**13C isotopic incorporation studies reveal alterations in glutamate-glutamine cycling in the hippocampus of aged Fischer 344 rats.**

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**Introduction**

Glutamate-glutamine cycling requires neuronal-astroglial interactions to produce and recycle glutamate. This cycle is coupled to neural activity and hippocampal activity decreases with age. Indeed, NMR data reveal that glutamate and glutamine labeling from isotopic glucose is reduced in cortical structures of aged rats. This decrease in neuronal activity could affect cortical function (e.g., cognition). Thus, the goal of this study was to examine age-related changes in glutamate-glutamine cycling and levels of key proteins in this process in hippocampal region CA1 and the dentate gyrus. LC/MS/MS was employed to determine isotopic enrichment of glutamate and glutamine resulting from metabolic incorporation of 13C-labeled acetate and glucose.

**Methods and Materials**

Animals:

Animals were housed with 95% O2/5% CO2 (pH 7.2-7.4). Using a vibratome, 400 μm-thick slices were cut from the CA1 and dentate gyrus. These slices were placed in an incubation chamber for 10 min. The incubation chamber contained 1.3 MgSO4, 1.25 NaH2PO4, and 10 C6H12O6 equilibrated cerebral spinal fluid (aCSF) for 1 min. The aCSF was assessed for pituitary tumors (excluded if found).

**Results**

**Figure 1.** Schematic diagram of isotopic acetate and glucose incorporation into glutamate and glutamine via the neuron (light yellow) or astrocyte (light gray).

**Figure 2.** Isotopic enrichment studies with 13C acetate revealed an increase in 13C incorporation into glutamate, but not glutamine, in CA1 and the Dentate Gyrus of aged rats.

**Figure 3.** Immunoblotting revealed no significant difference in Glutamine Synthetase levels.

**Figure 4.** Immunoblotting revealed a significant decrease in Glut 3 levels in the CA1. A similar, but non-significant trend was seen in the DG of aged rats.

**Figure 5.** Isotopic enrichment studies with 13C glucose revealed no significant change in 13C incorporation into glutamate or glutamine in CA1 and the Dentate Gyrus of aged rats.

**Summary and Conclusions**

Isotopic enrichment of glutamate from the incorporation of 13C acetate is significantly greater in both the CA1 region and dentate gyrus from aged rats (Figure 1). However, glutamine synthetase protein levels do not change with age, suggesting that the capacity of astroglial glutamine to glutamate is not altered with age (Figure 2) and is unlikely to underlie the change in isotopic labeling of glutamate. This age-related change is likely to reflect an increased dependence of aged neurons on astroglially-derived glutamine to replenish their glutamate supplies.

Neuronal dependence on glial-derived glutamine with aging could reflect changes in the capacity of aged neural tissue to uptake and/or utilize glucose due to alterations in transporter levels. Indeed, immunoblot data indicate that Glut 3 is significantly reduced in CA1 of aged rats. The dentate gyrus exhibits a similar trend (Figure 3). Thus, glucose uptake is likely to be altered in aged neurons. To assess this possibility, isotopic incorporation studies using 13C glucose were performed (Figure 5). However, these studies did not reveal a significant change in isotopic enrichment of glutamate or glutamine in the aged hippocampus.

These apparently inconsistent findings could reflect: 1) variability in the aged population, or 2) a need for using more physiological (glucose) in isotopic labeling experiments (e.g., 1-2 mM vs the 10 mM that was used here).

Studies are currently underway to assess these possibilities.

**Acknowledgments**

Funding provided by the Center for Alzheimer’s Disease and Related Disorders (CADRD) and Center for Integrated Research in Cognitive & Neural Sciences (CIR-CNS) at SIU

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1. Small S, Chawla MK, Buonocore M, Rapp PR, Barnes CA (2004) Imaging correlates of brain function in the aged population, or 2) a need for using more physiological (glucose) in isotopic labeling experiments (e.g., 1-2 mM vs the 10 mM that was used here).

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