Comparison of Amino Acid Turnover Rates in Cortex and Isolated Nerve Terminals of the Anesthetized Rat

A. B. Patel[1], J. C. Lai[2], G. M. Chowdhury[3], D. L. Rothman[1], K. L. Behar[3]

[1]Diagnostic Radiology, Magnetic Resonance Research Center, Yale University, New Haven, Connecticut, United States, [2]Department of Pharmaceutical Sciences, Idaho State University, Pocatello, Idaho, United States, [3]Psychiatry, Magnetic Resonance Research Center, Yale University, New Haven, Connecticut, United States

INTRODUCTION: ^{13}C NMR studies of cerebral metabolism have established a linear relationship between rates of neurotransmitter cycling (V_{cyc}) and neuronal glucose oxidation ($CMR_{glc(ox)N}$) (1,2). These studies estimated metabolism of whole cortical tissue, reflecting metabolism at the cellular level without differentiation of cell body or nerve terminal. The compartmentation of neurotransmitter glutamate and GABA between metabolic and vesicular pools, with distinct metabolic rates was proposed in late 1960s. The potential significance of subcellular compartmentation of glutamate and GABA metabolism for the *in vivo* rates measured with MRS is not currently known. In the present study, we have investigated ^{13}C turnover of neurotransmitters in nerve terminals and compared it with values in cortical tissue.

MATERIALS AND METHODS: Sprague-Dawly rats, fasted overnight, were anesthetized with halothane, tracheotomized and ventilated (30% O_2/68.5%N_2O/1.5% halothane), and a femoral artery and vein were cannulated for measurements of blood gases, blood glucose, and infusion of ^{13}C labeled substrates. Rats were infused with either [1,6-$^{13}C_2$]glucose for different times (8, 20, 60, 120 min) or [2-^{13}C]acetate for 20 min. At the selected times rats were decapitated, brains were quickly removed and homogenized in an ice-cold isotonic buffered sucrose, and nerve terminals were isolated from whole forebrain (not including cerebellum or pons/medulla) using Ficoll-sucrose density gradient centrifugation (3). Nerve terminals were also prepared from halothane-anesthetized rats undergoing bicuculline-induced seizures while infused with [1,6-$^{13}C_2$]glucose for 8 min. Metabolite levels and ^{13}C enrichments were measured from lyophilized ethanol extracts (4) of the isolated nerve terminals and from cortical tissue (removed prior to homogenization) using ^{1}H-[^{13}C]-NMR spectroscopy at 11.7 T (Bruker AVANCE). A three-compartment (glutamate neuron, GABA neuron, astrocyte) metabolic model was fitted to the time course data to estimate metabolic fluxes in cortical tissue (5). Time constants ($T_{1/2}$) of ^{13}C labeling of nerve terminals glutamate and GABA were estimated using least square exponential fitting of the turnover data.

RESULTS AND DISCUSSION: The time courses of ^{13}C labeling of glutamate, GABA, and aspartate in the isolated nerve terminals and whole cortical tissue are depicted in Fig. 1. Rates of glucose oxidation in glutamatergic and GABAergic neurons, as revealed by metabolic modeling of the ^{13}C turnover of amino acids in the whole cortical tissue, were 0.328±0.029 and 0.151±0.039 μmol/g/min, respectively. Rates of neurotransmitter cycling for glutamate and GABA neurons in the whole cortical tissue were 0.297±0.027 and 0.149±0.035 μmol/g/min, respectively. Nerve terminal ^{13}C labeling was consistently lower than whole cortical tissue labeling for all three amino acids suggesting either a constant dilution or subcellular heterogeneity (e.g., non-turning over pools). The half times of neurotransmitter labeling from [1,6-$^{13}C_2$]glucose in nerve terminals (Glu_{C4}, 22.0±2.5; $GABA_{C2}$, 21.4±3.8; Asp_{C3}, 20.2±1.7 min) were longer than the respective values for cortical tissue (Glu_{C4}, 12.6±1.6; $GABA_{C2}$, 14.8±2.6; Asp_{C2}, 27.7±5.6 min). However, ^{13}C labeling of nerve terminal neurotransmitters from [2-^{13}C]acetate-infused rats exceeded the values in whole cortical tissue indicating that astroglial glutamine was the likely source of much of the nerve terminal dilution. The slower turnover rates of amino acids in the isolated nerve terminals compared to the cortex probably reflects their isolation from the whole brain which sampled regions with lower metabolic rates than the cortex. In nerve terminals isolated from rats during bicuculline seizures, ^{13}C labeling of the amino acids were increased 40-60% relative to the basal condition, which was similar to the increase (60-70%) measured in cortical tissue from the same preparation. These data suggest that the turnover rate of the nerve terminal pool of neurotransmitter glutamate and GABA is similar to the whole tissue rates assessed *ex vivo* or *in vivo* by NMR. The replenishment of most of the nerve terminal pool occurs directly via trafficking of astroglial glutamine.

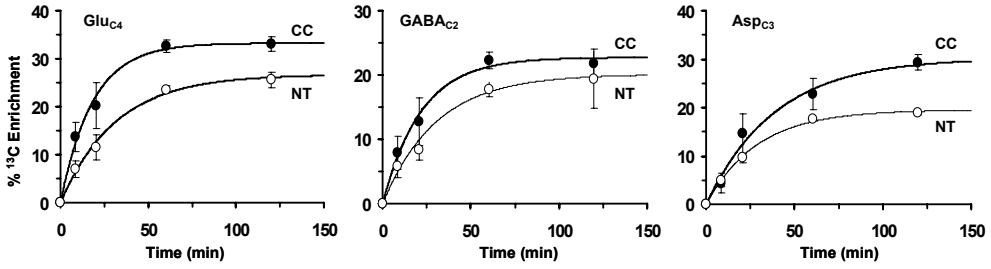

Fig. 1 ^{13}C Turnover of amino acids in nerve terminals and cortical tissue. Lines are drawn by exponential fitting of data

Table 1 The time constant ($T_{1/2}$) of ^{13}C turnover of amino acids in nerve terminals from [1,6-$^{13}C_2$]glucose

	Glu_{C4}	$GABA_{C2}$	Asp_{C3}
Cortex	12.6±1.6	14.8±2.6	27.7±5.6
Nerve Terminals	22.0±2.5	21.4±2.8	20.2±1.7

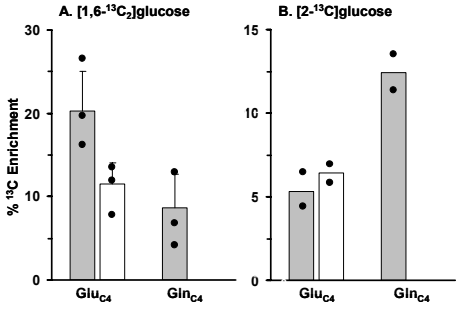

Fig. 2 ^{13}C Labeling of amino acid in nerve terminals (□), and cortical tissue (▨) from [1,6-$^{13}C_2$]glucose, and [2-^{13}C]acetate

REFERENCES: (1) Sibson N et al. *Proc Natl Acad Sci* **95**:316-321, 1998; (2) Patel AB et al. *J Cereb Blood Flow Metab* **24**:972-985, 2004; (3) Lai JCK and Clark JB. *Neuromethods* **11**:43-98, 1989; (4) Patel AB et al. *Brain Res* **919:**207-220, 2001; (5) Patel AB et al. *Proc Natl Acad Sci* **102:**5588-5593, 2005.

ACKNOWLEDGEMENTS: This study was supported by NIH grants NS34813 and DK27121.