Assessment of the Energetics of Glutamatergic and GABAergic Neurotransmission in Rat Cortex during Postnatal Development

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INTRODUCTION:

Glucose and oxygen consumption in rat brain increases substantially during the first postnatal month in conjunction with the maturation of synapses, receptors, neuronal-glial metabolic interactions, and the maturation of sensory functions and electrical activity (1). The repletion of neurotransmitter glutamate and GABA is linked to neurotransmitter cycles, glutamate/glutamine and GABA/glutamine, between neurons and astroglia involving glutamine. In the mature rat cortex the glutamate/glutamine and GABA/Gln cycle fluxes constitute a major fraction of glucose oxidation by glutamatergic and GABAergic neurons, respectively (2). However, relationship between these fluxes with maturation of nervous system is not known. The objective of current study was to quantify fluxes of glutamatergic and GABAergic neurotransmission and energy consumption by these two neuronal types in the developing cortex. We infused [2-^{13}C]acetate to determine the ratios, V_{cycle}/V_{TCA} both for the GABAergic and glutamatergic neurons. These ratios were then 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 in 10 day old (P10) and 30 day old (P30) rat cortex.

METHOD:

Two groups of SD rats: (A) 10 day old (18~22g, n=30) and (B) 30 day old (90~110g, n=30) were studied under urethane anesthesia (1.5g/kg, i.p). Rats were infused with [1,6-$^{13}C_2$]glucose for specific times 7, 15, 30, 60, 120 and 150 min with 3 to 5 rats measured per time point. In P30 rat's blood was sampled periodically to measure the concentration and ^{13}C enrichment of plasma glucose. In P10 rats' blood was sample from the heart at the end of each infusion. In addition both, P10 and P30 rats were also infused with [2-^{13}C]acetate for 3 hrs. At the end of the infusion the brain was frozen *in situ* with liquid. N_2. Cortical amino acids were extracted and ^{13}C enrichments were measured (3) 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 infused rats. The value of $V_{cycle(GABA/Gln)}/V_{TCA(GABA)}$ was calculated similarly by substituting Glu_NC4 with $GABA_NC2$. Time courses of Glu-^{13}C4, Gln-^{13}C4 and GABA-^{13}C2 were then fitted to a three compartment model (Glu neuron, GABA neuron, glia) to calculate the metabolic fluxes constrained by values of the ratio V_{cycle}/V_{TCA} obtained for glutamatergic and GABAergic neurons from the [2-^{13}C]acetate measurement (2).

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

	V_{cycle}/V_{TCA}	
	Glutamatergic	GABAergic
P10	0.48±0.24	0.23±0.14
P30	0.47±0.11	0.26±0.04

RESULTS AND DISCUSSION:

Cortical glutamate (P10, 5.8±0.7; P30, 12.1±0.9 μmol/g, P<0.01), glutamine (P10, 4.7±0.8; P30, 6.8±0.7 μmol/g, P<0.01) and GABA (P10, 1.6±0.2; P30, 2.4±0.3 μmol/g, P<0.01) levels were significantly higher in P30 compared to P10 cortex. As shown in Table 1, the respective ratios of V_{cycle}/V_{TCA} for glutamatergic and GABAergic neurons did not change over this period of postnatal development, suggesting a unitary mechanism coupling neurotransmission to energetics (4) within each neuronal system. In contrast to the flux ratios, absolute fluxes for both neurotransmitter systems increased substantially during development. Glutamatergic TCA cycle flux ($V_{TCA(Glu)}$) and $V_{cycle(Glu/Gln)}$ increased to the same degree, 3.5x and 3.6x respectively, between P10 and P30, consistent with the 4x increase seen in neuronal TCA cycle flux in a previous study of the same postnatal ages (5). GABAergic neurotransmission also increased between postnatal day 10 and 30, although the increase was greater than for glutamate (V_{TCA}, 4.6x and $V_{cycle(GABA/Gln)}$, 6x). Our findings indicate that both neurotransmitter cycles are tightly coupled to their energetics by at least postnatal day 10 and remain constant through maturity.

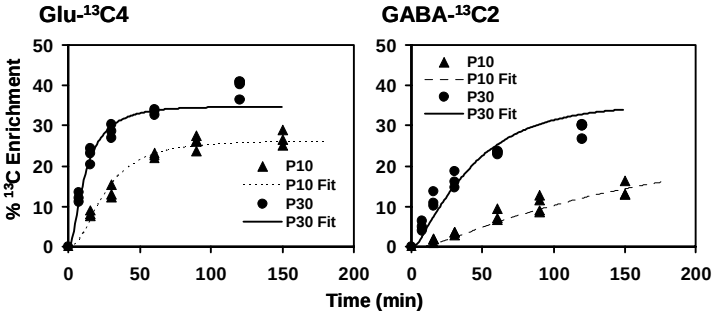

Fig. 1. Isotopic ^{13}C labeling of Glu-^{13}C4 and GABA-^{13}C2 in P10 and P30 rats following [1,6-$^{13}C_2$]glucose infusion

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