The relative distributions of N-acetylaspartate (NAA) + N-acetylaspartylglutamate (NAAG), creatine + phosphocreatine (Cr/PCr), and choline (Cho) in the gray and white matter of human brain were determined by utilizing proton magnetic resonance spectroscopic imaging (SI). The SI data was processed using an automated spectroscopic image processing algorithm, and image segmentation was performed using a supervised technique. Linear regression analysis indicated that the NAA + NAAG (2.01 ppm) and Cr/PCr (3.02 ppm) peaks are greater in gray matter compared with white matter. The large intersubject variation observed in the Cho (3.20 ppm) resonance prevented the assessment of its regional distribution with confidence.

Key words: brain; MRI; MRS segmentation.

INTRODUCTION

The strongest peaks seen in in vivo proton magnetic resonance spectroscopy (MRS) of neural tissue occur at 2.01, 3.02, and 3.20 ppm. These peaks are thought to arise mainly from N-acetylaspartate (NAA) + N-acetylaspartylglutamate (NAAG) with possible contributions from other acetyl-bearing moieties, creatine + phosphocreatine (Cr/PCr), and choline-containing moieties (Cho), respectively. The peaks at 2.01 and 3.20 ppm have attracted considerable interest because they appear to be markers for several central nervous system diseases. There is a significant controversy about the distribution of intensity of these peaks, particularly the 2.01 ppm peak (for the sake of brevity, this peak is referred to as the NAA peak throughout this manuscript) in gray matter (GM) and white matter (WM) (1–5). Part of this controversy arises because the estimation of these distributions have been based on relatively large spectroscopic voxels that contain variable and unknown amounts of GM and WM. However, it is possible to determine the relative GM and WM content in a given spectroscopy voxel by segmenting the high resolution MR images.

The purpose of this work was to determine the relative levels of the resonances at 2.01, 3.02, and 3.20 ppm within GM and WM in human brain tissue using spectroscopic imaging (SI) with a relatively small nominal voxel size of 0.84 cm³, and a supervised tissue segmentation based on dual echo images.

METHODS

MR Data Acquisition

Six normal volunteers (five male, one female) with ages between 25 and 35 years (mean age of 30 years) were recruited for these studies. MR studies were performed on a 1.5 T GE scanner (GE Medical Systems, Milwaukee, WI) equipped with a shielded gradient coil system operating under version 4.7 software. The volunteers were positioned supine in the magnet and dual-echo (TE = 30/80 ms, TR = 3000 ms), flow compensated, contiguous, interleaved 3 mm thick axial MR images were acquired for tissue segmentation. The imaging parameters were field-of-view (FOV) = 240 mm, image matrix size = 256 × 256, and number of excitations = 0.5.

Two-dimensional SI with 32 × 32 phase encoding steps was performed on a prelocalized volume-of-interest (VOI) of 110 (anterior - posterior) × 65 (right - left) × 15 mm (superior - inferior) just superior to the lateral ventricles using the stimulated echo sequence. The acquisition parameters were TE = 30 ms, TR = 1000 ms, and TM (mixing time) = 11.3 ms, spectral width = 1000 Hz, and number of averages = 2. SI data were also acquired without water suppression with 16 × 16 phase encoding steps, and zero filled to 32 × 32 for automatic phasing (6, 7), spectral alignment, and expressing the intensity of the peaks relative to the tissue water contained within the VOI (5, 8, 9). Based on our studies, the reduced number of phase encoding steps for suppressed data does not appear to cause any noticeable artifacts.

Postacquisition processing of the MR images included application of an anisotropic diffusion filter for reducing the low frequency noise without blurring the edges (10, 11), stripping of extrameningeal tissues based on 2D connectivity (12), and radio frequency (RF) inhomogeneity correction (13), (for a robust segmentation). About 40 pixels for each tissue, GM, WM, and cerebrospinal fluid (CSF), as well as background were identified on the images for generating the scatter plot of the intensities of the 30-ms echo images, versus the intensities of the 80-ms echo images. A two-dimensional feature map was determined from the scatter plot, using nonparametric Parzen window (14). Images were then segmented (15, 16) into the tissues (GM, WM, and CSF) and background using the feature map. As described by Narayana and Borthakur (17), a single feature map was used for all

From the Department of Radiology, University of Texas Medical School at Houston, Houston, Texas.
Received September 26, 1994; revised January 27, 1995; accepted January 27, 1995.
Address correspondence to: Ponnada Narayana, Ph.D., The University of Texas Medical School, Department of Radiology, 6431 Fannin, MSB 2.132, Houston, TX 77030.
This work was supported in part by National Institutes of Health (1 R01 NS31499) and Advanced Research Program by the Texas Higher Education Coordinating Board (011618-072).
0740-319X/95 $3.00
Copyright © 1995 by Williams & Wilkins.
All rights of reproduction in any form reserved.
subjects to eliminate the interoperator and intraoperator variabilities.

The individual volumes of GM, WM, and CSF in a given spectroscopy voxel were determined as follows. Initially the nominal spectroscopy voxel size was determined by dividing the FOV (240 mm) by the number of phase encoding steps (32). Based on the imaging parameters used in the present studies, each spectroscopy voxel contained $8 \times 8 \times 5$ pixels. The factor 5 accounts for the fact that the spectroscopy slab (15 mm thick) was comprised of five 3-mm thick high resolution images. The volumes of the individual tissues in a given spectroscopy voxel were determined by adding the total number of pixels for the corresponding tissue, estimated using the image segmentation, and multiplying by the pixel dimensions. To take into account the point spread function (PSF) of each spectroscopy voxel that resulted due to a finite number of phase encoding steps and spatial apodization of the SI data, the size of the spectroscopy voxels was increased by 43% (18). Tissue maps of GM, WM, and CSF were generated in which the pixel intensity corresponded to the percentage of the tissue in each voxel.

Spectroscopic data was analyzed using an automatic spectroscopy analysis software (19). Spectral processing included DC baseline correction, Fermi spatial apodization with a 12-point roll off center and 4-point width, 2D FFT, autophasing, water suppression, deconvolution difference baseline correction using an exponential function with 50 Hz line broadening, time apodization with $-1$ Hz exponential resolution enhancement and 6 Hz Gaussian line-broadening, zero-filling to 8 K complex points, and 1D FFT. The phased data of the water-suppressed spectra located within the spectroscopic VOI were fit with Gaussian line shapes. The unsuppressed spectra were fit with Lorentzian line shapes.

Regression analysis was performed for the estimation of the GM and WM percentage contributions to the 2.01, 3.02, and 3.20 ppm peaks using SI and segmentation data. Quantitative calculations were limited to a VOI in the parietal lobe of the brain just superior to the lateral ventricles, as this is the region where we are currently conducting longitudinal SI studies of multiple sclerosis (MS) patients (20).

These studies were approved by the Institutional Committee for the Protection of Human Subjects, and written consent was obtained from each subject.

RESULTS

Figure 1 shows an example of the quality of tissue segmentation obtained from an RF corrected short TE (30 ms) and long TE (80 ms) image pair. The effect of the RF correction on the high-resolution images in Figs. 1a and 1b was a better demarcation of the GM/WM boundary (17), as can be appreciated from the segmented image shown in Fig. 1c.

The distributions of GM, WM, and CSF in a 15-mm thick slice and a resolution corresponding to $32 \times 32$ are shown in Fig. 2. These maps were generated so that metabolic and tissue distributions could be directly correlated. In these images, the gray scale intensity represents the concentration of a given tissue, with black representing the background.

The spectroscopy data indicated excellent signal-to-noise ratio even for a nominal voxel size as small as $0.75 \times 0.75 \times 1.5$ cm$^3$. Figure 3 shows the observed spectrum from a representative voxel from a normal volunteer, along with the calculated (using the parameters determined by the fitting algorithm) and difference spectra (19). The metabolic maps from the six volunteers were...
Concentrations of Neurochemicals in Gray and White Matter

Figure 3. (a) Spectrum from a representative voxel with dimensions 0.75 × 0.75 × 1.5 cm³ of a normal volunteer. The spectrum calculated from the Gaussian line shape parameters from the fitting algorithm is shown in (b). The spectra were displaced for the sake of clarity. The difference between the observed and calculated spectra is shown in the upper portion of the window (c).

FIG. 4. (a) The GM image of a normal volunteer and the superimposed metabolic maps of (b) NAA, (c) Cr/PCr, and (d) Cho.

as marked a trend toward correlation of the Cho (3.20 ppm) peak with GM.

DISCUSSION

The reliability with which the quantitative distribution of the MRS-visible neurochemicals in the GM and WM can be determined is dependent on a number of factors. These include the spectroscopy voxel size, which determines the partial volume averaging, and spectral processing and tissue segmentation algorithms for robust analysis.

In our studies, we employed a relatively small spectroscopy voxel with a nominal volume of 0.84 cc. Most previous studies, which attempted to estimate the regional variation of the NAA, Cr/PCr, and Cho peak intensities, have utilized relatively large spectroscopic voxels of 2.7 to 27 cm³ (1–5). Our segmentation studies indicate that even with the relatively small voxels we have utilized, it is difficult to find more than one or two voxels that contain either pure white or gray matter.

In the present studies, we used a supervised segmentation technique to determine the tissue compositions of the SI voxels. The interstudy and intrastudy reproducibility of this semi-automated segmentation technique has been extensively tested and shown to be robust (17, 21, 22). Furthermore, RF inhomogeneity correction of the MR images produced better demarcation of the GM/WM matter boundaries. The result was improved segmentation of brain tissue (17) leading to more accurate deter-
The SI data processing algorithm used in our analysis was shown to be fully automatic, robust, and operator-independent (19). Based on our previous studies, the intrasubject variability in the estimated intensities of the 2.01, 3.02, and 3.20 ppm peaks was less than 10% (18).

These studies demonstrate a significant difference in the intensities of the NAA and Cr/PCr resonances between GM and WM, but do not indicate a definite trend for the Cho peak. For example, these studies indicate that the concentration of Cho in GM and WM (ref. 25, Table 2) is, on the average, about 93% higher in GM compared with WM. This discrepancy may be at least in part, due to their use of relatively large single voxels for the spectroscopy studies, resulting in a significant partial volume averaging. Our results also indicate that the concentration of Cr/PCr is, on the average, about 46% higher in GM compared with WM. This is somewhat higher than the 40–50% value reported by Hennig et al. (23) based on long echo MRS studies. Again, partial volume averaging effect may be, in part, responsible for this discrepancy. Other investigators reported the relative concentrations of NAA, Cr/PCr, and Cho in GM and WM that were contained in different parts of the brain (3–5) or were expressed as ratios relative to Cr/PCr (2). It is, therefore, difficult to compare our results with these studies.

The observed peaks in the in vivo proton MRS of brain, in general, have contributions from multiple compounds. For instance, the 2.02-ppm peak has contributions from NAA, NAAG, and other acetyl-bearing moieties. Similarly, the choline resonance at 3.2 ppm arises from a variety of compounds such as free choline, acetylcholine, phosphocholine, glycerophosphocholine, etc. (24). Therefore, it is more appropriate to compare our results with those of the high resolution NMR studies performed on tissue extracts of human brain rather than biochemical assays. The high field, high resolution NMR studies of Petroff et al. (25) indicate that the ratios NAA(GM)/NAA(WM) and Cr(GM)/Cr(WM) are 1.5 and 1.3, respectively, compared with 1.5 and 1.9 obtained in the present studies. Based on the conservative criterion, these authors concluded that "the gray-white matter differences for creatine, NAA were highly significant." More importantly, they did not observe any significant differences in the concentrations of Cho in GM and WM (ref. 25, Table 2). Thus, our results are in general agreement with theirs except for the numerical difference in Cr(GM)/Cr(WM) ratio.

Our studies indicate a significant intersubject variability in the GM/WM ratios of NAA and Cr/PCr. In part, this may be due to the intrinsic biological diversity among different individuals and uncertainties in the determina-

### Table 1

Summary of the Results of the Linear Regression of the Variation Metabolites in Gray and White Matter

<table>
<thead>
<tr>
<th>Subject</th>
<th>Metabolite</th>
<th>( R )</th>
<th>( p )</th>
<th>GM/WM ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NAA</td>
<td>0.42</td>
<td>0.003</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.58</td>
<td>&lt;0.001</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.07</td>
<td>0.616</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>NAA</td>
<td>0.60</td>
<td>&lt;0.01</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.64</td>
<td>&lt;0.001</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>−0.01</td>
<td>0.944</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>NAA</td>
<td>0.77</td>
<td>&lt;0.001</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.81</td>
<td>&lt;0.001</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.36</td>
<td>0.019</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>NAA</td>
<td>0.46</td>
<td>0.008</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.72</td>
<td>&lt;0.001</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>−0.21</td>
<td>0.252</td>
<td>0.89</td>
</tr>
<tr>
<td>5</td>
<td>NAA</td>
<td>0.82</td>
<td>&lt;0.001</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.68</td>
<td>&lt;0.001</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.40</td>
<td>0.009</td>
<td>1.07</td>
</tr>
<tr>
<td>6</td>
<td>NAA</td>
<td>0.59</td>
<td>&lt;0.001</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Cr/PCr</td>
<td>0.56</td>
<td>&lt;0.001</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.04</td>
<td>0.819</td>
<td>1.04</td>
</tr>
</tbody>
</table>

NAA 1.46 ± 0.2<sup>a</sup>
Cr/PCr 1.93 ± 0.54

<sup>a</sup>Average ± SD.

...
tion of peak areas, which are about 10% in our case (19). Partial volume averaging in MR images also contributes to the observed variability. In these studies, we have utilized MR images with 3-mm thick sections for segmentation. While utilization of such thin sections reduces the effect of partial volume averaging, it is not completely eliminated. The segmentation technique utilized in the present studies does not correct for the partial volume averaging effect and may contribute to these observed intersubject variabilities.

CONCLUSIONS
Our studies indicated a statistically significant higher concentration of NAA (2.01 ppm) and Cr/PCr (3.02 ppm) peaks in GM relative to WM. Although this trend seemed to hold for the six volunteers we studied, the subject-to-subject variation appeared to be substantial. The large intersubject variations observed in Cho (3.02 ppm) precluded the assessment of the relative distribution of this metabolite in GM and WM.

REFERENCES