Exploring Energy Metabolism in Zebrafish Brain: A ^{1}H-[^{13}C]-NMR Study

Anant Bahadur Patel[1], Madhavi Adusmilli[1], Sandeep Saxena[1], and Mohammed M Idris[1]
[1]*CSIR-Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India*

INTRODUCTION: Glutamate and GABA are the most abundant neurotransmitters in the cerebral cortex and responsible for the excitatory and inhibitory neurotransmissions in the matured central nervous system. *In vivo* ^{13}C NMR studies in rat and human brain have established that the glutamate-glutamine cycle accounts for major fraction (>80%) of glutamine synthesis. Moreover, experiments conducted in rat brain under conditions of graded anesthesia have shown that rates of neurotransmitter cycle and neuronal glucose oxidation are stoichiometrically coupled through the entire level of brain activity, thus indicating that neurotransmitter energetic is supported by oxidative glucose metabolism[1]. Zebrafish is emerging as vertebrate model system for understanding the mechanism of different diseases. However, cerebral metabolism is not explored in zebrafish. In the present study brain metabolism is investigated in the zebrafish by ^{1}H-[^{13}C]-NMR spectroscopy together with administration of [1,6-$^{13}C_2$]glucose.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the Institutional Animal Ethics Committee. Zebrafish (~1 year age) were used for the study. Zebrafish were anesthetized using Tricane (0.1% Tricane (in water) and [1,6-$^{13}C_2$]glucose (10 µl, 0.225 M) was administered into heart using a fine Hamilton syringe. The animals were allowed to recover in water and the brain tissues were collected at different time points (15, 60 and 90 minutes) post injection and snap frozen in liquid nitrogen. In addition, acute effect of ethanol on brain metabolism in zebrafish was also assessed. In this experiment, zebrafish were acclimatized in 1 % ethanol for 2 hours and brain metabolism was followed by administering [1,6-$^{13}C_2$]glucose and returning them into water containing 1% ethanol. Brain was removed 30 min post [1,6-$^{13}C_2$]glucose administration. Brain metabolites were extracted from 4-5 pooled brain as described earlier[2]. Concentration and percentage ^{13}C enrichment of cortical amino acids were measured in ^{1}H-[^{13}C]-NMR spectrum of brain extracts acquired at 600 MHz spectrometer[3].

RESULTS AND DISCUSSIONS: Typical ^{1}H-[^{13}C]-NMR spectrum from zebrafish brain is presented in Fig. 1A. Similar to rat[4] and mouse[5] brain, signals from glutamate (6.1±1.1 µmol/g), GABA (4.0±0.8 µmol/g), glutamine (3.4±0.7 µmol/g), NAA (8.1±1.0 µmol/g) are seen. In addition, strong resonance from NAAG (4.3±0.7 µmol/g) is also visible at 2.1 ppm. The ^{13}C labeling of brain amino acids was measured by ^{1}H-[^{13}C]-NMR spectroscopy in brain extract. Labeling of glutamate-C4, GABA-C2 and lactate-C3 could be seen in the 30 min spectrum, which increases with time (Fig. 1B). At longer duration (60-90 min), ^{13}C labeling of glutamate-C3/C4, GABA-C2/C3/C4 and glutamine-C4 are clearly seen. The percent ^{13}C enrichment of amino acids with time are depicted in Fig. 2. The turnover of amino acids decreased in the order glutamate-C4 > glutamine-C4 > GABA-C2 > glutamate-C4, which is very similar to the pattern that has been reported for rat[6] and mouse brain[7]. Moreover, the incorporation of ^{13}C label from [1,6-$^{13}C_2$]glucose into amino acids was reduced when fish is exposed with 1% ethanol, suggesting that energy metabolism in zebrafish is decreased by ethanol. Acute ethanol has been shown to reduce the glutamatergic and GABAergic metabolism in mouse brain[8]. In summary, ^{13}C labeling of amino acids in zebrafish follows the pattern similar to rat and mouse brain. Moreover, as the ^{13}C labeling of brain amino acids is modulated by ethanol, zebrafish would be useful alternate model to study mechanism of various neurological disorders and screening of drugs.

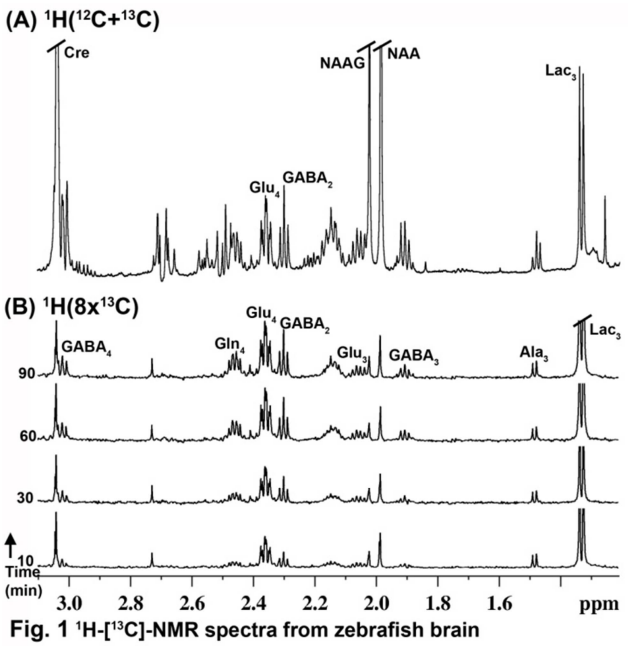

Fig. 1 ^{1}H-[^{13}C]-NMR spectra from zebrafish brain

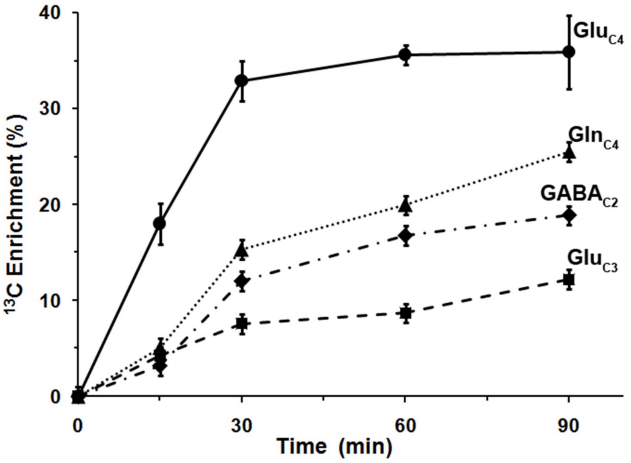

Fig 2. ^{13}C Turnover of brain amino acids from [1,6-$^{13}C_2$]glucose in zebrafish

REFERENCES: 1. Sibson *et al* (1999) *Proc Natl Acd Sic USA* **95**:316; **2**. Patel et al (2001) *Brain Res* **919**:207; **3**. de Graaf et al (2003) *Magn Reson Med* **49**:37; **4**. Tkác *et al* (1999) *Magn Reson Med* **41**:649; **5**. Tkác *et al* (2004) *Magn Reson Med* **52**:478; **6**. Duarte and Gruetter (2013) *J Neurochem* **126**:579; **7**. Tiwari *et al* (2013) *J Cereb Blood Flow Metab* **33**:1523; **8**. Tiwari *et al* (2013) *J Neurochem* doi: 10.1111/jnc.12508