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RAPID COMMUNICATION

On the *N*-Acetyl Methyl Resonance in Localized ^{1}H NMR Spectra of Human Brain *In Vivo*

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INTRODUCTION

Prerequisites for the quantification of cerebral metabolite concentrations by NMR are a proper assignment of pertinent resonances and the identification of components with overlapping signals. The purpose of this study is to more closely examine the nature of the strong *N*-acetyl methyl signal that is observed in ^{1}H NMR spectra of mammalian brain and commonly ascribed to *N*-acetylaspartate (NAA). It complements a previous publication on resonance assignments in short-echo time, localized ^{1}H NMR spectra of human brain *in vivo* at a field strength of 2.0 T.[1]

There has been considerable discussion about contributions from other *N*-acetylated compounds to the *N*-acetyl methyl signal at 2.01 ppm and the 'true' *in vivo* concentration of NAA.[1–4] Recently, a resonance of the dipeptide *N*-acetylaspartylglutamate (NAAG) has been identified at 2.05 ppm in high-field ^{1}H NMR spectra of perchloric acid extracts of rat nervous tissue.[5] Such results are in agreement with analytical biochemical studies of brain tissues from humans[6] and various species of animals[7] yielding NAAG levels in the mM concentration range. Here we report on the first *in vivo* detection of NAAG in localized ^{1}H NMR spectra of adult human brain. The present achievements are based on significant improvements in both signal-to-noise ratio (SNR) and spectral resolution using short-echo time stimulated-echo (STEAM) localization sequences.[8]

EXPERIMENTAL

All human studies were performed at 2.0 T (Siemens Magnetom) using image-localized, water-suppressed ^{1}H NMR spectroscopy of 12 young adult volunteers. Informed consent was obtained prior to the investigations. Spectra of 8–18 mL volumes-of-interest (VOI) were localized in paramedian parietal grey matter and parietal white matter regions with use of STEAM sequences as described previously.[8] Fully relaxed conditions were achieved at repetition times of 6000 ms ($TE = 20$ ms, $TM = 30$ ms). Measuring times were 6.5–13.0 min using 64–128 accumulations.

Spectral processing involved zero filling of the original 2 k complex data points to 32 k, Fourier transformation, and zero and first order phasing. Residual water signals were less than 10 times the *N*-acetyl methyl signal. No baseline correction or resolution enhancement were employed. The native linewidth of the NAA resonance without linebroadening was about 3 Hz (full width at half height). It transforms into a spectral resolution that allows the identification of individual resonances provided they are separated by about 0.03 ppm at 2.0 T. When applied, filtering consisted of a Gaussian multiplication of the time-domain data that lead to a 1.3 Hz linebroadening of the NAA resonance. ^{1}H NMR spectra of perchloric acid extracts of bovine brain and rat brain were acquired at a field strength of 7.0 T (Bruker MSL 300).

RESULTS

Figure 1 shows the result of a typical grey matter acquisition. The three spectra represent the same data but are processed with the use of a mild linebroadening in the bottom trace ($LB = 1.3$ Hz) and without any filtering in the middle and top traces ($LB = 0$). The top trace depicts an expanded section covering a 1 ppm range from 1.75–2.75 ppm of the ^{1}H NMR chemical shift scale. For the *in vivo* spectra the chemical shift position of the maximum *N*-acetyl methyl signal was set to 2.010 ppm as a frequency reference. As shown in Fig. 1, the *N*-acetyl signal yields an almost symmetric lineshape in grey matter without any indication of a further shifted resonance contributing to its singlet resonance.

Filtering of the raw data improves the SNR at the expense of reducing the spectral resolution and eventually obscuring further resonances. In contrast to grey matter, ^{1}H NMR spectra of parietal white matter frequently exhibit an upfield shoulder of the 2.010 ppm resonance as shown in the linebroadened spectra of both Figs. 2 and 3 (bottom traces, $LB = 1.3$ Hz). Without filtering (middle and top traces, $LB = 0$) the *N*-acetyl methyl signal splits into two resonances with frequencies of 2.010 ppm and 2.045 ppm, respectively. The latter value represents the average chemical shift (± 0.005 ppm) obtained from all volunteer studies. The two separated components become most obvious in the 1 ppm expanded sections shown in the top traces of Figs. 2 and 3.

Preliminary measurements of T_1 and T_2 relaxation

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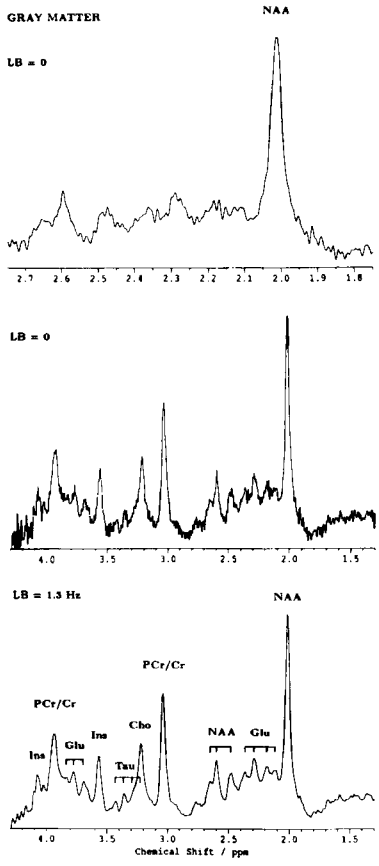

Figure 1. Fully relaxed ^{1}H NMR spectrum (2.0 T) of human grey matter localized in the paramedian parietal lobe of a 29 year old volunteer (18 mL VOI, STEAM, *TE* = 20 ms, *TR* = 6000 ms, 64 scans). Bottom trace: linebroadening by Gaussian multiplication (*LB* = 1.3 Hz), middle and top traces: no filtering (*LB* = 0), top trace: expanded 1.75–2.75 ppm section of the ^{1}H NMR chemical shift range.

times of this component were carried out by varying the repetition time or echo time of the STEAM sequence, respectively. The values of $T_1 = 1400$ ms and $T_2 = 360$ ms are close to those reported for the *N*-acetyl methyl protons of NAA.[9] Most importantly, however, the T_2 determinations confirm the singlet nature of the 2.045 ppm resonance by the absence of *J* modulation due to spin-spin coupling. According to its relaxation and chemical shift properties the signal is therefore assigned to the *N*-acetyl methyl resonance of NAAG. To further support this assignment we have performed extract studies in bovine brain which overcome the problem of low NAAG concentrations in rat cortex and also allow a more convenient separation of grey and white matter than in rat brain. Figure 4 shows pertinent high-field ^{1}H NMR spectra of perchloric acid extracts of bovine grey and white matter in comparison to rat cortex. The enhanced NAAG level in white matter as compared to grey matter is in agreement with the *in vivo* human studies.

DISCUSSION

The assignment of the 2.045 ppm resonance to NAAG is based on the chemical shift and relaxation properties, and on its ^{1}H NMR identification in various regions of both rat brain[5] and bovine brain in agreement with biochemical analyses.[6,7] Previous ^{1}H NMR studies of brain tissue extracts[10–12] focused on rat cortex where the NAAG concentration is low,[5] so that its small signal was overlooked or ascribed to the strongly coupled glutamate β-CH_2 multiplet. Although the *N*-acetyl moieties of gangliosides resonate at the same frequency as NAAG, significant contributions to the 2.045 ppm signal in white matter are unlikely since gangliosides are known to be enriched in grey matter. In fact, the observed enhancement of NAAG in white matter seems to be more pronounced *in vivo* than in early neurochemical analyses of human brain from autopsies.[6] Contributions from *N*-acetylglutamate may be excluded on the basis of its almost tenfold lower concentration in central nervous tissue.[13]

A retrospective examination of white matter spectra from a number of volunteers that were acquired in previous investigations under different conditions (*TR*, location, age) clearly supports the present findings when the data are processed without linebroadening. Unfortunately, the detection of an expected increase of NAAG in more caudal white matter regions of the brain, e.g., in the cerebellum, pons, and medulla,[5–7] is hampered by slightly larger magnetic field inhomogeneities.[14] Limited resolution as well as a low concentration also precluded the identification of NAAG in localized ^{1}H NMR spectra of rat brain *in vivo*[15] where native NAA resonance linewidths are of the order of 5 Hz (0.05 ppm at 2.35 T).

Additional information stems from the observation

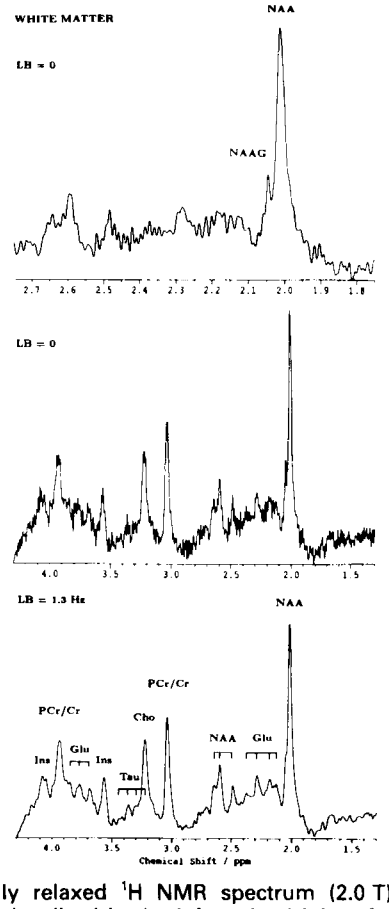

Figure 2. Fully relaxed ^{1}H NMR spectrum (2.0 T) of human white matter localized in the left parietal lobe of the same 29 year old volunteer as in Fig. 1 (8 mL VOI, STEAM, *TE* = 20 ms, *TR* = 6000 ms, 128 scans). Bottom trace: linebroadening by Gaussian multiplication (*LB* = 1.3 Hz), middle and top traces: no filtering (*LB* = 0), top trace: expanded 1.75–2.75 ppm section.

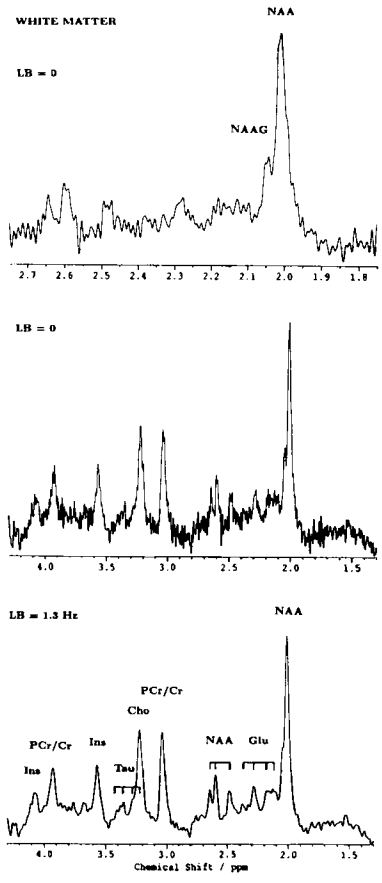

Figure 3. Fully relaxed ^{1}H NMR spectrum (2.0 T) of human white matter localized in the left parietal lobe of a 23 year old volunteer (12 mL VOI, STEAM, $TE = 20$ ms, $TR = 6000$ ms, 64 scans). Bottom trace: linebroadening by Gaussian multiplication ($LB = 1.3$ Hz), middle and top traces: no filtering ($LB = 0$), top trace: expanded 1.75–2.75 ppm section.

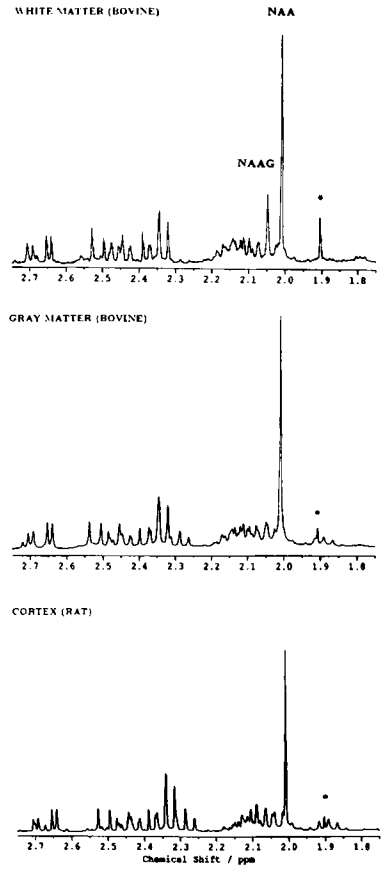

Figure 4. High-field ^{1}H NMR spectra (7.0 T) of perchloric acid extracts of rat cortex (bottom trace, *acetate reference at 1.915 ppm), bovine grey matter (middle trace), and bovine white matter (top trace). Similar to grey-white matter differences in humans, bovine brain shows significantly elevated concentrations of NAAG in white matter as indicated by its 2.05 ppm *N*-acetyl methyl resonance. The low NAAG concentration in rat cortex is in agreement with findings by Holowenko *et al.*[5]

that the 2.045 ppm resonance was not detectable in children below 10 years of age despite an even better resolution of such spectra. The absence of significant amounts of NAAG in white matter of young children parallels findings in young animals.[7] The NAAG concentration increases during brain maturation with a rostrocaudal gradient. In humans the time course for the ^{1}H NMR detectable increase of NAAG turns out to be rather slow reaching a plateau only at the age of 20, whereas the increase of NAA is found to be completed at about 2–3 years of age in close association with brain myelination (unpublished results). Finally, recent data on absolute concentrations of cerebral metabolites[16] indicate that the total *N*-acetyl methyl signal area in both grey and white matter amounts to about 10.5 mM. Assuming a NAAG contribution of 10–20% in white matter (see top traces in Figs. 2 and 3), the NAAG concentration would be 1–2 mM, while the NAA concentration reduces to about 8–9 mM.

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