Glucose and glial-neuronal metabolism in α-chloralose anesthetized rats measured by *in vivo* ^{13}C NMR spectroscopy

P. G. Henry[1], S. Crawford[1], G. Oz[1], K. Ugurbil[1], R. Gruetter[1]
[1]Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, United States

Carbon-13 labeling time courses for glutamate C4, C3, C2, glutamine C4, C3, C2 and aspartate C3, C2 were measured simultaneously in the rat brain during 7 hours of [1,6-$^{13}C_2$]glucose infusion under α-chloralose anesthesia. The isotopic enrichment of glutamate and glutamine at isotopic steady-state varied depending on carbon position, consistent with substantial pyruvate carboxylase activity and glutamine dilution. Labeling time courses were fitted by a metabolic model to derive quantitative metabolic fluxes, indicating a low rate of neurotransmission, high rate of anaplerosis and an important regulatory role for the malate-aspartate shuttle in the brain.

Introduction

Dynamic ^{13}C NMR spectroscopy is a unique tool to measure quantitative metabolic fluxes in the brain and investigate compartmentalized metabolism between neurons and glia. However, most ^{13}C studies in the rat brain have measured ^{13}C labeling time courses for a limited number of metabolites. The goal of the present study was to improve the accuracy and precision of metabolic modeling by measuring the entire range of ^{1}H-coupled ^{13}C resonances over an extended period of time. Temporally resolved ^{13}C labeling time courses for the C2 and C3 carbons of glutamate, glutamine and aspartate and the C4 of glutamate and glutamine were obtained simultaneously over 7 hours with excellent sensitivity and temporal resolution, and fitted by a metabolic model.

Methods

Five male Sprague-Dawley rats were intubated and both femoral veins and arteries were cannulated for glucose infusion and blood sampling. Blood gases and glycemia were measured every 15 min to ensure stable physiological conditions. Plasma glucose C1 and C6 fractional enrichment was rapidly raised from 1.1% to 70% and maintained at this level using [1,6-$^{13}C_2$]glucose. *In vivo* spectra were recorded at 9.4T (31cm horizontal bore) from a 400μl volume. At the end of *in vivo* measurements, brains were quickly funnel-frozen and metabolites were extracted with perchloric acid to determine the ^{13}C fractional enrichment of metabolites at the end of glucose infusion. *In vivo* ^{13}C spectra were analyzed in a fully automatic manner using LCModel, yielding labeling time courses of brain metabolites. Labeling time courses were fitted by a modified two-compartment model to derive metabolic fluxes (1).

Results and Discussion

The time courses of ^{13}C label incorporation into the C4 and C3 of glutamate (Glu_4 and Glu_3) and glutamine (Gln_4 and Gln_3) were analyzed from each rat (Figure). An infusion period of 7 hours was verified to achieve complete isotopic steady-state (not shown). The isotopic enrichment of each carbon position 7 hours after the start of the glucose infusion showed substantial differences between Glu and Gln (Table). The isotopic enrichment of Glu_4 (resp. Gln_4) was higher than that of Glu_3 and Glu_2 (resp. Gln_3 and Gln_2) ($p<0.01$). This was accounted for by adding terms for label dilution at the level of glutamine to the previous model (1), which provided excellent fits in each animal (Figure). Gln_2 was higher than Gln_3 ($p<0.01$), reflecting pyruvate carboxylase activity, V_{pc}, compared to glutamate/glutamine cycle V_{nt} ($V_{pc}/V_{nt}=0.37\pm0.22$, mean±SD, n=5), whereas Glu_3 and Glu_2 had the same isotopic enrichment ($p>0.5$). The different labeling pattern of Glu compared to Gln at steady-state was an indication that the glutamate-glutamine cycle (V_{nt}) was relatively low ($V_{nt}/CMR_{glc(ox)}=0.23\pm0.11$). Aspartate labeling was faster than that of Gln_4, suggesting a predominantly non-glial localization for aspartate. The exchange rate V_x between 2-oxoglutarate and glutamate was low ($V_x/V_{pdh}=1.02\pm0.20$), consistent with recent brain studies (1-3), emphasizing the role of the malate-aspartate shuttle in regulating oxidative energy metabolism in the brain.

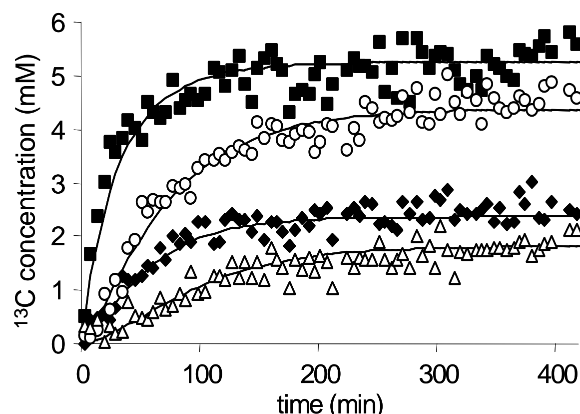

Figure. Labeling time courses of glutamate and glutamine C4 and C3 during 7 hours of [1,6-$^{13}C_2$]glucose infusion from a *single* rat with 5 min temporal resolution. Solid lines show the best fits from the metabolic modeling.

Glu_4	0.62 ± 0.02
Glu_3	0.52 ± 0.01
Glu_2	0.53 ± 0.01
Gln_4	0.56 ± 0.04
Gln_3	0.42 ± 0.02
Gln_2	0.48 ± 0.03
Asp_3	0.56 ± 0.01
Asp_2	0.55 ± 0.01

Table. Isotopic enrichments of individual carbon positions of glutamate, glutamine and aspartate at isotopic steady-state, measured from extracts (mean±SD, n=5).

Acknowledgements

Supported by NIH P41RR08079, NIH R01NS38672 and the Keck Foundation.

References

1) Gruetter et al., *Am. J. Physiol.*,281,E100 (2001)
2) Henry et al., *J. Neurochem.*,82,857 (2002)
3) Choi et al., *J.Cereb.Blood Flow Metab.*,22,1343 (2002)