Simultaneous Measurement of Neuronal and Astroglial Metabolism in Mouse Brain

A. B. Patel[1], P. Bagga[1], K. Varadarajan[1], and T. Rohith[1]
[1]NMR Microimaging and Spectroscopy, Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India

INTRODUCTION: *In vivo* experiments conducted by infusing ^{13}C labeled substrates (glucose and acetate) together with ^{13}C NMR spectroscopy have given important insight for the neuronal[1] and astroglial[2] metabolism. These studies have established that neurotransmitter cycle is stoichiometrically coupled to the neuronal glucose oxidation indicating neurotransmitter energetics is supported by oxidative glucose metabolism[3].However, these measurements were conducted with either glucose or acetate for the measurement of neuronal or astroglial function. It is desirable to investigate neuronal and astroglial metabolism together. In this study we have used a novel approach of infusion of [U-$^{13}C_6$]glucose and [2-^{13}C]acetate together with ^{13}C NMR spectroscopy to study neuronal and astroglial metabolism simultaneously.

MATERIALS AND METHODS: All animal experiments were performed under approved protocol by the CCMB Animal Ethics Committee. C57BL6 mice (2 months old) were used for the study. Overnight fasted mice were anesthetized with urethane (1.5 g/kg, i.p.) and tail vein was cannulated for the infusion of ^{13}C labeled substrates. Mice were infused with [U-$^{13}C_6$]glucose and [2-^{13}C]acetate for 10, 20, 40, 60, 90, 120 min as a bolus-variable rate infusion[4]. At the end of the experiment, brain was frozen *in situ* in liquid nitrogen. Different brain regions (cortex, cerebellum and sub cortex) were dissected under frozen conditions. Metabolites were extracted from frozen tissues[5]. The concentrations and percent ^{13}C enrichment of metabolites were determined from the ^{1}H-[^{13}C]-NMR spectrum of the extract acquired at 14T (Bruker AVANCE spectrometer)[6] . ^{13}C-[^{1}H]-NMR spectrum was recorded for the isotopomer analysis of Glu_{C4}, $GABA_{C2}$ and Gln_{C4}. The percentage ^{13}C enrichment of plasma glucose-C1 and acetate-C2 was measured using ^{1}H NMR peak of glucose-C1 at 5.2 ppm and acetate 1.91 ppm, respectively. The contribution of [U-$^{13}C_6$]glucose and [2-^{13}C]acetate for the total measured ^{13}C labeling of amino acids from ^{1}H-[^{13}C]-NMR spectrum was determined using isotopomer analysis of ^{13}C-[^{1}H]-NMR of tissue extract.^{13}C Turnover of amino acids from glucose and acetate was analysed for half time and initial rate of labeling using an exponential fitting script written in Matlab for all regions.

RESULTS AND DISCUSSION: Isotopomers of glutamate, glutamine and GABA obtained from [U-$^{13}C_6$]glucose and [2-^{13}C]acetate at different carbon positions were predicted using the biochemical knowledge. Predicted isotopomers for $GluC_4$ from [U-^{13}C]glucose (D_{45} and DD_{345}) and [2-^{13}C]acetate (S4 and D34) along with $GABA_{C2}$ and Gln_{C4} could be seen in ^{13}C-[^{1}H]-NMR spectra of different brain regions. The turnover curve of Glu_{C4} from [U-$^{13}C_6$]glucose in cortex and cerebellum obtained after de-convolution of measured total ^{13}C labeling using ^{1}H-[^{13}C]-NMR and isotopomers knowledge from ^{13}C-[^{1}H]-NMR is shown in Fig.1. Similar analysis has been carried out for the turnover of Glu_{C4} from [2-^{13}C]acetate as well as for $GABA_{C2}$ and Gln_{C4}. The calculated half time for turnover of amino acids is presented in Table 1. The half time for Glu_{C4} and $GABA_{C2}$ increased in the order; Cortex < SubCortex < Cerebellum while that of Gln_{C4} from [2-^{13}C]acetate is in the order; SubCortex < Cortex < Cerebellum. The initial rate of synthesis of Glu_{C4} and $GABA_{C2}$ from glucose is maximum in cortex and lowest in cerebellum indicating highest metabolic activity in the former (Fig. 2A). The highest rate for Glu_{C4} from acetate in cortex suggests higher neurotransmitter cycling flux in it (Fig. 2B). Further, higher value for Gln_{C4} rate from acetate in SubCortex and cerebellum compared to cortex indicates higher glial activity in former brain regions.

Table 1 Half time of ^{13}C turnover of amino acids from glucose & acetate

	Half Life Time (min)					
	Cortex		Sub Cortex		Cerebellum	
	Glucose	Acetate	Glucose	Acetate	Glucose	Acetate
Glu_{C4}	6.9±0.6	31.01±6.6	8.04±0.8	26.7±5.2	9.1±1.1	48.7±13.0
$GABA_{C2}$	10.4±1.5	66.3±29.7	16.6±3.4	22.7±8.8	18.9±4.6	32.9±13.7
Gln_{C4}	19.6±1.9	28.8±4.1	27.6±6.4	20.9±5.8	25.6±7.6	32.2±9.4

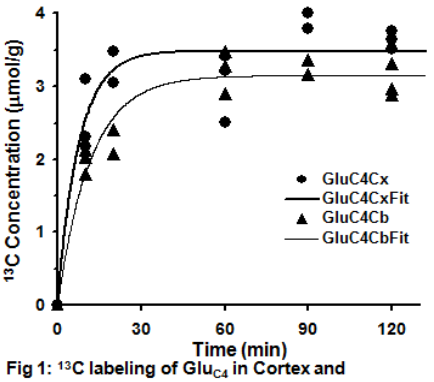

Fig 1: ^{13}C labeling of Glu_{C4} in Cortex and Cerebellum

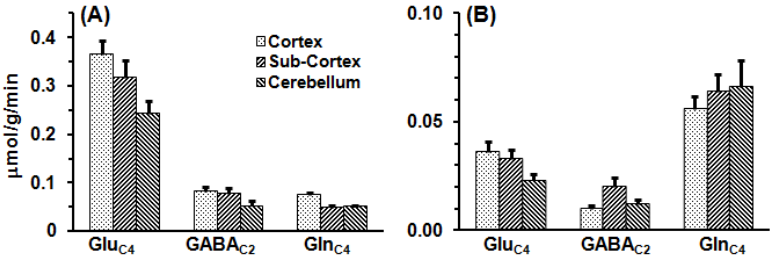

Fig 2: Initial rate of synthesis of amino acids from (A) [U-$^{13}C_6$]glucose, [B] [2-^{13}C]acetate. Rate for GABA and Gln are twice the actual values

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