NMR Investigations of Excitatory and Inhibitory Neurotransmission in Mouse Brain

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INTRODUCTION: Glutamate and GABA are major excitatory and inhibitory neurotransmitters in the mature central nervous system, respectively. It is well established that a neuronal-astrocytic substrate cycle exists in the brain. In this cycle, glutamate released from neurons into the synaptic cleft is taken up by astrocytes, converted to glutamine, and returned to neurons in synaptic inactive form[1]. ^{13}C NMR studies in the brain during ^{13}C-labeled substrate infusions have revealed new insights into the pathways of brain metabolism. It has been shown that neurotransmitter (glutamate/glutamine) cycle is coupled to neuronal glucose oxidation in 1:1 ratio[2]. Cerebral metabolism in mouse brain is very essential to understand the pathophysiology of aging and various neurological disorders in mice models. Cerebral metabolism in mouse brain is not clearly understood. In this study we have investigated excitatory and inhibitory neuronal TCA cycle and neurotransmitter cycle flux in different regions of mouse brain using ^{1}H-[^{13}C]-NMR spectroscopy.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by CCMB Animal Ethics Committee. Two months old C57BL6 mice (n=30) were used. Overnight fasted mice were anesthetized with urethane (1.5 g/kg) and tail vein was cannulated for the infusion of ^{13}C labeled glucose and acetate. Mice were infused with either [1,6-$^{13}C_2$]glucose for different times (7, 15, 30, 60, 90 min) or [2-^{13}C]acetate for ~90 min as a bolus-variable rate infusion[3]. At the end of the infusion, the brain was frozen *in situ* in liquid nitrogen. Different brain regions (cortex, cerebellum, striatum, hippocampus, thalamus-hypothalamus and olfactory bulb) were dissected in cryostat. Metabolites were extracted from frozen tissues[4]. The concentrations and percent ^{13}C enrichment of metabolites were determined from the ^{1}H-[^{13}C]-NMR spectrum of the extract recorded at 14T (Bruker AVANCE spectrometer)[5]. The percentage ^{13}C enrichment of plasma glucose-C1 was measured using ^{1}H NMR from glucose-C1 at 5.2ppm. The ratio $V_{cycle(Glu/Gln)}/V_{TCA(Glu)N}$ was calculated as follows: $V_{cycle(Glu/Gln)}/V_{TCA(Glu)} = Glu_{NC4}/(Gln_{AC4} - Glu_{NC4})$, where Glu_{NC4} and Gln_{AC4} are the ^{13}C enrichments of neuronal Glu and astroglial Gln, respectively at steady state in [2-^{13}C]acetate infused mice[6]. The value of $V_{cycle(GABA/Gln)}/V_{TCA(GABA)}$ was calculated similarly by substituting $GABA_{NC2}$ for Glu_{NC4}. A three compartments (glutamate neuron, GABA neuron, astrocyte) metabolic model was fitted to the measured ^{13}C time courses of amino acids from [1,6-$^{13}C_2$]glucose to obtain the metabolic rate in different brain regions[6].

RESULTS AND DISCUSSION: Level of cerebral metabolites measured in different brain regions is shown in Fig. 1. These data indicate that the concentration of metabolites was found to be distinct in different brain regions. Cortex being the higher neural density region has significantly higher glutamate and NAA level than other brain regions. Level of GABA and Taurine was higher in Olfactory bulb. The results of metabolic analysis for glutamatergic and GABAergic neurons are presented in Fig. 2. Glutamatergic rate was highest in cortex while it was lower in thalamus and hypothalamus (Fig. 2A). This is in consistence with higher synapse density in cortex observed under electron micrograph. In contrast, GABAergic neurotransmitter cycle rate was higher in cerebellum and olfactory bulb than thalamic and hippocampal rate, which is in agreement with the reported higher GABAergic neuronal density in olfactory bulb[7].

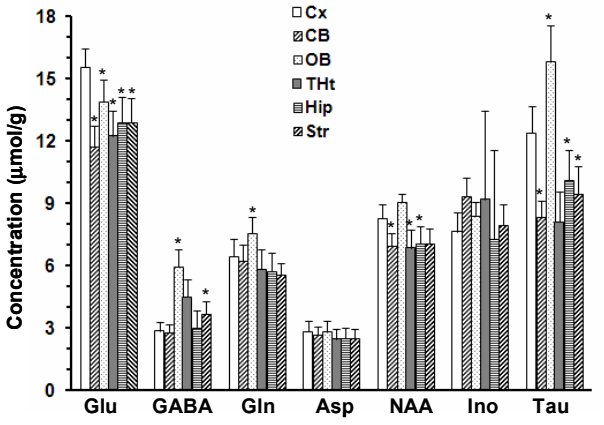

Fig. 1 Level of cerebral metabolites in different brain regions,* $p<10^{-5}$

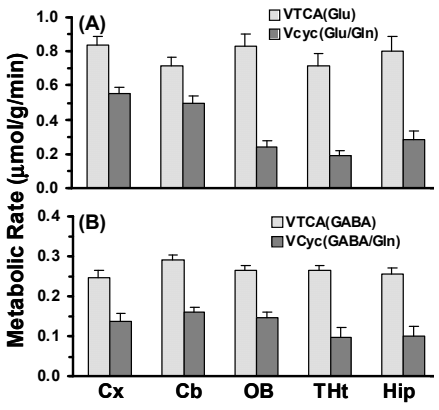

Fig. 2 TCA cycle and neurotransmitter flux: (A) Glutamatergic, (B) GABAergic neurons.

REFERENCES: 1. Benjamin and Quastel (1974) *J Neurochem* **23**:457; 2. Sibson et al (1998) *Proc Natal Acad Sci* USA **95**:316; 3. Fitzpatrick et al (1990) *J Cereb Blood Flow Metab* **10**:170; 4. Patel et al (2001) *Brain Res* **919**:207; 5. de Graaf et al (2003) *Magn Reson Med* **49**:37; 6. Patel et al (2005) *Proc Natl Acad Sci* **102**:5588; (7) Kuhn et al (2005) *Chem. Senses* **30(suppl 1)**:i109.

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