Diffusion characteristic of infused Acetate in the rat brain in vivo

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Target audience: *In vivo* NMR Spectroscopists and Neuroscience Researchers.

Purpose: Acetate (Ace), a glial-specific substrate, is an attractive alternative to glucose for the study of glial metabolism [1]. The objective of the study is to address the diffusion characteristic of Ace in the rat brain *in vivo* using localized diffusion weighed STEAM-based spectroscopic pulse sequence (DW-STEAM)[2]. The overlap of Ace ^{1}H MRS resonance with GABA resonances (at 1.89 ppm) hinders an accurate estimation of concentration and diffusivity of Ace. Therefore, the rationale was to suppress GABA signal and simultaneously to enable the measurement of the apparent diffusion coefficient (ADC) of Ace. The current study is based on the assumption that intracellular and extracellular metabolites experiences different diffusion behavior and may provide useful information on the localization of Ace. Therefore, the aim was to test whether Ace is distributed mainly in extracellular or intracellular space of the rat brain.

Methods: All experimental procedures involving animals were approved by the local veterinary authorities. Three adult rats (male, 200-235g) were prepared. Briefly, two femoral arteries were cannulated for animal physiology monitoring (blood gases, blood pressure, and glucose and lactate level) and two femoral veins for intravenous infusion of α-chloralose for anesthesia and Ace. The Ace infusion protocol was optimized to reach a steady-state concentration of Ace in brain in less than 30 min. All experiments were performed on a 14.1T/26cm horizontal magnet with a 12-cm gradient coil insert (400mT/m, 120µs). A home-built quadrature transceiver with two physically decoupled 12mm-diameter loops was used. ^{1}H-MRS data were acquired using the DW-STEAM sequence. GABA resonance at 1.89 ppm could be suppressed *in vivo* using a longer echo time of 50 ms and a mixing time of 49 ms. Diffusion weighting was applied along three orthogonal diffusion gradient directions (i.e. x, y and z) to compute the trace of apparent diffusion coefficient, so called mean diffusivity (MD). *In vivo* data was acquired with gradient duration of δ=5 ms, gradient separation of Δ=75 ms and gradient strength of 0,10,15 and 20 G/cm which corresponds to b-values ranging from 0 to 7 ms/µm^2 in a voxel of 240 µl enabling sufficient SNR at high b-value. Spectral analysis was carried out using LCModel for metabolite concentrations. The ADC of metabolites was estimated by a linear fit of the logarithm of the normalized signal attenuation for every diffusion direction and MD was calculated through trace of ADC for every metabolite. Diffusion behavior of Ace was compared with known primarily intracellular metabolites like NAA.

Results: The observed signal of GABA at 1.89 ppm was negligible at echo time of 50 ms and mixing time of 49 ms which is consistent with previously reported J-modulation of GABA[3] (Fig 1). The measurements of Ace time-course in brain showed that Ace reaches a stable level after 20 min (not shown). The acquired spectra without diffusion enabled the quantification of 11 cerebral metabolite concentrations such as NAA, Cr and Ace which were indicated in detail in Fig 2 (with CRLB <15). The remarkable sensitivity and spectral resolution of localized ^{1}H MRS at 14T resulted in the determination of metabolite diffusivity of five presumed intracellular metabolites (table 1). The signal attenuation of NAA and Ace using diffusion gradient in direction x were plotted in Fig 3.

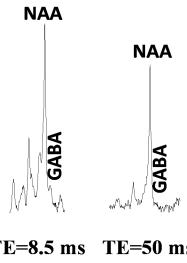

Fig 1. *In vivo* spectra of rat brain at short and long echo time (TE). The noticeable shoulder corresponding to Gamma-aminobutyric Acid (GABA) at 1.89 ppm was minimized at TE of ~50 ms.

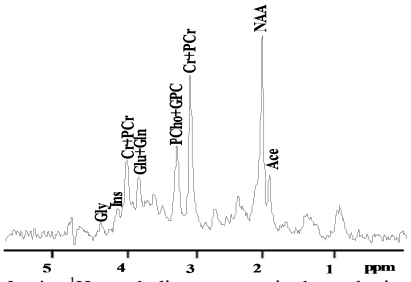

Fig 2. *In vivo* ^{1}H metabolite spectrum in the rat brain at TE of 50ms.
Abbreviations: Ace: Acetate; Cr: Creatine; Glu: Glutamate; Gln: Glutamine; Gly: Glycine; GPC: glycerophosphocholine; Ins:myo-inositol; NAA:N-acetylaspartyle; PCr:Phosphocreatine; PCho:Phosphocholine.

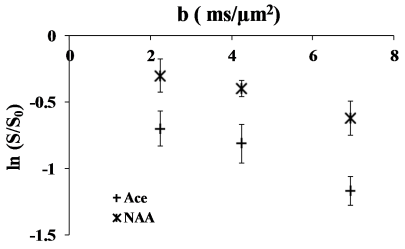

Fig 3. The diffusion attenuated ^{1}H NMR intensities of the metabolites NAA and Ace using diffusion gradient in direction x in the rat brain *in vivo* (averaged over three animals). The error bars indicate the standard deviation in the log linear plot. S and S_0 are signal intensity with and without diffusion gradients.

Discussion: Minimizing GABA peak contribution at 1.89 ppm in MR spectra acquired using DW-STEAM sequence improved the accuracy of the measurement of Ace time course and the Ace diffusion in brain *in vivo*. ADC values of intracellular metabolites in table 1 are in good agreement with previously reported values [4]. The difference between attenuation of primarily intracellular metabolite and Ace suggests a significant concentration of Ace in the extracellular space.

Table 1. Apparent Diffusion Coefficient (ADC) of metabolites in ^{1}H NMR spectra in the rat brain in vivo.

Metabolite	Ace	NAA	Glu	Ins	Cr
MD(µm^2/ms)	0.23	0.12	0.12	0.11	0.12
Standard Deviation	0.02	0.02	0.01	0.02	0.01

Conclusion: We conclude that the significantly larger ADC of Ace compared to intracellular metabolites indicates a substantial concentration of acetate in the extracellular space of rat brain during Ace infusion.

References

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