Measurement of the Astrocytic TCA Cycle Flux in Humans Using ^{13}C Labeled Acetate

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Introduction

Radioisotope and stable isotope labeling experiments have shown that cerebral metabolism is characterized by two distinct metabolic compartments, i.e. neuronal and glial cells (1,2). Recently the crucial role of glial metabolism for brain function has been demonstrated (3). NMR allows for non-invasive measurement of metabolic fluxes in human brain based on the detection of ^{13}C label from infused [1-^{13}C] glucose. However glucose, which is consumed by both neurons and glia, does not allow for a specific measurement of glial metabolism. Neuronal and glial metabolism may be distinguished using labeled acetate as a substrate since only astrocytes are capable of transporting acetate for further metabolism (4). In this study we measured ^{13}C incorportation into brain glutamate and glutamine during the infusion of [2-^{13}C] acetate in humans. Our purpose was to develop a mathematical model of ^{13}C labeling in order to derive the astrocytic TCA cycle flux from the NMR measurement.

Methods

Protocol

NMR data were collected in the parietal-occipital lobe of 8 healthy subjects using a 2.1T whole-body system equipped with a home-built RF probe consisting of a 8.5-cm diameter surface ^{13}C coil and 2 circular quadrature ^{1}H coils. ^{13}C NMR spectra were acquired 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 and glutamine C4 and C3 were measured after Gaussian multiplication, Fourier transformation and baseline subtraction.

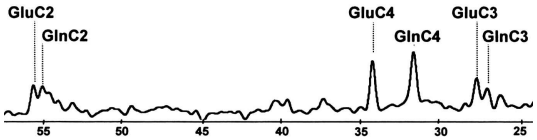

Fig 1. ^{13}C spectrum obtained from one volunteer at steady state.

Mathematical model

A preliminary analysis of the data showed that ^{13}C label from acetate is incorporated from the glial into the neuronal TCA cycle through the neuronal/astroglial glutamate/glutamine cycle (analysis submitted as another abstract): [2-^{13}C] acetate is converted into [2-^{13}C] acetyl-CoA in the glial compartment which labels glial TCA cycle intermediates and subsequently, glutamate and glutamine C4 in glia. Then the neuronal pool of glutamate C4 is labeled from glutamine C4. Following the 2nd turn of TCA cycle, C2 and C3 of glutamate and glutamine become labeled. The coupled differential equations describing the model are:

$$d[Gln_gC4]/dt = (Vcycle+Veff)\cdot Glu_gC4 - (Vcycle+Veff)\cdot Gln_gC4$$
$$d[Gln_nC4]/dt = Vcycle\cdot Gln_gC4 - Vcycle\cdot Gln_nC4$$
$$d[Glu_nC4]/dt = Vcycle\cdot Gln_nC4 - (Vcycle+VTCA_n)\cdot Glu_nC4$$
$$d[Glu_gC3]/dt = (VTCA_g+Veff)\cdot OAA_gC3+Vcycle\cdot Glu_nC3 - (Vcycle+Veff+VTCA_g)\cdot Glu_gC3$$
$$d[Gln_gC3]/dt = (Vcycle+Veff)\cdot Glu_gC3 - (Vcycle+Veff)\cdot Gln_gC3$$
$$d[OAA_gC3]/dt = \tfrac{1}{2}VTCA_g(Glu_gC3+Glu_gC4) - (VTCA_g+Veff)OAA_gC3$$

where $VTCA_n$ and $VTCA_g$ are the neuronal and glial TCA cycle fluxes, Vcycle the glutamate/glutamine cycle flux and Veff the glutamine efflux from the brain (= glial pyruvate carboxylase flux). Glx_yCz is the fractional enrichment (FE) of the metabolite Glx in carbon atom position z, in cellular compartment *y* (glial or neuronal pool).

The differential equations can be solved analytically under steady state conditions, leading to the following relationships between Vcycle, $VTCA_n$ and $VTCA_g$:

$$Vcycle = VTCA_n\cdot Glu_nC4 / [Gln_gC4 - Glu_nC4]$$
$$VTCA_g = 2[(Vcycle+Veff)\cdot Gln_gC3 - Vcycle\cdot Glu_nC3]/[Gln_gC4 - Gln_gC3]$$

Results

Fig. 1 shows a ^{13}C spectrum acquired in one volunteer at the end of [2-^{13}C] acetate infusion. Glutamate and glutamine exhibit significant enrichments in the C4, C3 and C2 positions.

Assessment of glial glutamate concentration

Conversion of NMR signal into glutamate FE requires the knowledge of the astrocytic glutamate concentration. Indeed the astrocytic pool can not be neglected due to its high FE during ^{13}C acetate infusion. The size of the astrocytic glutamate pool was calculated from a detailed analysis of the time course of the FE of glutamate C4. This time course presents a fast component reaching steady state within 5-min of acetate infusion, followed by a slower component delayed by about 30 minutes (Fig. 2). The rapid component is very likely to reflect the small glial glutamate pool. The time course was fitted using the model described above. The glial glutamate concentration was iterated and converged to $[Glu_g] = 0.5\pm0.1$ mM.

Calculation of VTCAg

Glutamate and glutamine C4 and C3 NMR signals were converted into FEs after correction for the concentrations of glutamate in neurons and astroglia. Using $VTCA_n$ = 0.80 micromol/g/min and Veff = 0.04 micromol/g/min as previously measured with ^{13}C glucose (5), the steady state equations yielded Vcycle = 0.32±0.07 micromol/g/min and $VTCA_g$ = 0.14±0.06 micromol/g/min.

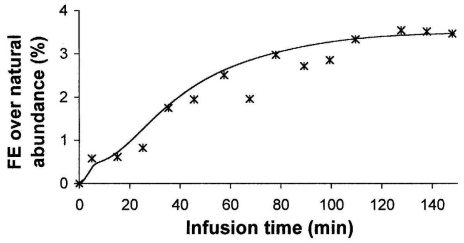

Fig 2. Time course of glutamate C4 fractional enrichment fitted by a two-compartment model (glia, neurons).

Discussion

The astroglial TCA cycle flux, which represents the complete TCA cycle as opposed to just glial PDH flux, measured in this study is 2-3 times higher than may be inferred from two previous studies using [1-^{13}C] glucose (5). The difference most likely reflects the relative insensitivity of the [1-^{13}C] glucose experiment to this flux. In the current study it was possible to measure astrocytic glutamate directly and thus, correct $VTCA_g$ for the contribution of astrocytic glutamate. In conclusion, the use of [2-^{13}C] acetate allows for in vivo measurement of astroglial oxidative metabolism. Astroglia accounts for ~15% of cerebral oxygen consumption in the human brain.

References

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