Acetate Utilization is Rate-Limited in the Rat Brain

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Introduction

Acetate is a glial-specific substrate, and is therefore an attractive alternative to glucose for the study of neuronal-glial interactions [1-3]. However *dynamic* metabolic modeling of ^{13}C labeling time courses obtained during ^{13}C-acetate infusion has been hampered by the fact that the kinetics of acetate uptake and metabolism are not well characterized. The goal of this study was to determine whether brain acetate metabolism depends on the rate of acetate infusion, and to also identify potential rate-limiting steps in the uptake and metabolism of acetate.

Methods

Experiments were performed on a 9.4T/31cm bore magnet interfaced to a Varian spectrometer. Overnight fasted male Sprague-Dawley rats were infused with 3.0M [2-^{13}C] acetate (pH 4.0) under morphine sulfate and pancuronium anesthesia. Localized *in vivo* ^{13}C-edited ^{1}H NMR spectra were acquired from the rat brain (VOI of 405µl) using the ACED-STEAM sequence [4]. The infusion protocol was as followed: a bolus of 0.625 g/kg of 99%-enriched labeled [2-^{13}C]acetate was administered with an infusion rate decreasing exponentially over 8 min, followed by a constant continuous infusion rate for 2 hours in each animal. The latter infusion rate was divided into four different groups (groups 1, 2, 3, 4; n = 2 in each group) corresponding to a continuous infusion rate of 0.31, 0.62, 0.94 and 1.09 g/kg/hr respectively. The acquired spectra were analyzed using LCModel with a simulated basis set including the effect of strong coupling [5]. Physiological condition and temperature were monitored and maintained throughout the experiment. Blood samples were collected to measure the plasma acetate concentration and enrichment.

Results

The summed $^{1}H\{^{13}C\}$ spectrum (Fig. 1) from one animal in group 3, during 2 hours acetate infusion shows ^{13}C label incorporation into brain acetate-C2 (at 1.90 ppm), glutamate-C4 and glutamine-C4 (both clearly resolved), and glutamate and glutamine C2 and C3 (not resolved). Analysis of both unedited and edited ^{1}H-^{13}C spectra allowed direct determination of isotopic enrichments.

After a bolus of ^{13}C-acetate, [2-^{13}C]acetate was quickly detected in the brain with an isotopic enrichment close to 100%. The time course of [2-^{13}C]acetate concentration in the brain depended on the rate of continuous acetate infusion (Fig. 2A). With a low infusion rate (0.31 g/kg/hr), acetate decreased to undetectable levels within 60 min. At 0.62 g/kg/hr, brain acetate also decreased but more slowly. At 0.94 g/kg/hour, the brain ^{13}C-acetate signal was stable, indicating that the infusion rate matched total (body) acetate uptake. Finally, at a high infusion rate (1.09 g/kg/hr), acetate concentration increased steadily, suggesting that the rate of infusion exceeded the rate of body acetate uptake.

Following [2-^{13}C]acetate uptake into the brain, ^{13}C label was readily incorporated into glutamine (Fig. 2B) and glutamate (not shown) via the glial TCA cycle. Interestingly, glutamine ^{13}C labeling curves (Fig. 2B) were nearly identical for groups 2, 3 and 4, indicating that the labeling of glutamine from [2-^{13}C]acetate was nearly *independent* of brain acetate concentration, as long as acetate is present in the brain. Glutamine labeling began to decrease in group 1 at ~40 min when brain acetate concentration approached zero in this group.

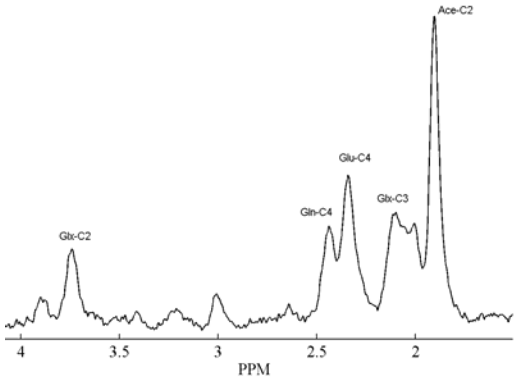

Figure 1: ^{13}C-edited ^{1}H spectrum acquired for 2 hours from one rat in group 3 (TR=2.5s, TE=7.7ms, TM=20ms) during infusion of [2-^{13}C] acetate.

Discussion and Conclusion

Our results demonstrate that transport of acetate through the blood-brain barrier (BBB) is fast and not rate-limiting. Rather, they show that metabolism of acetate is rate-limited *after* entry of acetate into the brain, as evidenced by the fact that glutamine ^{13}C labeling curves are independent of brain acetate concentration (as long as acetate concentration remains elevated in the brain). The rate-limiting step might actually be the synthesis of acetyl-CoA from acetate. In conclusion, this study provides new insights into the kinetics of acetate uptake and metabolism in the brain. This opens the way to dynamic metabolic modeling of glutamate and glutamine ^{13}C labeling time courses obtained during acetate infusion. We expect that such dynamic modeling using [2-^{13}C]acetate as a substrate will lead to more reliable estimates of the glutamate-glutamine cycle rate than those obtained when using [1-^{13}C]glucose or [1,6-$^{13}C_2$]glucose as a substrate [6].

References

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Acknowledgments

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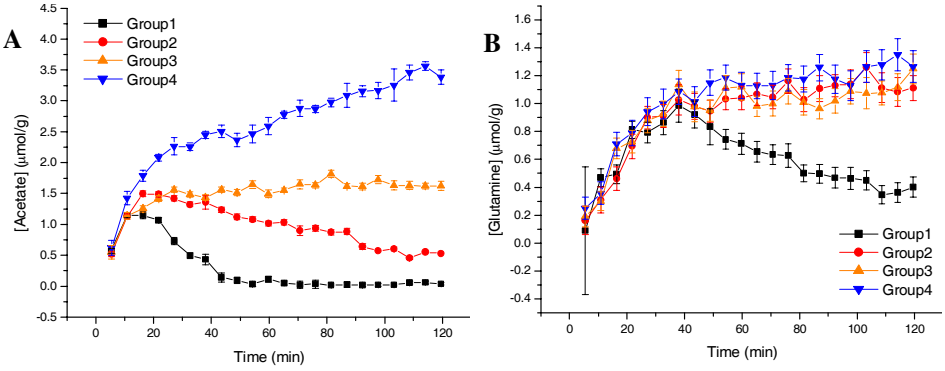

Figure 2: Comparing the effect of four different infusion groups on the *in vivo* ^{13}C time courses of acetate-C2 (A) and glutamine-C4 (B) after infusing [2-^{13}C] acetate in the rat brain. Temporal resolution was ~5.3min.