Quantitation of Proton NMR Spectra of the Human Brain Using Tissue Water as an Internal Concentration Reference

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The use of cerebral water as an internal intensity standard for the quantitation of spatially localized proton spectra of the human brain is investigated. The method is validated on standard samples of *N*-acetyl aspartate (NAA) and lactate, and possible sources of error are discussed. Using the STEAM pulse sequence, concentrations of choline, creatine and NAA in frontal lobe white matter are found to be 1.9±0.5, 10.6±1.3 and 16.6±2.3 μmol/g wet wt, respectively, in 10 normal volunteers. In the thalamus, the concentrations are 2.0±0.4, 11.6±2.0 and 17.2±1.3 μmol/g wet wt, respectively. Choline and creatine concentrations are in good agreement with conventional biochemical values: NAA concentrations are found to be three-fold higher, suggesting overlap of the NAA signal with other compounds. Quantitation relative to tissue water is a convenient and rapid means of quantitating proton spectra of the human brain.

INTRODUCTION

The quantitation of *in vivo* NMR spectra is an issue that has received much attention in the literature.[1] Since the amplitude of the NMR signal can depend in a complex manner on a large number of variables,[2] the majority of spectroscopic studies report results as ratios of signal intensities. These ratios may be converted into concentration units if a concentration of a particular compound is known or assumed. In the context of NMR spectroscopy as a tool for clinical investigation, quantitation is an issue of key importance for several reasons. First, it facilitates the comparison of data recorded at different sites, which may be obtained on a variety of instruments using various localization sequences and spectroscopic acquisition parameters. Second, it allows correlations to be made with conventional biochemical findings. Third, it removes possible ambiguities in ratio data, where it may be unclear whether one compound is elevated or another decreased. A routine and reliable quantitation method is required for clinical spectroscopy, where time limitations may preclude time-consuming calibration procedures which are possible in other experiments.

Quantitation methods fall into two categories; those which use an external reference sample, and those which use an internal reference. Each method has its own advantages and disadvantages. The chemical composition of external references can be carefully controlled, but systematic errors can occur due to inhomogeneities of both the B_0 and B_1 magnetic fields. It is also important to accurately determine the volumes of both the external reference and the sample under study. Internal references are insensitive to variations in B_0 and B_1 fields, but it may be difficult to determine exactly the concentration of the reference compound. There may also be variations in the reference concentration under pathological conditions.

For proton spectra of the human brain, previously published concentration values have been based either on the use of an external reference,[3] or an assumed value for either total creatine (creatine and phosphocreatine) or *N*-acetyl aspartate (NAA).[4,5] Creatine and NAA, however, are not particularly suitable as internal references since there is some controversy as to their absolute concentrations. There may also be overlap between these signals and those from other compounds, and pathological concentration variations can be large.

Cerebral water, however, appears to be a suitable internal reference signal.[6] Cerebral water content is relatively uniformly distributed, and pathological changes in water content vary over a relatively small range.[7] Quantitation relative to water has been used for some time in ^{31}P NMR spectroscopy.[1,6] In this case, because the experiment is heteronuclear, care has to be taken to carefully calibrate the ratio of spectrometer sensitivities at the different observation frequencies, and flip angles need to be carefully calibrated. Also, a correction factor has to be applied if coil loading is significantly different between the calibration solutions and the sample. For ^{1}H NMR spectroscopy, the implementation is somewhat simpler since no corrections are needed for frequency dependent variations in spectrometer sensitivity, and the potential for miscalibration of flip angles is virtually eliminated. Since the same spatial localization sequence can be used to record both the water signal and the water-suppressed spectrum, virtually identical sample volumes are observed. This removes the need to reshim or recalibrate flip angles which might be necessary when external standards are used.

In this paper, the use of cerebral water as an internal

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Abbreviations used: NAA, *N*-acetyl aspartate.

0952–3480/93/010089–06 $08.00

Received 13 January 1992
Accepted (revised) 5 May 1992

intensity reference is explored as a means of quantitating proton spectra of the human brain. After validating the methodology on phantom samples, concentration values are derived for the three major resonances in the spectrum (choline, creatine and NAA) in the grey and white matter of 10 normal adult subjects. These concentrations are compared with values in the literature determined both by conventional biochemical techniques and using NMR spectroscopy.

METHODS

Ten normal subjects (seven males, three females), aged 20–46 years (average = 34 years, SD = 9), were studied in a 1.5 T General Electric Signa scanner (General Electric Medical Systems, Milwaukee, WI, USA) equipped with actively shielded field gradient coils. Using the commercial quadrature head coil supplied, water signals and water-suppressed ^{1}H NMR spectra were recorded with the STEAM sequence from a 27 cm^3 volume of frontal lobe white matter, and an 8 cm^3 volume of tissue from the thalamus. Water signals consisted of four scans, with $TR = 10$ s, $TM =$ 80 ms and $TE = 50,100,150$ and 200 ms. Water-suppressed spectra were recorded with a range of TR and TE values in order to estimate relaxation times using 16, 64 or 128 scans ($TR = 10$, 3 or 1.5 s, $TM = 80$ ms, $TE = 30,60,90,120,200$ and 270 ms). Water suppression consisted of frequency selective single 90° CHESS pulses[8] applied prior to the STEAM sequence, and between the second and third slice selection pulses of the STEAM sequence. The CHESS pulses were single lobe sinc functions of 15 ms duration, giving a saturation bandwidth of $\Delta\nu_{1/2} \sim 140$ Hz.

All spectra were processed with an exponential filter corresponding to a line broadening of 2 Hz. In addition, a convolution difference filter of the type $S_f(t) = S(t)[1 - k\exp(-t\pi b)]$ was also used to baseline correct the spectra. The convolution difference broadening factor b was set at 40 Hz, and the subtraction factor k varied from 0.8 for short echo time spectra ($TE = 30$ ms) to 0.4 for long echo times ($TE = 270$ ms). Following Fourier transformation and phase correction, spectra were inverse Fourier transformed to yield a real, filtered and 'phase-corrected' FID which was used as the input to a time-domain least squares fitting program.[9] The program minimizes the difference between the experimental data and a model function consisting of the sum of a set of exponentially decaying oscillators. The phase of each resonance was held fixed (at zero), and the first three points of the FID were discarded in order to suppress residual broad signals. The T_1 and T_2 relaxation times of water and metabolite signals were estimated by linear regression from the experimental data.

All signal intensities, S, were corrected for relaxation losses according to the formula:

$$S_0 = \frac{S(RD, TE, TM)}{(1 - \exp(-RD/T_1))\exp(-TE/T_2)\exp(-TM/T_1)} \quad (1)$$

where TM is the delay between the second and third pulses of the STEAM sequence, and $RD = TR - TM - TE/2$. Metabolite concentrations were calculated from

Table 1. Comparison of NMR and actual concentrations (mmol/L) for standard samples

Sample	Lactate (NMR)[a]	Lactate (Actual)	NAA (NMR)[a]	NAA (Actual)
A	20.5±4.2	20.0	10.4±0.9	10.0
B	33.0±0.9	30.0	4.5±0.8	5.0
C	7.1±0.8	5.0	24.0±1.9	20.0
D	10.1±2.2	10.0	15.9±1.8	15.0
E	–[b]	1.0	30.1±0.7	30.0
F	42.5±2.4	40.0	–[b]	1.0

[a] NMR values are mean±SD, $n = 3$ or 4.
[b] Signal too small to quantitate.

$$[M] = \frac{2S_0(M)}{S_0(H_2O)nc} \times [H_2O] \times 10^{-A/20} \quad (2)$$

where n is the number of protons per functional group, c is a correction factor due to partial saturation of the water-suppression pulses and A is the attenuation value (in dB) used to record the water signal. Significant c correction values were only required for the choline and creatine signals (0.79 and 0.89, respectively). Calibration of the Signa receiver attenuator revealed differences of 1.5–2.0 dB between the nominal and actual attenuation over the values used in this study (20–30 dB). The water concentration was assumed to be 39.2 μmol/g wet wt for white matter and 46.8 μmol/g wet wt for grey matter.[10]

In order to check the accuracy of the quantitation procedure, five solutions containing differing mixtures of NAA and lactate in the concentration range of 1–40 mM were prepared by accurate weighing, and analyzed using the same methodology as above. In order to estimate the *in vitro* lactate T_2 relaxation times, TE values of multiples of 135 ms were used in order to avoid signal modulation due to scalar coupling, and a low TM value (11 ms) was used to minimize evolution of zero-quantum coherence.

RESULTS

Table 1 shows the relationship between the calculated and actual concentrations for the standard samples. Figure 1 shows representative T_1 weighted MR images ($TR = 500$ ms, $TE = 20$ ms) from one subject, indicating the volumes selected by the STEAM sequence from the frontal lobe white matter and thalamus, respectively. Figure 2 shows spectra from each of these regions recorded at short echo times ($TE = 30$ ms), and the corresponding output from the time-domain least squares fitting program for the three major resonances in the spectrum. Figure 3 shows simulations of the signal intensity of the STEAM sequence using Eqn (1) as a function of T_1 and T_2 for some commonly used TR and TE values. Concentrations of choline, creatine and NAA from each region and T_2 values are given in Table 2.

DISCUSSION

Methodology

Table 1 shows that excellent agreement was obtained from the standard samples and the NMR determined concentration values. However, a number of factors

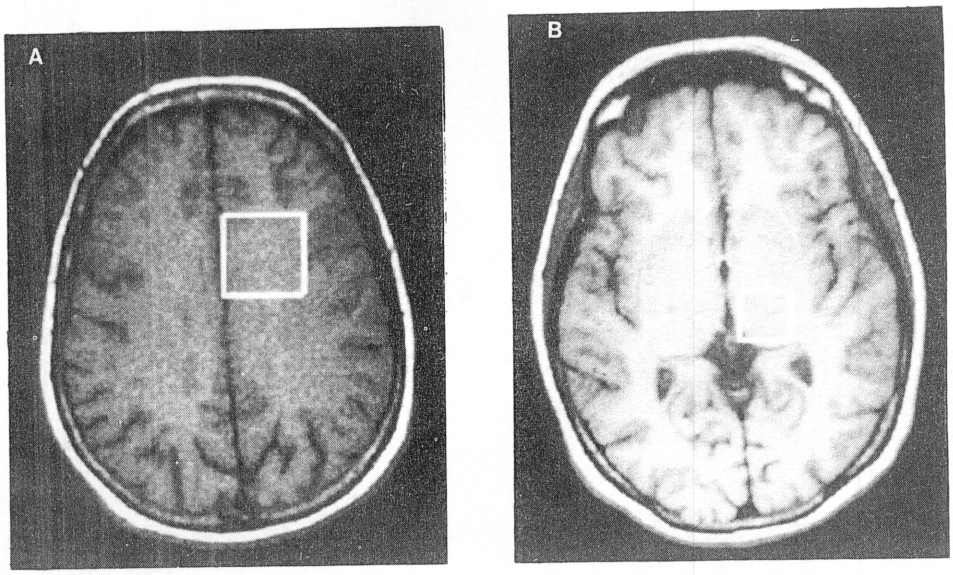

Figure 1. T_1 weighted MRI images ($TR = 500$ ms, $TE = 20$ ms) showing representative sample voxels: (A) frontal lobe white matter, 27 cm^3; (B) thalamus (predominantly grey matter), 8 cm^3.

can affect the accuracy of the quantitation procedure. It is important that the receiver attenuator and water-suppression bandwidth are accurately calibrated. Also, there is a small shift in voxel location due to the chemical shift difference between the water and metabolite signals. For studies at 1.5 T with high bandwidth slice selection pulses these shifts will be small. In the present study the RF pulse bandwidth was 4 kHz, so the voxel shift of NAA [~2.7 ppm (173 Hz) upfield from water] was ca 1.3 mm for the voxel of linear dimension 30 mm, and 0.8 mm for a 20 mm voxel.

Since most spatial localization sequences for ^1H spectroscopy involve at least some degree of T_1 and T_2 weighting, it is also important to correct for signal losses due to relaxation time effects. Figure 3(A) models the signal intensity of the STEAM sequence as a function of T_2 for a range of commonly used echo times. It can be seen that short echo times give both larger signals and reduced sensitivity to T_2 variations. For instance, a change in T_2 from 200 to 400 ms changes the signal intensity from 0.24 to 0.47 for $TE = 270$ ms, but from 0.80 to 0.86 for $TE = 30$ ms. However, spectra are often recorded with long echo times because they have less interference from lipid and residual water signals, and flatter baselines. A TE value of 270 ms is commonly used because there is no modulation of the lactate signal due to scalar coupling at this TE (see below). Figure 3(B) shows the variation of the STEAM

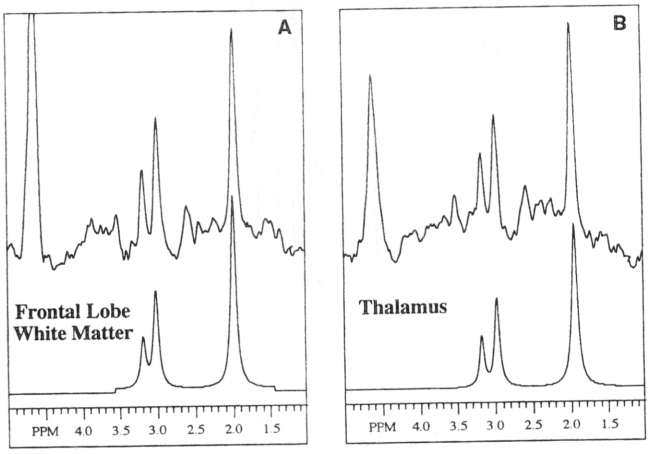

Figure 2. Corresponding STEAM proton spectra of the sample volumes indicated in Fig. 1 ($TR = 3$ s, $TE = 30$ ms, $TM = 80$ ms), and the output from the non-linear least squares fitting program for the peaks of interest: (A) frontal lobe white matter; (B) thalamus.

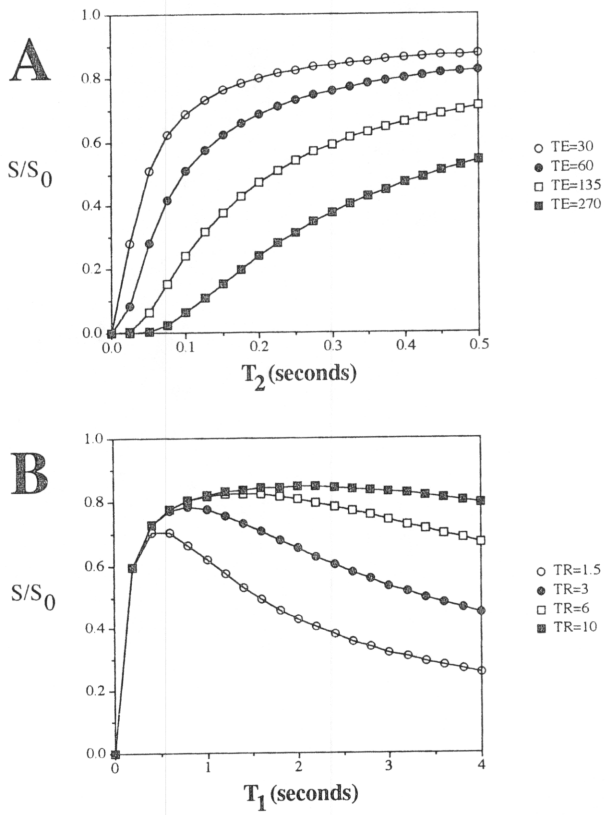

Figure 3. Simulations of the signal intensity [using Eqn (1)] of the STEAM sequence for non-coupled spins as a function of TR, TE and relaxation times: (A) T_2 variation, $TR = 6$ s, $TM = 80$ ms, $T_1 = 1.5$ s; (B) T_1 variation, $TE = 30$ ms, $TM = 80$ ms, $T_2 = 250$ ms.

Table 2. Concentrations[a] and apparent T_2 values[b] of creatine, choline and NAA in human brain

	Choline		Creatine		NAA	
	Conc.	T_2	Conc.	T_2	Conc.	T_2
White[c]	1.9±0.5	0.36±0.14	10.6±1.3	0.21±0.06	16.6±2.3	0.43±0.17
Grey[d]	2.0±0.4	0.35±0.14	11.6±2.0	0.18±0.03	17.2±1.3	0.32±0.07

[a] Concentrations in units of μmoles per gram wet weight, mean±SD, $n=10$.
[b] T_2 values are in seconds, mean±SD, $n=10$.
[c] Frontal lobe white matter.
[d] Thalamus.

signal intensity as a function of T_1 for a range of typical *TR* values. *TR* values of 6 s or more virtually eliminate T_1 weighting over typical ranges of *in vivo* proton relaxation times (for instance 1–2 s), but it should also be noted that losses can occur during *TM* if T_1 is very short. Equation (1) can be used to estimate how errors in relaxation times will propagate into metabolite concentration errors. Assuming $RD=3$ s, $TE=30$ ms, $TM=80$ ms, $T_1=0.515$ s and $T_2=0.129$ s (cerebral water), if T_1 and T_2 both have 13% error, then the propagated error in the ratio S/S_0 is only 4%. If the error in T_1 and T_2 is increased to 50%, the propagated error in S/S_0 increases to only 14%. However, if $TE=270$ ms, the propagated error in S/S_0 is 104% when T_1 and T_2 have a 50% error.

It should be noted that the T_2 measured with the STEAM sequence is often lower than the true T_2 (for instance as measured by the Carr–Purcell–Meiboom–Gill method),[11] since the STEAM sequence (when implemented with larger crusher gradients) is sensitive to both the diffusion and/or bulk macroscopic motion of spins.[12] The T_2 values reported here, and used in Eqns (1) and (2), therefore include a degree of apparent diffusional weighting. For pure water, with the pulse sequence used in the current study, assuming an apparent diffusion constant of 2×10^{-9} m^2/s,[13] the signal loss due to diffusion is estimated to be 7%. Assuming a typical intracellular apparent diffusion coefficient of 2×10^{-10} m^2/s,[14] the diffusion loss is 0.7%. However, provided that the quantitative spectra and relaxation time measurements are recorded using the same pulse sequence, no error is introduced due to differences in apparent diffusion coefficient between the solute and solvent, since this is incorporated into the T_2 values. In addition, it has also been noted that it is often the bulk macroscopic translation of the sample, rather than the molecular diffusion, which dominates the signal loss due to motion.[12] Under these circumstances, motional losses of solvent and solute are virtually identical.

It should also be recognized that the STEAM or PRESS localization sequences give complicated signal intensities as a function of *TE* and *TM* for coupled spin systems such as lactate or glutamate.[15] The signal is modulated both by the evolution of scalar coupling during *TE*, and, in the case of the STEAM sequence, of zero-quantum coherence during the *TM* period. For the lactate methyl group, scalar coupling is refocused at echo times of multiples of 135 ms (J_{HH}^{-1}). However, zero-quantum effects during *TM* are also important when *TE* is an odd multiple of 135 ms;[15] these can be minimized by using the shortest possible *TM*. Certain *TM* values (e.g., $TM=80$ ms) will result in almost no detectable lactate signal with $TE=135$ ms. For glutamate, which has a very complicated multiplet pattern, quantitation is difficult, but perhaps may be attempted from spectra recorded with very short *TE* (and *TM*) delay times.

Water content

The validity of the quantitation procedure depends on an accurate knowledge of the cerebral water content. Under pathological conditions, there may be small changes in water content and quite large changes in water relaxation times. Water relaxation times can be measured in a short period of time, and it is possible (as discussed above) to minimize their influence on the STEAM sequence by appropriate choice of acquisition parameters. Changes in cerebral water content are usually comparatively small (10–20% at most), and may also be estimated from spin density MR images. It has also been suggested that water content can be calculated from T_1 measurements.[16]

In the present study, an assumed water content was used in units of μmoles per gram tissue wet weight. The ratio of the NMR signal intensities therefore also yields calculated concentrations in the same units. However, if water content was determined from MR images using an external intensity standard, the cerebral water concentration would then be determined on a moles per unit volume basis (i.e., molarity). Molarity and wet weight concentration measurements may be related by a knowledge of the tissue density. In both cases, the concentration is expressed as a macroscopic measure, and may not reflect actual intracellular concentrations. It should be noted that if the localized volume contains appreciable contributions from fluid filled cavities such as ventricles or cysts, wet weight measurements will underestimate intracellular concentrations.

Metabolite concentration values

There is reasonable consensus between different groups concerning the ratios of peak intensities of proton spectra of the normal human brain for the major signals of NAA, creatine and choline. However, there is more disagreement concerning the absolute concentration values.[17–20] For instance, NAA values varying as widely as 5.9 to 17 mM have been reported,[3,5] whereas the conventional biochemical value from human brain is ca 4.9 μmol/g wet wt.[21,22] The following section reviews the concentrations of creatine, NAA and choline as determined in this study and compares these values with those available in the literature.

Total creatine. Using *in vivo* NMR spectroscopy and an external concentration standard, total creatine (creatine and phosphocreatine) has been determined to be

8.9±1.9 μmol/g in white matter and 9.2±1.8 μmol/g in grey matter.[3] In perchloric extracts of human brain from patients undergoing temporal lobectomy for epilepsy,[23] total creatine was determined to be in the range of 7–9 μmol/g, depending on the exact location of the biopsy sample. Biochemical assays of perchloric acid extracts of normal canine brain gave a total creatine value of 11.0 mM, corresponding to a phosphocreatine concentration of ca 5.0 mM and a creatine concentration of 6.0 mM.[24] This is in good agreement with recent determinations of human cerebral phosphocreatine levels using ^{31}P NMR spectroscopy [5.18 mM±0.89(SD)].[25] In rat brain, total creatine has also been determined to be ca 10 mM.[26,27] In the current study, creatine values of 10.6±1.3 and 11.6±2.0 μmol/g wet wt were found for white and grey matter, respectively, which are in good agreement with conventional biochemical values.

NAA. Tallan measured NAA to be 5.5 μmol/g in human brain[28] using a biochemical assay, and an average value of 4.9 μmol/g has been reported based on a wide range of references.[21] Using NMR spectroscopy and an external intensity reference, NAA values in human brain have been reported as 5.9±1.9 μmol/g in white matter and 10.5±2.1 μmol/g in grey matter.[3] In perchloric extracts of human brain from patients undergoing surgery for epilepsy, NAA values were found to be 5.97±0.26 μmol/g for cortical samples and 3.99±0.20 μmol/g for white matter.[23] The values for NAA in the current study (16.6±2.3 μmol/g for white matter, 17.2±1.3 μmol/g for the thalamus) are considerably higher than any of these values. However, they are in reasonably good agreement with the values originally reported by Frahm *et al.*[5] (13 mM for cerebellum and thalamus, 17 and 18 mM for white and grey matter, respectively) who quantitated relative to an assumed total creatine concentration of 10 mM in white matter. The numbers reported by Narayana *et al.*[3] for white matter are difficult to interpret, since the NAA is lower than the creatine concentration, whereas most other groups report NAA/creatine ratios greater than one *in vivo*.[5]

The origin of these wide variations remains to be determined. In the comparison of the *in vivo* studies, a number of technical factors will contribute to the uncertainty. In the *in vitro* studies,[23] the low NAA values may reflect either pathological conditions or changes occurring during the extraction procedure. The data presented here suggest that the peak at 2.0 ppm, which is assigned to the *N*-acetyl CH_3 of NAA, must also contain contributions from other compounds. A number of groups have speculated as to the origin of other *N*-acetyl groups, for instance *N*-acetyl aspartyl glutamate[29] or membrane associated sialic acid (*N*-acetyl neuraminic acid).[30] In addition, *in vivo* at 1.5 T, appreciable overlap will exist between NAA, glutamate and glutamine, which resonate at around 2.1 ppm.

It has also been observed that the NAA peak is decreased, and may in some cases be completely absent, in certain pathological conditions such as infarction or brain tumors.[31,32] Since NAA is believed to be primarily neuronal in origin,[22] it is generally accepted that these observations reflect the absence of viable neurons from these tissue types. However, if the 2.0 ppm peak consists of other unidentified compounds, then one must also consider that these compounds are also only associated with normal, viable brain tissue.

Choline. For choline, *in vivo* values of 1.9 mM (for insular grey matter), 2.2 mM (for white matter) and 2.7 mM (for thalamus) were reported by Frahm *et al.*[5] who quantitated relative to 10 mM creatine. The *in vitro* studies of epilepsy patients gave values of 0.49±0.08 and 0.55±0.09 μmol/g for grey and white matter, respectively.[23] The present study yielded values of 2.0±0.4 and 1.9±0.5 μmol/g for grey and white matter, respectively, which are in reasonable agreement with the values of Frahm *et al.* The composition of the choline peak has recently been reviewed by Miller.[33] Summing the biochemically determined concentrations of known water-soluble choline-containing compounds (phosphocholine, glycerophosphocholine, choline, cytidine-diphosphatyl-choline, acetylcholine, and choline-plasmalogen) yields a total trimethylamine concentration of 1.6 mM, which is fairly close to the values determined here. Any other signal contributing to this peak may result from lipid-soluble sphingomyelin and phosphatyl-choline, which would not be seen in perchloric acid extracts.

SUMMARY

Quantitation of human cerebral proton spectra using the internal water signal is a simple and convenient means of determining metabolite concentrations. The time required to record the reference water signal is negligible compared to the time of the rest of the examination. Creatine concentrations measured by this technique are in good agreement with accepted biochemical values. Choline concentrations are in reasonable agreement with biochemical values when all water soluble compounds containing trimethylamine groups are considered. Tissue biochemical NAA concentrations are some three-fold lower than the NMR determination, suggesting that the 2.0 ppm peak contains considerable signal intensity from other compounds.

The similarity between the spectra of the thalamus and frontal lobe white matter may reflect some averaging of both grey and white matter in the sample volumes. Studies at higher spatial resolution may reveal significant differences in metabolic concentrations between grey and white matter. Finally, quantitation relative to tissue water should be readily extendable from single-voxel localization methods to chemical shift imaging.

Acknowledgements

Presented in part at the 10th Annual Meeting of the Society of Magnetic Resonance in Medicine, San Francisco, CA, 10–16 August 1991. The authors would like to thank Dr Chrit Moonen and Dr Peter van Zijl (NIH *in vivo* NMR Center, Bethesda, MD, USA) and Dr Joseph Giller (Pittsburgh NMR Institute, Pittsburgh, PA) for the STEAM pulse sequence which was used to record the spectra in this study, and Dr Steven Breiter and Dr Richard Kelley for help in preparing standard samples.

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