Absolute Quantification of GABA/Glutamine and Glutamate/Glutamine Cycle Fluxes in the Rat Cerebral Cortex: An In Vivo 13C NMR Study

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SYNOPSIS: The objective of this study was to measure the absolute values of the GABA/Gln and Glu/Gln cycle fluxes. We have used ^{13}C NMR spectroscopy together with infusion of labeled glucose and acetate to determine the metabolic flux under mild halothane and isoelectric pentobarbital anesthesia. The GABA/Gln cycle was highly active accounting for 22% of total neurotransmitter cycling. Isoelectricity eliminated neurotransmitter cycling and resulted in a 6 and 9 fold reduction of glucose oxidation in both glutamatergic and GABAergic neurons, respectively indicating that in both cell types the primary energetic costs are associated with activity.

INTRODUCTION: Glutamate (Glu) and γ-aminobutaric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the cortex. Neuron-astrocyte substrate cycles exist between neuronal Glu and GABA, and glial glutamine (Gln). Recently, we have shown that the Glu/Gln cycle ($V_{cycle(Glu/Gln)}$) flux and neuronal glucose oxidation ($CMR_{glc(ox)N}$) changed proportionately over the entire range of neuronal activity above isoelectricity (1, 2). However, there is no such measurement for the GABA/Gln cycle. In the present study we quantified Glu/Gln and GABA/Gln cycle fluxes under pentobarbital-induced isoelectricity and under halothane anesthesia. The objective of this study was to determine the relationship between the GABA/Gln and Glu/Gln cycle fluxes and for this purpose we have used [2-^{13}C]acetate infusion to allow the separate determination of these fluxes. The steady state ratios of the neurotransmitter cycle fluxes to their respective TCA cycle fluxes was then used in fitting the time courses of Glu C4, Gln C4 and GABA C2 labeling measured using [1,6-$^{13}C_2$]glucose.

MATERIALS AND METHODS: SD Rats were anesthetized with halothane, tracheotomized and ventilated (30% O_2/67.5%N_2O,1.5% halothane), and a femoral artery and both veins were cannulated for blood gas assessment and labeled isotope infusions. Two groups of SD rats (160-190g, fasted overnight) were studied: (A) Pentobarbital, 120 mg/kg, i.p. injected over 60min to induced an isoelectric EEG with supplementary doses of 20 mg/kg every 20min. These animals were infused with [1,6-^{13}Cglucose and acetate for 30 mins. (B) Halothane (0.5-1%) anesthesia. *In vivo* experiments were performed at 7Tesla (Bruker AVANCE spectrometer) using a surface coil over the rat's head. First and second order shims were adjusted using FASTMAP. After acquiring the baseline spectrum, infusion of [1,6-$^{13}C_2$]glucose and acetate began and ^{1}H-decoupled, ^{13}C NMR spectra were acquired serially using a polarization transfer sequence from a localized volume of 10x3x12 mm^3. In addition rats were also infused with [1-^{13}C]glucose and acetate on the bench for 7 mins and 20 mins (or [2-^{13}C]acetate and glucose for 2 hours) and the GABA enrichment was measured in extracts of the cortex prepared from the in situ-frozen brain. Amino acids were extracted from the frozen cortex and ^{13}C enrichments of of the different carbon positions were determined using POCE NMR spectroscopy at 11.7 Tesla (AM-500 Bruker Avance). 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 fractional enrichments at steady state in [2-^{13}C]acetate and glucose infused animals. The value of $V_{cycle(GABA/Gln)}/V_{TCA(GABA)}$ was calculated similarly. The in vivo time courses of Glu C4 and Gln C4 and the ex vivo time courses of Glu C4, Gln C4 and GABA C2 were then fitted to a three compartment model (glutamatergic neurons, GABAergic neurons, astroglia) to calculate the metabolic fluxes.

RESULTS: The GABA/Gln cycle and Glu/Gln cycle fluxes under halothane anesthesia was 0.10±0.01 and 0.35±04 μmol/g/min, respectively. The rate of glucose oxidation in GABAergic and glutamatergic neurons was 0.07±0.01 and 0.41±03 μmol/g/min, respectively. Under isoelectric conditions total neurotransmitter cycling (Glutamate+GABA) was reduced, consistent with the flat-line isoelectric condition measured by EEG. Oxidation of glucose under isoelectric conditions by GABAergic and glutamatergic neurons was 0.008±0.003 and 0.074±021 μmol/g/min, respectively.

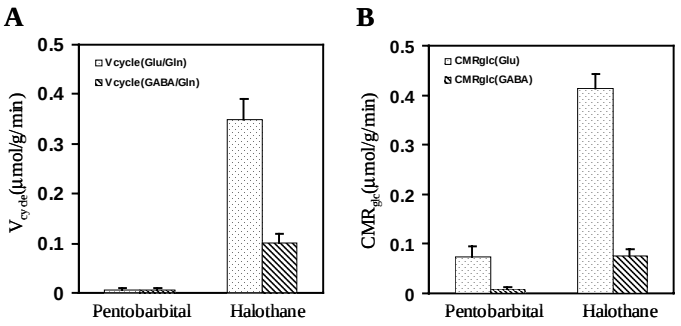

Fig.1 (A) GABA/Gln and Glu/Gln cycle flux with increasing activity, (B) Glucose oxidation by GABAergic and Glutamateric neurons with increasing activity

DISCUSSION: Under halothane anesthesia GABA/Gln cycling flux associated with GABAergic neurons accounted for 22% of total (GABA + glutamate) neurotransmitter cycling, indicating that this is a major pathway of GABA neurotransmitter repletion. Suppression of neuronal activity during pentobarbital-induced isoelectricity eliminated both neurotransmitter cycling fluxes, providing further evidence for the validity of the MR measurements. Glucose oxidation was suppressed ~6x and 9x in glutamatergic and GABAergic neurons, respectively indicating that for both cell types even under halothane anesthesia the major energy cost is associated with neuronal activity.

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REFERENCES: 1. Patel et al. (2002) Abst. Soc. Neurosci. 2. Sibson et al. (1998) Proc. Natl. Acad. Sci. 95:316-321.