NMR Investigations of Acetate Transport and Metabolism in the Rat Brain *In Vivo*

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INTRODUCTION: Although glucose is the major source of energy in the mature CNS, other substrates can be metabolized in brain at significant rates. The short-chain free fatty acid, acetate, is well known as a glial-specific substrate. Extensive investigations using cultured cells have established that acetate is preferentially transported and utilized by astroglia and that metabolism in neurons is minimal (1). ^{13}C NMR studies in rats and humans *in vivo* with intravenous infusions of [2-^{13}C]acetate have shown substantial incorporation of ^{13}C label into cerebral amino acids with higher enrichment in glutamine than glutamate (2,3), consistent with a small rapidly turning over pool of glutamate precursors for glutamine synthesis (4) as shown in early studies using ^{14}C isotopes. Analysis of the dynamic ^{13}C turnover curves of cerebral amino acids from ^{13}C-acetate to extract metabolic rate information requires knowledge of the kinetics of acetate transport as well as metabolism. Currently, little information is available on brain acetate transport kinetics *in vivo.* In this study we report our findings concerning the dynamics of acetate transport and metabolism in the anesthetized rat brain.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the Yale IACUC. Overnight fasted Sprague Dawley rats (160-180g) were anesthetized with halothane, tracheotomized and artificially ventilated (30%O_2/68.5%N_2O, 1.5% halothane). Arterial and venous catheters were placed for measurement of blood gases, blood pressure, and the infusion of [2-^{13}C]acetate and unlabeled glucose. *In vivo* experiments were performed at 7T and 9.4T (Bruker AVANCE spectrometer) using a surface transceiver coil placed on the rat's head for ^{1}H detection and an orthogonal pair of coils, coupled in quadrature, for ^{13}C RF transmission. Shimming was optimized using FASTMAP (5). ^{1}H-[^{13}C]-NMR spectra were obtained every 23s from a localized volume (7x4x7 mm^3) during the infusion of glucose and [2-^{13}C]acetate (6). Arterial blood samples were taken periodically for the analysis of plasma acetate concentration and ^{13}C enrichment. In addition rats were infused with [2-^{13}C]acetate at different rates, and the steady state brain and plasma acetate levels were measured. In bench experiments rats were infused with [2-^{13}C]acetate and glucose for 40 min to evaluate steady state Gln-C4 labeling. At the end of the experiment, the brain was frozen *in situ* in liquid nitrogen and metabolites were extracted from the frozen cortical tissue (7). Metabolite concentrations and ^{13}C enrichments in extracts were measured at 11.7T using ^{1}H-[^{13}C]-NMR (for cerebral metabolites) or ^{1}H NMR (for plasma acetate-C2 only).

RESULTS AND DISCUSSION: Infusion of [2-^{13}C]acetate led to a rapid increase (within 5 min) in the plasma acetate concentration (baseline, 0.23 ± 0.l0 mM) and percentage ^{13}C enrichment to constant values of 19.4 ± 2.2 mM and 94 ± 4%, respectively. Brain acetate levels also increased rapidly and reached a plateau within 10-15 min, indicating that the acetate was rapidly transported into the brain. To define the kinetics of acetate transport further, brain acetate levels were measured at different plasma acetate concentrations. Brain acetate levels increased linearly with plasma acetate concentrations (N=6, R^2=0.85), and most of the data fell within the 95% confidence interval of the predicted line (Fig. 1). These data indicate that acetate transport is not saturated even at very high (50 mM) plasma acetate levels. Infusion of [2-^{13}C]acetate led to rapid appearance of ^{13}C label into Gln-C4 followed by Glu-C4, suggesting that acetate is exclusively transported and metabolized in astroglia. Analysis of the steady state cortical Gln-C4 labeling at different levels of plasma acetate indicates that acetate oxidation in the astroglial TCA cycle reached saturation at plasma acetate concentrations ≥15 mM with further increases in plasma acetate having no effect on Gln-C4 labeling. Metabolic modeling using a two-compartment model (7) and assuming reversible symmetric Michaelis-Menten kinetics for transport and metabolism, yielded values of K_M and V_{max} for transport of 21 ± 7 mM and 1.1 ± 0.4 μmol/g/min, respectively, and for acetate utilization a V_{max} of 0.14 ± 0.03 μmol/g/min and K_M < 0.25 mM. The slow acetate transport led to a 5-min delay for acetate oxidation to reach 95% of its steady-state rate. The neuronal TCA cycle and glutamate-glutamine cycling rates were 0.91 ± 0.06 and 0.57 ± 0.08 μmol/g/min, respectively, and the total astroglial TCA cycle rate (includes oxidation of acetate and non-acetate substrates), was 0.15 ± 0.07 μmol/g/min, or 14 ± 7% of the total TCA cycle rate. The relative enrichments of glutamate, glutamine, and acetate also showed that under saturating conditions, acetate supplied carbon for 94% of the astroglial TCA cycle rate.

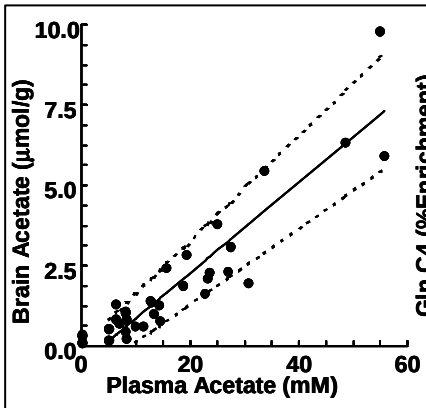

Fig. 1 Correlation of steady-state brain acetate level with plasma.

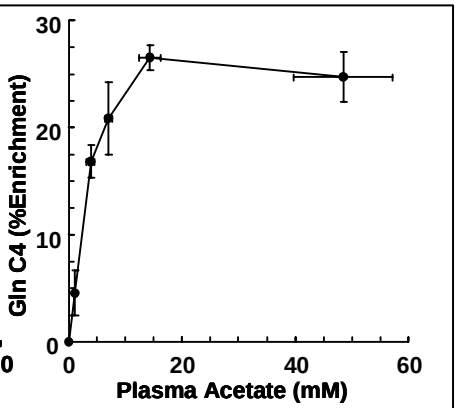

Fig. 2 Relationship of steady state Gln C4 labeling with plasma acetate.

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