Measurement of Anaplerosis in Rat Cortex During Intense Synaptic Activity: An *In Vivo* ^{13}C NMR Study

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INTRODUCTION:

It has been established that neuron-astrocyte substrate cycles exist between neuronal glutamate (Glu) and GABA and glial glutamine (Gln). As a consequence of mass balance, the only pathways of net Gln synthesis are the neurotransmitter (Glu/Gln) cycle and anaplerosis, the de novo synthesis of oxaloacetate from pyruvate and CO_2 via pyruvate carboxylase pathway. Anaplerosis accounts for ~20 % (0.09 μmol/g/min) of the total Gln synthesis rate (1) in deeply anesthetized rat cortex. Recently it was shown that the Glu/Gln cycle flux increased 2x during bicuculline induced seizures (2). However, the effect of increasing brain activity on the rate of anaplerosis was not determined. In the present study we infused [2-^{13}C]glucose to determine whether anaplerotic flux changed during bicuculline-induced seizures.

MATERIALS AND METHODS:

Two groups of Wistar rats (160-180g, fasted overnight) were studied: (A) baseline (no seizures), (B) bicuculline-induced seizures. Rats were anesthetized (1% halothane), tracheotomized and ventilated (30%O_2/69% N_2O), and arterial and venous catheters placed for monitoring of blood gases, blood pressure and infusion of [2-^{13}C]glucose. Rats were immobilized using D-tubocurarine chloride. *In vivo* experiments were performed on a 7T Bruker AVANCE spectrometer using a surface RF coil placed on the animal's head. Shimming was optimized using FASTMAP. POCE NMR (3) spectra were obtained from a localized volume (7x4x7 mm^3) centered in the cortex during infusion of [2-^{13}C]glucose. Seizures were induced by injection of bicuculline (1 mg/kg, i.v.), after the first 3 min of [2-^{13}C]glucose infusion. Arterial blood samples were taken periodically for the analysis of plasma glucose concentration and percent ^{13}C enrichment. *In vivo* peak intensities were quantified using LCModel (3). At the end of the experiment, the brain was frozen *in situ* with liquid N_2. Metabolites were extracted from the frozen cortical tissue (2). The concentrations and ^{13}C enrichment of amino acids were determined from the POCE spectrum of cortical extracts obtained at 11.74T (Bruker AM-500).

RESULTS AND DISCUSSION:

Consistent with a previous study of seizures (2), the concentrations of Glu (control 11.8±0.2 μmol/g vs. seizures 8.9±1.3 μmol/g) and Asp (control 3.1±0.2 μmol/g vs. seizures, 2.3±0.2 μmol/g) decreased, whereas Gln (control 6.5±0.1 μmol/g vs. seizures, 8.1±1.0 μmol/g) and GABA (control 2.2±0.1 μmol/g vs. seizures, 4.2±0.1 μmol/g) were increased. The sum of the concentrations of these amino acids did not change during seizures. The total amount of ^{13}C labeling of Gln and Glu during seizures followed the same time course as for the controls suggesting no significant change in the anaplerotic flux during seizures. Since our previous studies have shown that glutamate/ glutamine cycling flux increases > 2x during seizures (2) the current finding indicate that anaplerosis is not linked to glutamate/glutamine cycling. The anaplerotic flux previously measured under deep anesthesia remaining constant with activity, becomes a small fraction (~10%) of glutamate/glutamine cycling flux at higher levels of cortical activity. This finding also suggests that glutamine synthetase has sufficient activity to support the > 2x increase in glutamate release during seizures.

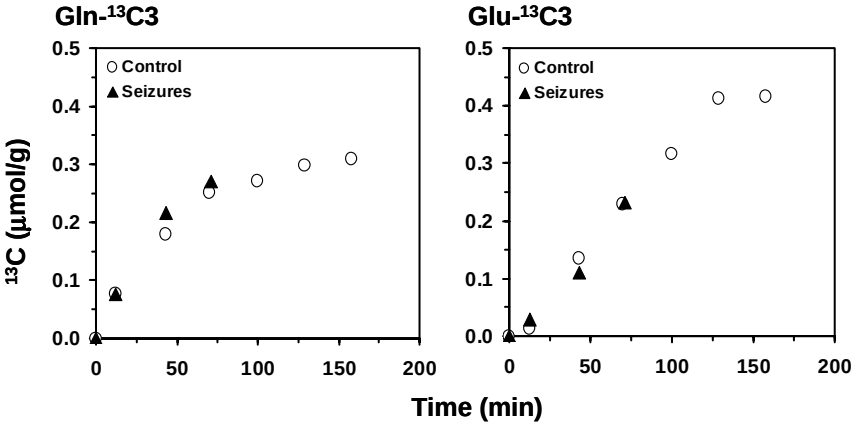

Fig. 1. Isotopic ^{13}C labeling of Gln-^{13}C3 and Glu-^{13}C3 during [2-^{13}C]glucose infusion in cortex of control and bicuculline-treated rats.

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