroscopy at ultrahigh magnetic field strength has made it possible to simultaneously detect glutamate, glutamine, and gamma-aminobutyric acid turnover in brain from intravenously infused ^{13}C-labeled substrates using Proton-Observed, Carbon-13 Edited (POCE) spectroscopy methods. In this study, we measure the time courses of [4-^{13}C]glutamate, [4-^{13}C]glutamine from the somatosensory cortex of α-chloralose anesthetized rats with and without forepaw stimulation to evaluate the effect of focal neuronal activation on the turnover of [4-^{13}C]glutamate and [4-^{13}C]glutamine.

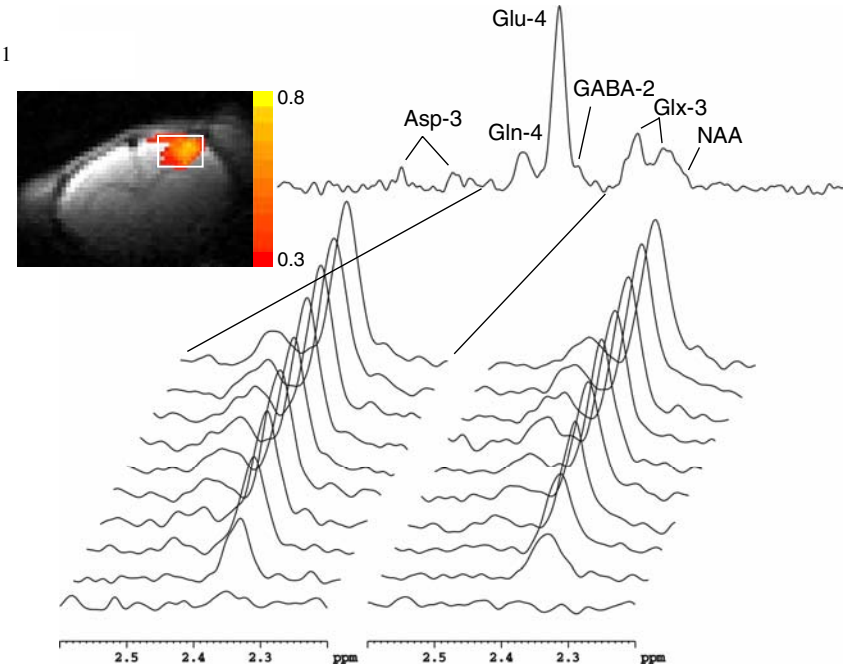
Methods

Male Sprague-Dawley adult rats fasted for 24 hours were studied. Control group (n = 5) received no forepaw stimulation and were infused with [1,6-$^{13}C_2$]glucose for measuring the kinetics of [4-^{13}C]glutamate and [4-^{13}C]glutamine turnover in the somatosensory cortex. The stimulated group (n = 6) were studied with forepaw stimulation. 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. α-Chloralose (initial dose: 80 mg/kg supplemented with a constant infusion of 26.7 mg/kg/hr throughout the experiment) and [1,6-$^{13}C_2$]glucose were infused intravenously. Electrical pulses (3 Hz, 0.3 ms, 5 mA) were generated using an external stimulator connected to needles inserted in between digits 1, 2 and 3, 4 under the skin, respectively. All measurements were performed at 11.7 Tesla from a 3.5 x 2.5 x 4 mm^3 (35 µL) voxel located at the primary somatosensory cortex. The intravenous glucose infusion protocol consists of an initial bolus of 162 mg/kg/min of 1.1 M [1,6-$^{13}C_2$]glucose in the first five minutes followed by constant-rate infusion of the same glucose solution to raise the total plasma glucose level rapidly to ~11.0 mM measured 10 min after the start of [1,6-$^{13}C_2$]glucose infusion. Plasma glucose condition was maintained approximately constant at an averaged concentration of 17.3 mM. The POCE signals were quantified based on in vitro high-resolution NMR spectra of brain PCA extracts. A two-compartment metabolic model describing the glutamate-glutamine cycling flux between glutamatergic neurons and glia was used to analyze the [4-^{13}C]glutamate and [4-^{13}C]glutamine time courses.

Results

Fig. 1 shows the time courses of in vivo POCE spectra. Left stack: with forepaw stimulation; right stack: control without forepaw stimulation. The expanded POCE difference spectrum from the end point spectrum in the left stack was shown. ^{13}C label incorporation into aspartate C3 (Asp-3), glutamine C4 (Gln-4), glutamate C4 (Glu C4), gamma-aminobutyric acid C2 (GABA-2), glutamate and glutamine C3 (Glx-3), and N-acetyl aspartate C6 (NAA) were indicated. The Gln-4 at 2.46 ppm and GABA-2 at 2.30 ppm were spectrally resolved from Glu-4 at 2.35 ppm at 11.7 Tesla. The inset shows the BOLD map overlaid on a 64 x 64 single-shot EPI image. Color bar indicates the scale of cross-correlation coefficient. In controls, the tricarboxylic acid cycle (TCA) rate V_{TCA} was determined to be 0.49 ± 0.10 µmol/g/min, (mean ± SD, n = 5), which is significantly different from the stimulated group (with forepaw stimulation, V_{TCA} = 0.68 ± 0.11 µmol/g/min, mean ± SD, n = 6, $p < 0.05$, unpaired t test). No statistically significant difference was found in the rate of the glutamate-glutamine cycling flux ($V_{Glu\text{-}Gln\text{-}cyc}$ = 0.19 ± 0.07 µmol/g/min, mean ± SD, n = 6, for the stimulated group vs. $V_{Glu\text{-}Gln\text{-}cyc}$ = 0.15 ± 0.07 µmol/g/min, mean ± SD, n = 5, for the non-stimulated group, $p > 0.4$, unpaired t test). The stimulated group's endpoint fractional enrichments (FEs) of glutamate and glutamine determined from brain PCA extracts are significantly greater than the corresponding FEs in non-stimulated group: FE(Glu-4) = 0.68 ± 0.02 (n = 6) vs. 0.64 ± 0.02 (n = 5, mean ± SD, $p < 0.05$); FE(Gln-4) = 0.48 ± 0.03 (n = 6) vs. 0.44 ± 0.03 (n = 5, mean ± SD, $p < 0.05$). Inclusion of a simultaneous fit of the GABA turnover time course into the cost function was not used due to the much larger scatter of the GABA-2 time course data.

Fig. 1



focal activation control

Discussion

The relative change in cerebral TCA cycle rate ($\approx \Delta CMRO_2/CMRO_2$) during functional stimulation has been inconsistent with the reported values ranging from 30% to ~200% (e.g., 1, 2 and refs therein). None of the previous reports separated [4-^{13}C]glutamate from the overlapping [4-^{13}C]glutamine and [2-^{13}C]GABA. With the significant technical improvement achieved at 11.7 Tesla, we found a moderate but statistically significant increase (~39%) in the rate of the TCA cycle due to focal activation. The results presented in this study are in reasonable agreement with $\Delta CMRO_2/CMRO_2$ values calculated from changes in BOLD and CBF during forepaw stimulation (3). Our results also closely match the POCE results obtained from the visual cortex of conscious human brain during photic stimulation (1). From the 35 µL voxel, we were unable to detect from the much smaller [4-^{13}C]glutamine and [2-^{13}C]GABA signals a statistically significant change in the rate of the glutamate-glutamine cycling flux or the GABA synthesis flux in the primary somatosensory cortex of α-chloralose anesthetized rats although a non-significant rising trend was seen in $V_{Glu\text{-}Gln\text{-}cyc}$ of the stimulated group. The scatter in $V_{Glu\text{-}Gln\text{-}cyc}$ can be significantly reduced by the use of intra-subject comparison (1, 4), which, in this case, requires simultaneous data acquisition from the ipsilateral somatosensory cortex. The quality of the data was sufficient to detect the change in V_{TCA} without the use of the ipsilateral control experiment, which, without dynamic shimming, would cause a significant degradation of data quality due to B_0 inhomogeneity. No statistical difference in the endpoint FE(GABA-2) was found between the two groups.

References

1. Chen et al, MRM 45:349 (2001). 2 Smith et al, PNAS 99:10765 (2002). 3. Liu et al, MRM 52:277 (2004). 4. Xu et al, NeuroImage 28:401 (2005).