On the *in vivo* ^{13}C NMR measurement of the cerebral TCA cycle rate from label incorporation into a single resonance

R. Gruetter[1], P-G. Henry[2]

[1]Radiology and Neuroscience, University of Minnesota, Minneapolis, MN, United States, [2]Radiology, University of Minnesota, Minneapolis, MN, United States

Introduction

The majority of ATP required for brain function is normally generated oxidatively via glycolysis of glucose to pyruvate, followed by metabolism in the tricarboxylic acid (TCA) or Krebs cycle. When administering ^{13}C labeled glucose, incorporation of the label into amino acids can be measured, which may serve as an indicator of the rate of the TCA cycle (1). However, for label to be incorporated into amino acids such as glutamate, which are mainly located in the cytosol, it must be transferred out of the mitochondrion. Therefore, the rate of label incorporation into cerebral amino acids is intrinsically a function of the TCA cycle rate, V_{tca}, as well as tbe apparent rate of label exchange across the mitochondrial membranes, V_x. The purpose of this study was to determine the robustness of the measurement of V_{tca} from the label incorporation into three resonances measurable by ^{13}C NMR in the brain.

Methods

Experimental data acquired from three experimental groups that differed in their energy status (deep pentobarbital (From Ref. 2), light α-chloralose and morphine sulfate anesthesia), each containing at least 5 rats was analyzed. All in vivo data was acquired using direct-detected ^{13}C NMR spectroscopy from the rat cortex using localization (400-500µl) with surface coil detection at 9.4 Tesla (2). In all experimental groups the following average time courses were analyzed: the C4 and C3 of glutamate, $Glu_4(t)$ and $Glu_3(t)$ respectively, and the C3 of aspartate, $Asp_3(t)$, each measured over 300 min. The time course for each resonance was fitted to a single TCA cycle compartment model (1), by fitting the TCA cycle rate, V_{tca} and the label dilution, V_{out}, while V_x was fixed. Two modeling assumptions were compared: (I) $V_x=V_{tca}$ (3-5) and (II) V_x=50µmol/g/min (1). As input, the measured precursor enrichment of glucose was used. The pool size of Asp and Glu was fixed during fitting.

Results and Discussion

First, the goodness of the fit to $Asp_3(t)$ (not shown) was unaffected by either assumption. V_{tca} was largely independent of the choice of V_x (line 1 in Table 1), providing an accurate measure of V_{tca} from $Asp_3(t)$, as reported (3).

Second, when fitting the $Glu_4(t)$, the goodness of fit (not shown) was also independent of the assumed V_x, as shown previously (4). The value of V_{tca}, however, was underestimated by 100±22% when comparing assumption II to I (2nd line in Table 1).

Third, when fitting the $Glu_3(t)$ only, assumption II underestimated V_{tca} by 21±2% (line 3 in Table 1) compared to assumption I. However, in all three experimental groups the fit to $Glu_3(t)$ clearly exposed systematic errors in the fit when assuming V_x=50µmol/g/min (solid line in Fig. 1), resulting in a poor fit especially at the early time points. Fitting with $V_x=V_{tca}$ resulted in an excellent approximation of the experimental data, consistent with previous results (5).

In conclusion, the *accuracy* of the measurement of V_{tca} is highest for $Asp_3(t)$, then for $Glu_3(t)$ with the commonly used $Glu_4(t)$ being the least robust. Given that the *precision* of $Asp_3(t)$ is lower due to its low concentration, it is suggested that $Glu_3(t)$ can be used to obtain reliable estimates of V_{tca} in the brain, which supports recently reported changes in V_{tca} from $Glu_3(t)$ (6).

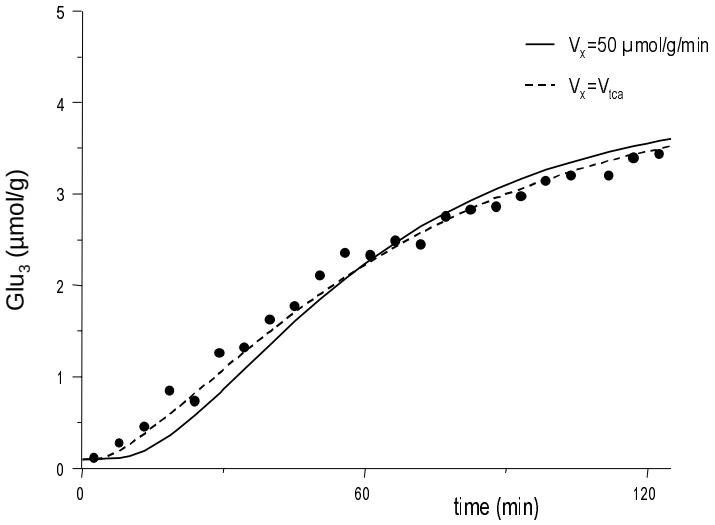

Fig.1 Labeling time courses of glutamate C3 in rat brain during [1,6-$^{13}C_2$]glucose infusion under α-chloralose anesthesia (closed circles) and fit of model with two assumptions.

Measured variable	$\frac{V_{tca}(V_x=V_{tca})}{V_{tca}(V_x=50)}$
$Asp_3(t)$	1.02±0.04
$Glu_4(t)$	2.00±0.22
$Glu_3(t)$	1.21±0.02

Table 1. Effect of V_x on the Measurement of the TCA cycle rate, Vtca, expressed as a ratio of the two assumptions evaluated.

References

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