Increased Inhibitory Neurotransmission Observed During Intense Neuronal Activation in Rat Cortex: An *Ex Vivo* ^{13}C NMR Study

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INTRODUCTION:

Glutamate (Glu) and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the cortex, respectively. It has been established that neuron-astroglia substrate cycles exist between neuronal Glu and GABA, and glial glutamine (Gln). Previous studies showed that Glu/Gln cycle flux and neuronal glucose oxidation changed proportionately over a large range of neuronal activity above isoelectricity (2, 3). Recently, GABAergic neurotransmission and energy consumption has been shown to be a significant fraction (20 %) of total (Glu+GABA) cycling and energy consumption, respectively (1). However, the relationship of GABAergic transmission with increased activity is not known. In the present study we measured Glu/Gln and GABA/Gln cycle fluxes under baseline and bicuculline-induced seizures. The objective of this study was to determine the relationship between the inhibitory and excitatory neurotransmission fluxes under intense synaptic activity. The ratio V_{cycle}/V_{TCA} was determined for GABAergic and glutamatergic neurons following an infusion of [2-^{13}C]acetate. The ratios were used as constraints in fitting the ^{13}C labeling time courses of Glu-^{13}C4, Gln-^{13}C4 and GABA-^{13}C2 measured during [1,6-$^{13}C_2$]glucose infusion to determine the absolute fluxes under baseline and seizures.

MATERIALS AND METHODS:

Two groups of halothane (1%) anesthetized Wistar rats (160-180g, fasted overnight) were studied: (A) baseline and (B) bicuculline-induced seizures. Rats were anesthetized, tracheotomized and ventilated (30% O_2/69%N_2O), and a femoral artery and both veins were cannulated for blood gas assessment and labeled isotope infusions. Rats were infused with [1,6-$^{13}C_2$]glucose+unlabeled acetate for specific times (baseline: 7, 17 and 90 min; seizures: 7, 17 and 55 min) or unlabeled glucose+[2-^{13}C]acetate, the latter long enough for ^{13}C the labeling of amino acids to reach a steady state value. Seizures were induced by injection of bicuculline (1 mg/kg, i.v.), 3 min after the start of the labeled substrate infusion. At the end of the infusion periods the brain was frozen *in situ* with liquid N_2 while ventilation continued. Cortical amino acids were extracted and ^{13}C enrichments were measured using POCE NMR spectroscopy at 11.74 Tesla (AM-500 Bruker Avance spectrometer). 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+unlabeled glucose infused rats (1). The value of $V_{cycle(GABA/Gln)}/V_{TCA(GABA)}$ was calculated similarly substituting $GABA_NC2$ for Glu_NC4. Time courses of Glu-^{13}C4, Gln-^{13}C4 and GABA-^{13}C2 were then fitted to a three compartment model (glutamatergic neurons, GABAergic neurons, astroglia) to calculate the metabolic fluxes (1).

RESULTS:

The ^{13}C turnover of Glu-^{13}C4 and GABA-^{13}C2 from [1,6-$^{13}C_2$]glucose (Fig. 1) was faster during bicuculline-induced seizures compared to baseline indicating both glutamatergic and GABAergic TCA cycle fluxes were increased during seizures. On the other hand, the ratios, V_{cycle}/V_{TCA}, relating the respective neurotransmitter cycles to their TCA cycle flux as obtained from the steady state measurement using [2-^{13}C]acetate, were similar (P>0.1, n=5) between glutamatergic and GABAergic neurons both at baseline and during seizures (Table 1). Together these results suggest that the respective neurotransmitter cycling (Glu/Gln and GABA/Gln) and TCA cycle fluxes increase proportionately in glutamatergic and GABAergic neurons in response to bicuculline-induced seizures.

Table 1: Ratios V_{cycle}/V_{TCA} for Glutamatergic and GABAergic neurons during seizures.

	V_{cycle}/V_{TCA}	
	Glutamatergic	GABAergic
Control	0.452±0.038	0.492±0.036
Seizures	0.445±0.067	0.526±0.046

DISCUSSION:

Electrical recording in slices has suggested that conductance of inhibitory neurotransmission increases with excitatory neurotransmission (4). Stimulation of inhibitory parallel fibers simultaneously with stimulation of excitatory climbing fibers leads to a further rise in the local field potential and cerebral blood flow (5) suggesting increased inhibitory activity increases energy consumption. In accordance with these findings our data indicate that both neurotransmission and energy consumption by inhibitory and excitatory neurons increases proportionately with increasing cortical activity.

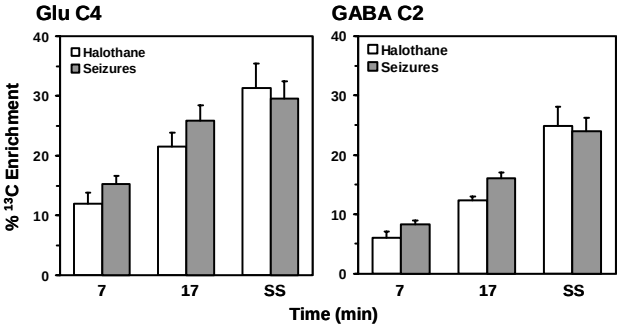

Fig. 1. Isotopic ^{13}C labeling of Glu C4 and GABA C2 in control and seizing rats following [1,6-$^{13}C_2$]glucose+acetate infusion

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