The Use of [2-^{13}C] Acetate to Determine the Pathway for Neurotransmitter Glutamate Repletion in the Human Brain

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Introduction

Glia has been shown to play a critical role in brain excitatory neurotransmission and metabolism (1). ^{13}C labeled acetate is ideal for probing glial metabolism (2) because it is metabolized specifically in glia (3). The aim of this study was to measure glial metabolism in humans using ^{13}C labeled acetate as a precursor: ^{13}C incorporation into brain glutamate and glutamine was detected by NMR spectroscopy during an IV infusion of [2-^{13}C] acetate in healthy volunteers. Three distinct pathways of neurotransmitter glutamate repletion were modeled and tested: 1) the glutamate/glutamine (Glu/Gln) cycle 2) the glutamate/alpha-ketoglutarate (Glu/alphaKG) cycle and 3) direct neuronal reuptake in which the glutamate/glutamine cycling would represent internal glial exchange.

Models of neuronal glutamate repletion

1) Glu/Gln cycle: In this model, neuronal glutamate is taken up by astrocytes and converted to glutamine by the astrocyte specific enzyme glutamine synthetase. Glutamine is transported to the extracellular fluid where it is taken up by neurons and converted back to glutamate. The flux through this cycle is Vcycle (Fig. 1).

2) Glu/alphaKG cycle: A 2nd model for glutamate repletion is based on the potential participation of alpha-ketoglutarate in terms of a Glu/alphaKG cycle between astrocytes and neurons (4) at the rate Vcycle'.

3) Internal exchange: In this model both Vcycle and Vcycle' have 0 flux. The rapid labeling of glutamine reported from [1-^{13}C] glucose is due to internal isotopic exchange in the glia.

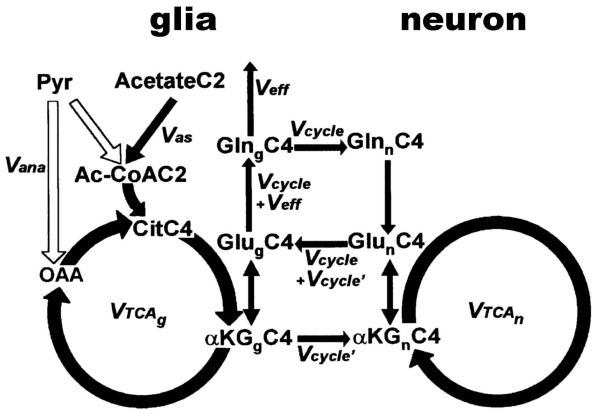

Fig 1. Label incorporation from [2-^{13}C] acetate. Vas = flux through the acetyl-CoA synthetase, $VTCA_g$ and $VTCA_n$ = glial and neuronal TCA cycle fluxes, Vana = anaplerotic flux, Veff = glutamine efflux from the brain.

Material and Methods

Eight healthy volunteers were studied after informed consent was obtained. NMR studies were performed on a 2.1T whole-body system equipped with a home-built RF probe consisting of one circular ^{13}C coil (8.5-cm diameter) and 2 circular quadrature ^{1}H coils. ^{13}C NMR spectra were acquired in the parietal-occipital lobe for 10-min before and during a 160-min [2-^{13}C] acetate infusion (sodium salt, 3 mg/kg/min). An ISIS localized adiabatic ^{13}C{^{1}H} polarization transfer sequence was used (voxel size ~95ml). Amplitudes of glutamate C4 and C3 (34.3 and 27.7 ppm, respectively) and glutamine C4 and C3 (31.7 and 27.1 ppm, respectively) were measured after Gaussian multiplication, Fourier transformation and baseline subtraction.

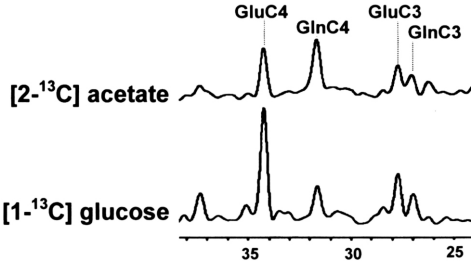

Fig 2. Steady state ^{13}C spectra obtained in a volunteer during IV infusions of [2-^{13}C] acetate and [1-^{13}C] glucose (glucose spectrum from (5)).

Results

Fig. 2 shows a ^{13}C spectrum acquired in one volunteer at the end of [2-^{13}C] acetate infusion. Comparison with a spectrum obtained under identical experimental conditions in the same volunteer during a [1-^{13}C] glucose infusion shows differences in the labeling pattern.

At steady state, the average FE ratio in the C4 positions was GluC4/GlnC4 = 0.28±0.05 (n=8, mean±SD). The differential equations describing ^{13}C incorporation into glutamate and glutamine yields the theoretical ratio of C4 fractional enrichment at steady state:

$$Glu_nC4/Gln_gC4 = (Vcycle+Vcycle') / (VTCA_n+ Vcycle+Vcycle') \quad [1]$$

Vcycle and $VTCA_n$ have been measured in the human parietal-occipital cortex during an infusion of [1-^{13}C] glucose (5,6). Glucose based measurements of glutamate trafficking rely on the dynamics of ^{13}C incorporation into glial glutamine from glutamate. Thus these measurements are only sensitive to the Glu/Gln cycling. When acetate is used, label is incorporated from glial alphaKG into glutamate, leading to an equal sensitivity to both Glu/Gln and Glu/alphaKG cycling at steady state, as shown by equation 1. Based on the Vcycle and $VTCA_n$ values measured with glucose (0.32 and 0.80 micromol/g/min, respectively) and on our experimental FE ratio (0.28±0.05), Eq. 1 leads to Vcycle' = 0.01±0.07 micromol/g/min. The possibility that the labeling in glutamate C4 was from the glial pool, indicating significant internal label exchange, was ruled out based on the time course which indicated much more rapid glutamine C4 labeling.

Discussion

The comparison between steady state labeling from [2-^{13}C] acetate and [1-^{13}C] glucose is consistent with previous in vitro findings from animal studies that acetate and glucose are metabolized primarily in different compartments in human brain, astroglia and neurons, respectively. Mathematical modeling of ^{13}C incorporation through glia shows that Vcycle accounts for the large majority of total neuronal/glial glutamate trafficking, indicating a minor role for the Glu/alphaKG cycle under normal physiological conditions. This finding strongly supports the Glu/Gln cycle as being a major metabolic flux, and glutamine as the primary source of neuronal glutamate replenishment in human brain.

References

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