Effect of acetate concentration on its cerebral metabolism studied by hyperpolarized ^{13}C MRS

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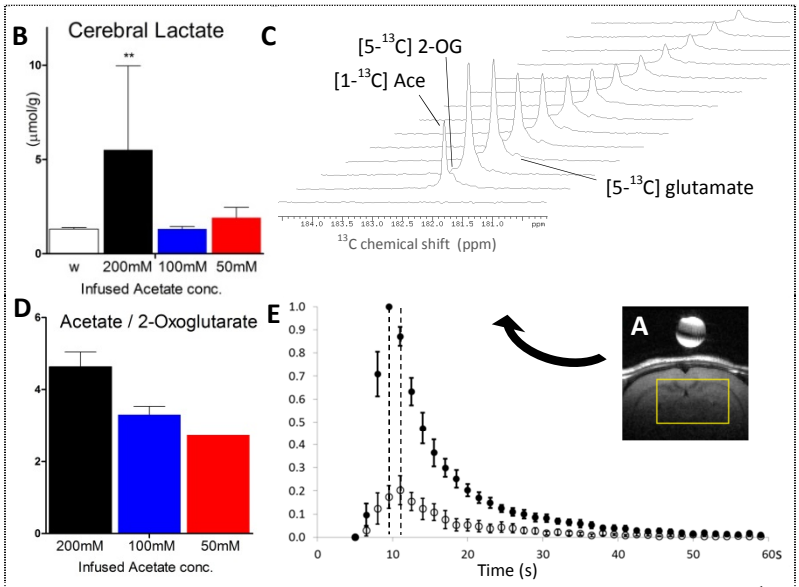
PURPOSE: Hyperpolarized ^{13}C MRS enables highlighting intermediate states that are invisible using its thermally polarized counterpart [1]. It was shown that hyperpolarized [1-^{13}C]acetate (Ace), an astrocyte specific precursor, can be used to detect [5-^{13}C]2-oxoglutarate (2OG), a tricarboxylic acid (TCA) cycle intermediate, in an intact rat brain [2]. This observation provided a way for direct analysis of glial TCA cycle activity. In this study, we aimed at examining the effect of hyperpolarized Ace concentration on its cerebral metabolites and its influence on the neurochemical profile.

METHODS: [1-^{13}C]Ace samples were prepared using previously described formulations [3] and hyperpolarized using a custom-designed DNP polarizer operating at 7 T/1±0.05 K for 180 min. Following solid-state polarization, samples were rapidly dissolved in 5 ml of super-heated D_2O (170°C) to obtain ~ 200, 100 and 50 mM Ace aqueous solutions. A bolus of 1.5 ml was injected into the femoral vein of male Sprague-Dawley rats (n=9, 250-275g, fasted 12 hr) 3 s after dissolution using an automated protocol [4], leading to blood concentrations of 17.1, 8.6 and 4.3 mM. Animals were anesthetized using a gas containing 1.5% isoflurane and their physiology was monitored. MR measurements were carried out on a 9.4 T/ 31 cm actively shielded animal scanner (Varian/Magnex) using a home-built single loop ^{13}C/^{1}H quadrature surface coils. The voxel of interest (VOI) was located in the frontal cortex (**A**). Field inhomogeneity was corrected using the FASTMAP protocol. To characterize the neurochemical profile before and after the Ace injection, ^{1}H spectra were acquired using the SPECIAL [5] sequence (TR/TE = 4000/2.8ms, VOI = 5x5x5mm^3 in 20 blocks of 16 scans). Metabolites were quantified by the LCModel-based fitting routine [6]. ^{13}C MRS spectra were acquired every 1.5 s in the same VOI using the SIRENE scheme [7] starting 5 s after the beginning of the [1-^{13}C]Ace injection. Cardiac rhythm was monitored and arterial blood gases, plasma lactate and glucose were measured before and after the injection.

RESULTS: Following the injection of the 200 mM Ace bolus, a large increase in plasma and cerebral lactate was observed in 2 animals, possibly originating from cerebral hypoxia or general tissue damages. In contrast, rats injected with lower Ace doses did not show significant changes in any quantified metabolites (**B**). For all substrate concentrations, hyperpolarized [1-^{13}C]Ace (182.18ppm) and [5-^{13}C]2-OG (182.05ppm) were detected (**C**). We observed an additional peak at 181.87 ppm in several experiments, which was tentatively assigned to [5-^{13}C]glutamate (**C**). The ratio between maximal [1-^{13}C]Ace signal and maximal [5-^{13}C] 2OG signal was calculated and found to be dependent on the initial substrate concentration (**D**). Finally, the observable peaks were fitted with Lorentzian functions, and the area under the curves were plotted as a function of time. Two distinct kinetics were observed (**E**): the maximum Ace signal appears 9.5 s after the completion of the injection, whereas the 2-OG signal is maximum 1.5 s later.



In vivo real-time Ace metabolism. **A**: The voxel of interest is shown in yellow on the ^{1}H image **B**: Changes in neurochemical profile upon Ace injection at different concentrations measured by ^{1}H MRS **C:** *In vivo* ^{13}C MRS spectra measured in the VOI as a function of time at 9.4T following the injection of a 100 mM bolus of hyperpolarized [1-^{13}C]Ace. The spectra were acquired in a single experiment with a series of 30° adiabatic pulses applied every 1.5 s starting after the completion of the injection. **D:** Mean [1-^{13}C]Ace/[5-^{13}C]2-OG signal ratio measured at the time of the maximal signal area of the two peaks for each animal (n=3,3,1) **E:** The open and full circles correspond to the mean values of the normalized signal integrals measured in three different animals injected with 100 mM Ace solution.

DISCUSSION: Our study demonstrate that, in contrast to what has been observed following long Ace infusions [8], a bolus injection of Ace does not influence the highly concentrated endogenous metabolites of the rat front cortex. However, an exceedingly large concentration of Ace increases the risk of physiological impairments manifested by lactate production. A lower Ace concentration is therefore preferable for animal physiology but also to improve acetate dynamics range in relation to its metabolic products, which has been shown with Ace/2OG ratio decreasing proportionally to infused Ace dose. The different kinetics of the resonance observed at 182.05 ppm as compared to Ace and its dependence on substrate concentration supports its assignment to the downstream metabolite 2OG. The maximum signal of Ace and 2OG appeared 2.5 s earlier than previously reported [1], which is possibly due either to higher oxidative metabolic rate and thus faster acetate metabolism inherent to the younger animals used in the present study [9] or to shorter time repetition in localized ^{13}C MRS accelerating acetate signal decay. Finally an additional peak at 181.87 ppm was observed which was tentatively assigned to [5-^{13}C]glutamate. A polarization transfer scheme following the injection of [1,2-^{13}C]Ace could be used to confirm the assignment[10].

CONCLUSION: From the results described above, we conclude that optimized sample formulation combined with lower Ace dose improves the detection of 2-oxoglutarate.

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