Lactate Metabolism in Human Brain Measured by Dynamic ^{13}C MRS

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Introduction

Studies in isolated neurons and animal models have shown that lactate is a potential fuel for brain energy metabolism. However the conditions under which lactate becomes a net fuel for human brain metabolism are not well characterized, and the relative rates of consumption of lactate by neurons and glia are not known. In order to determine the capacity of normal human cortex to transport and oxidize lactate localized ^{13}C MRS and infusions of [3-^{13}C]lactate were used to detect the entry and utilization of lactate in the brain. With a plasma concentration ($[Lac]_P$) being in the 0.8-2.8 mmol.L^{-1} range, the tissue lactate concentration ($[Lac]_B$) was assessed as well as the fractional contribution of lactate to brain energy metabolism (CMRlac) using reversible Michaelis-Menten kinetics for transport (V_{MAX}, K_T) and a neuronal-glial model to establish the neuronal and astrocytic TCA cycle fluxes (V_{TCAn},V_{TCAa}).

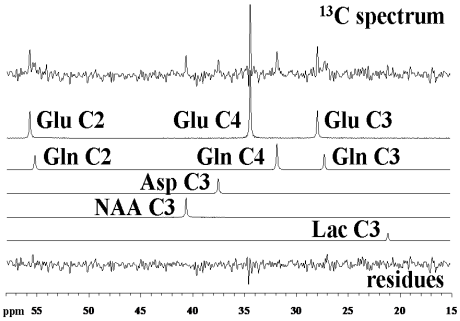

Fig. 1. Localized ^{13}C spectrum acquired from a subject during the last 32 minutes of a 2-hour [3-^{13}C]lactate infusion study and its decomposition by LCModel.

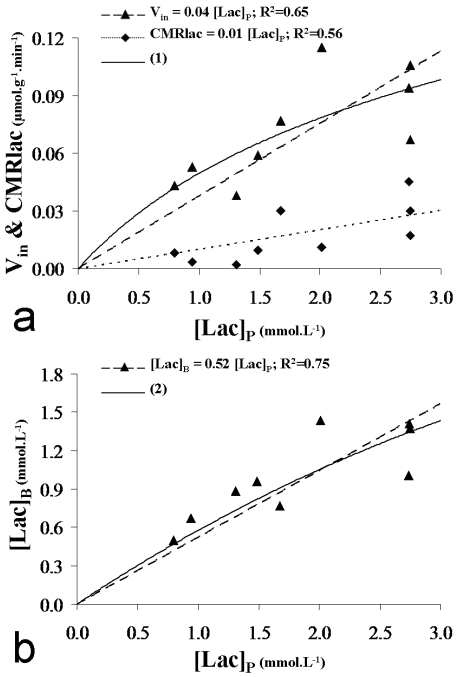

Fig. 2. A. Lactate influx and net consumption plotted as a function of the plasma lactate concentration. B. Brain lactate as a function of the plasma lactate concentration. The solid curve lines represent the best fit obtained from a least-square minimization using expression (1) and (2). The dashed straight lines are linear regression.

Materials and Methods

MRS acquisition. [3-^{13}C]lactate experiments were conducted on 7 young healthy volunteers (aged 24±1 years, BMI 24±1 kg/m^2, three men and four women). In two cases, subjects were studied twice at two different concentrations of plasma [3-^{13}C] lactate. Subjects lay supine in a 4.0 T whole-body magnet (Bruker Instruments, Billerica, MA) with the head lying on top of one 8.5-cm-diameter ^{13}C circular coil and two ^{1}H quadrature coils for ^{1}H acquisition and decoupling. After tuning, acquisition of scout images, shimming with the FASTERMAP procedure [1], and calibration of the decoupling power, ^{13}C MRS spectra were acquired with polarization transfer [2] from a 50x40x45 mm^3 volume located in the occipital-parietal lobe before and during infusion of [3-^{13}C]lactate (TR=2500ms, 128 transients). During the acquisition, blood samples were collected every 5-10 minutes to measure glucose and lactate plasma concentration and ^{13}C fractional enrichment.

MRS spectral analysis. The scans were added in running averages of 15 min. Spectra were analyzed using LCModel 6.1 [3] (Stephen Provencher Inc., Oakville, Ontario, Canada) with modified input parameters and a simulated basis set generated as described by Henry et al. [4]. Concentrations for the different isotopomers of glutamate and glutamine were obtained relatively to the signal of natural abundance NAA assuming a concentration of 11µmol.g^{-1} (see Fig.1).

Metabolic modeling analysis. ^{13}C Labeling time-courses for Glu C4, C3 and C2, and Gln C4, C3 and C2 were fitted according to a neuronal-astrocytic metabolic model [7-9]. Metabolic modeling was performed using Matlab 7.0 (The MathWorks Inc., Natick, MA) and CWave [7].

Modeling of Lactate transport kinetics. We included a reversible Michaelis-Menten blood brain transport of lactate [5,6] in the two-compartment metabolic model (V_d=0.77). At steady state, the model results in the following expressions for the influx (V_{in}) and the brain lactate concentration ($[Lac]_B$):

$$(1)\quad V_{in} = V_{MAX}\frac{[Lac]_P}{K_T + [Lac]_P + [Lac]_B/V_d}\quad ; \qquad (2)\quad [Lac]_B = V_d\frac{\left(\frac{V_{MAX}}{CMRlac} - 1\right)[Lac]_P - K_T}{\frac{V_{MAX}}{CMRlac} + 1}$$

Results and Discussion

The fe_{GlnC4}/fe_{GluC4} labeling ratio was 0.78 ± 0.04 and the neuronal and astrocytic TCA fluxes were V_{TCAn} = 0.50 ± 0.02 µmol.g^{-1}.min^{-1} and V_{TCAa}= 0.15 ± 0.02 µmol.g^{-1}.min^{-1} (mean ± SD, n=9) consistent with values reported in the literature. The fe_{GlnC4}/fe_{GluC4} ratio is very similar to what has been reported with [1-^{13}C]glucose as a substrate, suggesting that blood lactate is metabolized in both neurons and glia similarly to glucose. First, a preliminary fit of the metabolic model was done assuming a half-saturation constant K_T = 2 mmol.L^{-1}, which lies in the range of published values for monocarboxylic acids. For each experiment, $[Lac]_B$, V_{MAX} and CMRlac were estimated and the rate of lactate influx V_{in} calculated for each subject were plotted as a function of $[Lac]_P$ (Fig.2a). In a similar way, the values of $[Lac]_B$ were plotted as a function of $[Lac]_P$ (Fig.2b). As shown by the relations (3) and (4), the calculated values of V_{in} and $[Lac]_B$ are insensitive to the K_T assumed:

$$(3)\quad \frac{fe[GluC4]}{fe[LacC3]_P} = \frac{fe[LacC3]_B}{fe[LacC3]_P} = \frac{V_{in}}{2.CMRglc + V_{in}}\quad ; \qquad (4)\quad [Lac]_B = \frac{[LacC3]_B}{fe[LacC3]_B}$$

However, K_T and V_{MAX} can be determined from Fig. 2a and b using the relations (1) and (2). A least-square minimization algorithm was used, yielding: V_{MAX} = 0.30 µmol.g^{-1}.min^{-1} and K_T = 4.3 mmol.L^{-1}. The value of K_T was then used for a more precise run of the metabolic modeling, leading to individual determinations of V_{MAX} and CMRlac. The final result for V_{MAX} was 0.29 ± 0.03 µmol.g-1.min-1 (mean ± SD, n=9), with a range of 0.20 to 0.46 µmol.g^{-1}.min^{-1}. The values of CMRlac are plotted on Fig. 2a as a function of $[Lac]_P$.

Conclusion

In this study, we report the first use of [3-^{13}C]-labeled lactate in order to elucidate its transport kinetics directly and estimate its contribution to oxidative human brain energy metabolism *in vivo*. Our results demonstrate that the human brain uses circulating lactate, and that its use is modest in normal condition. However the calculated V_{MAX} is compatible with a capacity to consume lactate at a rate up to 40 % of the total rate of brain oxidative energy synthesis, and even at the lowest level studied it can potentially be a significant net energy source if glucose levels are low enough to drop intracerebral production. The same approach could be used to investigate *in vivo* the role of lactate metabolism in sustaining brain function especially in conditions such as fasting, hypoglycemia, hypoxia/ischemia or neurodegenerative processes.

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