In Vivo Differentiation of N-Acetyl Aspartyl Glutamate From N-Acetyl Aspartate at 3 Tesla

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A method is described that allows the in vivo differentiation of N-acetyl aspartate (NAA) from N-acetyl aspartyl glutamate (NAAG) by in vivo MR spectroscopy (MRS) at 3 Tesla (3T). The method, which is based on MEGA-point-resolved spectroscopy (PRESS) editing, selectively targets the aspartyl spin system of one species while deliberately removing the other species from the spectrum. This allows quantitative measurements of NAA and NAAG without the need for fitting of unresolved peaks. White matter concentrations of NAA (6.7 ± 0.3 mM) and NAAG (2.2 ± 0.3 mM) were measured in 10 healthy volunteers to demonstrate the method. Magn Reson Med 57:977–982, 2007. © 2007 Wiley-Liss, Inc.

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N-acetylaspartylglutamate (NAAG) is the most abundant peptide neurotransmitter in the human brain (1). As an agonist at the type 3 metabotropic glutamate receptor (mGluR3), it acts to reduce cyclic AMP levels, inhibits synaptic release of GABA, and regulates GABA receptor expression. Levels of NAAG have been suggested to be abnormal in patients with schizophrenia (2) and reduced in amyotrophic lateral sclerosis (ALS) (3). Inhibition of glutamate carboxypeptidase, which interconverts NAA and NAAG, has been studied in models of chronic pain (4), ischemia (5), and schizophrenia (6).

Structurally, NAAG consists of N-acetyl aspartate (NAA) with a peptide bond to glutamate (Glu). It is difficult to differentiate NAAG from NAA and Glu by in vivo 1H MR spectroscopy (MRS), due to the similarity of the structure and spectra of these three compounds. The most intense signal in the NAAG spectrum, that of the N-acetyl protons, is separated by only 0.03 ppm from the corresponding NAA signal (7), a difference that only allows resolution of the peaks at very high fields (8) or at 2 Tesla (2T) or 3T with unusually good shimming and field stability (9,10). There are two possible approaches to the quantification of NAAG: either the Glu portion of the NAAG molecule must be differentiated from free Glu and glutamine (Gln), or the aspartyl or acetyl signals must be differentiated from NAA. The largest chemical shift difference (approximately 0.2 ppm) is between the aspartyl α-protons of NAA and NAAG, although these resonances cannot usually be directly detected in vivo because they lie in the region of the spectrum that is normally saturated by water-suppression pulses. However, it is possible to take advantage of this frequency difference by j-editing with the MEGA-point-resolved spectroscopy (PRESS) technique (11). Applying frequency-selective inversion pulses to the aspartyl α-protons of NAAG significantly affects the evolution of coupling to the β-protons at approximately 2.6 ppm, which can be observed. However, since the chemical shift difference of the aspartyl α-protons is relatively small, the frequencies of the editing pulses have to be chosen carefully (and placed symmetrically around the NAA α-proton resonance) to avoid coediting of NAA. Symmetrical editing pulses were previously suggested by Henry et al. (12) for the detection of GABA (while removing signal associated with macromolecules) using ISIS localization, and first applied to the MEGA-PRESS sequence for GABA-editing by Terpstra et al. (13).

THEORY

The MEGA-PRESS experiment has been successfully used for the in vivo detection of GABA (11,13), glutathione (14), and ascorbate (15). In this work we demonstrate that it can also be used to detect and differentiate between NAA and NAAG. The aspartate spin systems of NAA and NAAG are suitable candidates for MEGA editing since they both have coupled spins with significant chemical shift dispersion. At an echo time (TE) of 140 ms, the β-proton signals of NAA and NAAG are both largely inverted due to the evolution of coupling to the α-proton. Refocusing of the coupling evolution, by applying an inversion pulse to the passive spins, gives a positive signal. The MEGA-PRESS methodology consists of one experiment in which the selective inversion pulse is applied to the NAAG spins (the “On” experiment), and one in which it is not (the “Off” experiment). The experiments are then subtracted to remove from the spectrum all signals that are unaffected by the editing pulse.

In attempting to selectively edit NAA and NAAG, it is important to consider the inversion profile of the editing pulse. Figure 1a shows the amplitude modulation of the sinc-Gaussian editing pulse used. The inversion profile generated, which is shown in Fig. 1b, was judged to represent a good trade-off between the narrowness of the central inversion band and the small size of the side-
bands. An editing pulse of duration 35 ms (as used in this study) is insufficiently selective to edit NAAG to the exclusion of NAA at 3T. A dotted line is superimposed on the inversion profile at the offset of NAA, and at this offset (28 Hz at 3T) the inversion profile has not returned to $\frac{1}{2}$H (corresponding to zero inversion), so coediting of NAA would occur in a simple MEGA-PRESS experiment. However, the frequency of the pulse in the Off experiment can be chosen to affect the NAA spins to the same extent; that is, the frequency of the editing pulses in the On and Off NAAG experiments can be set to be symmetrical around the frequency of the NAA spins, as shown in Fig. 1c. If a spin system is treated identically in the On and Off experiments, its signals will be removed from the spectrum on subtraction along with all of the uncoupled signals (12). Conversely, the experiment to exclusively acquire NAA signal places the editing pulses symmetrically about the NAA resonance.

FIG. 1. MEGA-editing to distinguish between NAA and NAAG. a: A 35-ms sinc-Gaussian editing pulse is not sufficiently selective to edit for one species without disturbing the other. When the pulse is set for the NAAG On experiment (represented by the dashed line superimposed on b), significant coediting of NAA will occur (dotted line on b) unless the NAA signal is suppressed by symmetrical positioning of the Off pulse. c: The editing frequencies for the NAAG experiment superimposed on simulated spectra of the NAAG (black) and NAA (gray) aspartyl $\alpha$-proton. By placing the editing pulses for the On and Off NAAG experiments symmetrically about the NAA resonance, any coediting NAA signal is subtracted from the spectrum. d: Conversely, since the editing pulses for the On and Off NAA experiments symmetrically about the NAA resonance, any coediting NAAG signal is subtracted from the spectrum.

Editing in the MEGA-PRESS experiment was achieved using two sinc-Gaussian inversion pulses of length 35 ms, as shown in Fig. 1a. The On and Off experiments of the MEGA-editing sequence were performed in an interleaved fashion, with the editing frequencies set as in Fig. 1. Prior to MEGA-PRESS, magnetic field homogeneity was optimized using the FASTMAP routine and shim correction up to second order, as well as optimization of chemical shift-selective (CHESS) water suppression.

Quantitation
To quantify absolute NAA and NAAG concentrations in vivo, a two-step process was used. Edited NAA and NAAG spectra were acquired to calculate the ratio of signal intensity of NAA to NAAG. This can be interpreted as the concentration ratio, since NAAG and NAA edit with the same efficiency. Absolute quantitation of NAA + NAAG (total NAA) was achieved through the acetyl methyl signal, compared to a reference scan acquired without water suppression. This approach bypasses the need to measure the in vivo relaxation parameters of the $\beta$-protons, relying instead on established parameters for the methyl signal (16). However, the assumption is made that the transverse and longitudinal relaxation times of the aspartyl spin system of NAA and NAAG are not significantly different.

In Vivo Measurements
Measurements were performed on 10 healthy volunteers (six females and four males, average age = 33.1 years). The study was approved by the local institutional review board and informed consent was obtained. Experiments were performed on a $3 \times 3 \times 5$ cm$^3$ volume located in predominantly white matter at the level of the centrum semiovale. Two MEGA-PRESS experiments, optimized for NAA and
NAAG, were performed with $TE = 140$ ms, $TR = 2$ s, 256 transients, spectral width = 2 kHz, 2048 data points, and experiment time = 8 min. For quantitation, a PRESS experiment in the same region was performed (16 transients) both with and without water suppression. The PRESS-excited volume was surrounded by six saturation slabs of thickness 30 mm. A new $F_0$ calibration was performed before each edited experiment in order to ensure that editing selectivity was not degraded by magnetic field drift over time.

Spectra were processed with 1.5 Hz line-broadening and eightfold zero-filling. The On and Off experiments were subtracted as raw time-domain data, retaining the separate datasets, so that appropriate phase parameters for the difference spectra could be determined. The amplitude of edited signals was determined without baseline correction, which was not found to be necessary.

The total NAA (NAA+NAAG) concentration was calculated using water as an internal standard (17). This method requires the following parameters to be known: the in vivo water concentration, $[\text{water}]$; the relaxation time constants ($T_1$ and $T_2$) of the water signal, $T_{1\text{w}}$ and $T_{2\text{w}}$; the relaxation time constants of the N-acetyl signal, $T_{1\text{NA}}$ and $T_{2\text{NA}}$; the integral of the N-acetyl peak and the corresponding water peak, $I_{\text{NA}}$ and $I_{\text{w}}$; and the number of the number of scans in the water, $n_{\text{w}}$, and metabolite, $n_{\text{NA}}$, experiments. The total NAA+NAAG concentration was calculated as:

$$2I_{\text{NAM}}n_{\text{NA}}\exp\left(-\frac{TE}{T_{2\text{w}}}(1-\exp(-\frac{TR}{T_{1\text{w}}}))\right)[\text{water}]$$

$$\frac{3I_{\text{w}}n_{\text{NA}}\exp\left(-\frac{TE}{T_{2\text{NA}}}(1-\exp(-\frac{TR}{T_{1\text{NA}}}))\right)}{3I_{\text{w}}n_{\text{NA}}\exp\left(-\frac{TE}{T_{2\text{NA}}}(1-\exp(-\frac{TR}{T_{1\text{NA}}}))\right)}.$$

RESULTS

Figure 2 shows the results of four MEGA-PRESS experiments: the NAA experiment applied to a pure NAA phantom and a pure NAAG phantom, and the NAAG experiment applied to the two phantoms. The specificity of both experiments is good, with cross-talk of NAA signal into the NAAG experiment and NAAG signal into the NAA experiment of 8% and 9%, respectively. Due to the high ratio of NAA:NAAG in vivo, the effect of contamination of NAA signal into the NAAG experiment will be increased. Figure 2c shows the in vivo NAA and NAAG MEGA-PRESS spectra of one volunteer. There appear to be a number of compounds coediting with NAAG at other frequencies, but (to our knowledge) no other compound coedits with signal at 2.6 ppm. This is discussed further below.

The average total NAA+NAAG concentration over the group is 8.9 mM with a standard deviation (SD) of 0.5 mM, and the average NAA:NAAG ratio is 3.1 with a SD of 0.4. These values are combined to give an average NAA concentration of $6.7\pm0.3$ mM and an average NAAG concentration of $2.2\pm0.3$ mM. The relatively small variation in results over the group suggests that the method is reproducible and can be used to make accurate measurements of NAAG and NAA concentrations in vivo. The lineshape and linewidth of edited spectra for all 10 volunteers were found to be consistent.

DISCUSSION

The results presented in this paper clearly demonstrate that it is possible, with very good reproducibility, to dis-
criminate in vivo between NAA and NAAG at 3T. Previously, it has generally not been possible to achieve this separation reliably using in vivo proton spectroscopy because of the extensive overlap of the N-acetyl resonances normally used for measurement. The two main limitations of the current method are the need to set the MEGA-PRESS selective pulse frequencies with great care, and the lower signal-to-noise ratio (SNR), since the observed aspartyl resonance is of much smaller amplitude (than the N-acetyl resonance). The deliberate exclusion of a known coediting component by careful choice of editing frequencies (placed symmetrically about the passive spin of the coediting spin system) was previously suggested to exclude the macromolecular contribution to edited GABA signal (12). However, we believe that this is the first application of this method for the detection of NAAG.

Absolute Values

The values of total NAA+NAAG concentration are within accepted limits (18). The majority of prior quantitative MRS studies of human brain referred to the combined NAA and NAAG signal as “NAA,” and there have been fewer reports of separate NAA and NAAG determinations. Separate quantification of NAAG was previously performed by LCModel analysis of single-voxel spectra at 2T (9). Fitting of the methyl peak shoulder is heavily reliant on shimming quality and is demanding on the fitting routine; therefore, this approach generally may yield more variable results than an editing approach. In the Pouwels and Frahm (9) study, parietal white matter NAAG concentrations were measured as 2.7 ± 1.2 mM, which is in good agreement with (but slightly higher than) the measurements presented here (2.2 ± 0.3 mM). This may be due to a lower proportion of gray matter in the voxel selected (which is possible for smaller volume measurements), or simply to the larger variability of the results.

Although the intense peak at 2 ppm is generally assigned as NAA (which is responsible for the greater part of the signal), and in this work is assumed to correspond only to NAA and NAAG, any small N-acetyl molecules in the brain will contribute to the peak. Small contributions from other N-acetyl species (e.g., N-acetylglutamate) will result in an overestimation of NAA and NAAG concentration.

One possible weakness of our method is the reliance on accurate suppression of NAAG signal in the NAA scan, and particularly of NAA signal in the NAAG scan (due to the higher concentration of NAA). Phantom measurements suggest that the discrimination is very good, and it appears from the reproducibility of the in vivo results that there is very little variation in the degree of cross-talk between experiments. This does not rule out reproducible errors in the method, possible sources of which include the measured chemical shifts of the α-protons, which are known to be pH-sensitive (19), and the chemical shift of water, which is temperature-sensitive (20). It was necessary to recalibrate $F_0$ between the two MEGA-PRESS experiments to get these results. Changes in frequency during the scans are addressed further below.

Coediting

Figure 3a shows a plot of the efficiency (and polarity) of coediting of other species with coupled protons in the 4–5-ppm range. The curve shown corresponds to the NAAG experiment, although the corresponding curve for the NAA experiment can be plotted. The chemical shifts of various coupled CH spins are marked, including NAA,
which gives zero signal intensity, glutathione (GSH), which strongly coedit, and lactate (Lac), which coedit with negative intensity. Some of the coediting signals in the spectrum are marked in Fig. 3b, and assignments are based on chemical shift and editing polarity.

It is important for the integrity of this method that no other signals coedit and result in signal at 2.6 ppm. While it is not possible to rule out this possibility absolutely, we believe that this is the case. Published chemical-shift tables of known metabolites do not suggest any possible conflicts (7,19), although the authors do not claim an exhaustive list. Of the chemically similar candidates, NAA itself is targeted for suppression and aspartate does not coedit as the α-proton chemical shift is 3.9 ppm (as shown in Fig. 3b).

If NAA signal appears in the in vivo NAAG experiment with the same intensity as in the phantom experiments (8% of maximum), and assuming a 3:1 concentration ratio of NAA:NAAG, NAA cross-talk may be responsible for 20% of the total edited NAAG signal. This will lead to an overestimation of the NAAG concentration unless appropriate spectral analysis techniques are used. Further studies should be performed to determine the origin of the residual cross-talk and its effect on the quantitation of NAAG.

It is interesting to note that the Glu moiety of NAAG coedit negatively into the NAAG spectrum at 2.1 ppm, whereas free Glu does not. This peak was not used for quantitation of the NAAG concentration in the current analysis; however, alternative analysis methods, such as generating an edited basis set to analyze the spectrum with LCModel as in Ref. 21, could be applied.

Field Drift

Although the reference frequency is calibrated before each scan, any drift in the magnet frequency during the scan can lead to cross-contamination, and this is a possible weakness of the method. Figure 3d shows the change in resonant frequency of the NAA methyl peak during two of the MEGA-PRESS experiments. In this analysis the experiments were not time-averaged over the entire duration of the experiment, but in 16 groups of 16 scans (grouped as full phase cycles) with no editing subtraction. In these scans, which are typical of the group, frequency drift over the 8-min experiment was less than 1 Hz. Figure 3c shows the effect of frequency drift on cross-talk: the response is very close to linear in this regime, with a 1-Hz offset resulting in 4.5% cross-talk. The total cross-talk (e.g., the percentage of the maximum NAA-edited signal that seeps into the NAAG experiment) that occurs during the experiment can be estimated by averaging the drift offset (because 5c is linear), and in these two cases it is estimated as -2% and +0.9%. Assuming an NAA:NAAG ratio of 3:1, NAA signal would be added to the NAAG experiment at the level of -6% and +2.7%, as a percentage of the NAAG-edited signal.

Subtraction Artifacts

In the edited spectra, an artifact of variable intensity and lineshape appears at the N-acetyl methyl frequency (2.0 ppm). This is probably caused by imperfection in the editing subtraction due to system instability or subject motion. As a percentage of the total methyl signal, this is small (~1%), and presumably subtraction artifacts of similar magnitude will be present for the edited signal measured at 2.6 ppm.

Field drift and subtraction artifacts may occur to a more noticeable extent in patients who are unable to remain still for the duration of the experiment. A post hoc check of the methyl signal drift during the experiment would be useful to catch these cases.

Further Developments

A number of further developments in the quantitation procedure are possible. As mentioned above, LCModel analysis of the NAAG-edited spectrum may improve the accuracy of measurements. It may also be possible to quantify NAAG from a single edited spectrum without comparing intensity to the NAA-edited spectrum, although this would require careful measurement of the relaxation parameters of the coupled aspartyl resonances in vivo.

To a first approximation, cross-talk of the deliberately suppressed species will be independent of linewidth, provided that the lineshape is symmetrical, because the extent of inversion of the suppressed species in the On and Off experiments will change in equal amounts. However, further investigation will be required to determine how much asymmetric linewidths affect cross-talk. When the spectral linewidth approaches the frequency difference of the α-aspartyl resonances of NAA and NAAG (0.2 ppm, or approximately 25 Hz at 3T), editing will not be possible. However, linewidths of this magnitude should not normally be encountered in most brain regions, with the exception of the inferior frontal lobe and some parts of the temporal lobe.

It should be noted that the unedited measurement made in this study is redundant, as the NAA methyl signal can be quantified by adding, rather than subtracting, the MEGA-PRESS data. However, the additional experiment is relatively short and it can be performed at a shorter TE and longer TR in order to reduce the relaxation dependency of measurements of total N-acetyl.

CONCLUSIONS

In conclusion, it has been shown that NAAG and NAA can be discriminated through appropriate application of the MEGA-PRESS method. In normal control subjects, the method appears reliable and provides robust separation of the spin systems. The main limitation of the method is its low SNR, which requires relatively large brain volumes to be interrogated. However, the method shows promise for use in global or diffuse neurological or psychiatric diseases in which NAAG is believed to be involved (22,23).

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REFERENCES


