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Metabolite ^1H Relaxation in Normal and Hyponatremic Brain

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Abstract

Proton spin relaxation rate constants in normal and hyponatremic rat brain were measured to determine the sensitivity of metabolite relaxation properties to cytotoxic edema and to quantify metabolite concentration in normal and edematous brain. Relaxation rate constants for protons of water and spectral regions with dominant contributions from methyl protons of cholines (Cho), creatines (Cr), N-acetylaspartate (NA), and lactate (Lac), and for methylene protons of glutamate (Glu) were measured at 7 T. Changes in metabolite relaxation properties associated with cytotoxic edema were a decrease in the Cr longitudinal rate constant, from $0.63 \pm 0.02 \text{ s}^{-1}$ (mean $\pm$ SE) in controls to $0.50 \pm 0.03 \text{ s}^{-1}$ in edematous brain, and an increase in the transverse rate constant of NA from $5.3 \pm 0.2 \text{ s}^{-1}$ in controls to $6.6 \pm 0.3 \text{ s}^{-1}$ in edematous brain. Four hours after induction of hyponatremia, there was a 14% reduction in summed metabolite concentrations of Cho, Cr, and NA, and a 200% increase in Lac signal intensity. It is concluded that changes in both metabolite spin relaxation and detectable spin concentration accompany the cerebral pathology of cytotoxic edema complicated with secondary ischemia.

Keywords

quantitative magnetic resonance spectroscopy; cytotoxic edema; rat brain

INTRODUCTION

In vivo spectroscopy studies are often acquired with significant delays between spin excitation and detection to improve water and lipid suppression, and at pulse recycle rates too high to allow for complete longitudinal spin relaxation to maximize accumulated signal. To quantitate signal intensities acquired under these T_1 - and T_2 -weighted conditions, accurate knowledge of metabolite spin relaxation properties is required. Although the relaxation properties of major proton metabolites from normal brain are relatively well established (1–3), the dependencies of these properties on tissue pathologies are not. This is in contrast to the concentration and relaxation properties of tissue water protons which have been studied extensively, and it is the sensitivity of these properties to tissue pathology that make MRI a powerful clinical tool. Because metabolites have a more discrete spatial distribution than water, being localized primarily within intracellular volumes, changes in either their concentration or relaxation properties could provide a selective and complementary measure of tissue pathology. Changes in proton spectra have been reported for several disease states (4–8), and although the observed

differences are usually attributed to concentration effects, in most cases a change in spin relaxation properties could not be ruled out. In a recent report, apparent proton spin relaxation properties of cerebral metabolites were substantially altered in edematous ischemic stroke and in regions of peritumor edema (9). To improve the ability to interpret MRS data, it is important to establish the sensitivity of metabolite relaxation properties and concentration to various tissue pathologies.

The purpose of this study was to investigate the sensitivity of metabolite proton relaxation properties to the common cerebral pathological condition of cytotoxic edema. We used this data to establish quantitative estimates of metabolite concentrations in normal and edematous rat brains. In addition, we catalog the longitudinal and transverse proton relaxation properties of major metabolite resonances and H₂O in rat brain at 7 T.

METHODS

Animal Preparation

Female Sprague-Dawley rats weighing between 220 g and 250 g were intubated with a 16-gauge angiocatheter and maintained on 1.5%–2% isoflurane and a 3:7 mixture of O₂ and N₂O using a constant-volume respirator. An intraperitoneal injection of pancuronium bromide (1 mg/kg/h) was used to reduce spontaneous respiration and voluntary muscle movement. The femoral artery was catheterized and blood pressure was recorded continuously. Mean arterial blood pressure (MABP) was calculated from the diastolic pressure plus one-third pulse-pressure (10). Animal temperature was monitored using a rectal probe and maintained at 37°C–38°C by heating the bore of the magnet with warm air. Dilutional hyponatremia (DH) was induced *in situ* by an intraperitoneal injection of 15 ml/100 g animal weight of distilled water, split into two injections of 10 ml/100 g and 5 ml/100 g separated in time by 40 min, as described by Melton and Nattie (11). Seventeen animals were included in this study. Eleven animals were studied in a paired fashion for all NMR measurements—normal state data were acquired, and then hyponatremia was induced. The other animals were studied in one state; either control or hyponatremic. Three hours after the water injection NMR data acquisition from the hyponatremic state was initiated. After completion of the NMR measurements, the rats were killed by decapitation while still under anesthesia. Their brains were quickly removed, blotted dry, weighed, and placed in a vacuum oven at a temperature of 110°C for 3 days. Wet and dry weights were used to determine brain water content. In some animals, a cardiac puncture was used before decapitation to obtain 2 ml of blood for determination of serum electrolyte levels.

MR Instrumentation

All ¹H NMR studies were carried out on a QUEST 4400 imaging spectrometer (Nalorac Cryogenics Corp., Martinez, CA) operating at 300 MHz and equipped with an actively shielded gradient set. Gradient performance was optimized by adjustment of gradient pre-emphasis on direct and B₀ channels using a modification of the method proposed by Lowe and co-workers (12). A 6.7-cm highpass linear birdcage coil was used for spin excitation and a 2-cm single-turn surface coil was used for signal detection. Rats were securely mounted in a prone position with the center of the surface coil placed directly over the bregma. The volume coil was rotated to an orthogonal position with respect to the surface coil to reduce the interaction between the two coils. Further isolation was achieved by active surface coil detuning using a PIN diode switching network gated to the transmitter pulse.

MRI Acquisition

Magnetic resonance images were obtained using a 30-mm field of view and a 2-mm slice thickness. Gradient echo images were used for initial positioning, followed by proton density

and T_2 -weighted spin echo images. The matrix size was 128×128 and was zero-filled to 256 in both dimensions for display.

Measurement of H_2O Relaxation

In vivo H_2O proton relaxation properties were obtained using a PRESS (13) sequence to select a $5 \times 5 \times 5\text{-mm}^3$ volume at the center of the rat brain. The on-resonance RF pulses used for volume selection were of sinc shape and were applied in the presence of 10 mT/m gradients. The spatial profile of the 90° sinc pulse was 4.4 mm at 90% intensity, 5.0 mm at 50% intensity, and 5.8 mm at 10% intensity. The spatial profile of the 180° sinc pulse was 3.3 mm at 90% intensity, 5.0 mm at 50% intensity, and 6.6 mm at 10% intensity. Longitudinal relaxation rate constants (R_1) were measured using a progressive saturation technique with $TE = 0.020$ s, TR values that ranged from 0.050 s to 20 s, and a variable number of dummy scans (from 50 for $TR = 0.050$ s to 0 for $TR = 20$ s) to ensure a steady state. Transverse relaxation rate constants (R_2) were measured with TE values that ranged from 0.02 s to 0.3 s in 0.01 s increments, with a TR of 10 s. One signal was acquired for each TE or TR .

Measurement of Metabolite Relaxation

Two pulse sequences were used to acquire metabolite relaxation data. A modified PRESS technique, [water suppression]- $90_{ss}\text{-}\tau\text{-}180_{ss}\text{-fd-}180_{ss}\text{-fd-}2662\text{-}\tau\text{-acquire}$, was used to acquire metabolite relaxation data from a fully localized volume completely within the brain. The ss subscript indicates that the pulse was applied in the presence of a gradient, fd indicates a fixed delay, and the 2662 represents a binomial refocus pulse, used to provide additional water suppression, with refocus maxima centered 60 Hz downfield of the NA resonance and null at H_2O resonance. From these data sets several spectral regions could be analyzed quantitatively with little contamination from scalp regions. The second method was a simple spin echo, [water suppression]- $90_{ss}\text{-}\tau\text{-}2662\text{-}\tau\text{-acquire}$, which provided localization in one dimension only. Because data collected with this method contained signal from scalp regions, relaxation data only from the NA resonance was analyzed.

A $5 \times 5 \times 5\text{-mm}^3$ volume of interest (VOI), positioned at the center of the brain, was selected using the modified PRESS sequence. This large volume was used so that metabolite relaxation data could be acquired with good signal-to-noise and temporal resolution. We did not attempt to address the question of region-specific changes in metabolite relaxation properties in this study. The H_2O resonance was suppressed with three Gaussian-shaped pulses of bandwidth 80 Hz that were applied before the first slice-selective pulse. This sequence employed the same slice-selective sinc pulses used to measure H_2O relaxation. These pulses were applied in the presence of 10 mT/m gradients, resulting in a chemical shift artifact of 7×10^{-4} m/ppm. The excitation frequency of the slice-selective RF pulses was positioned at the center of the metabolite spectral region of interest-750 Hz upfield from the water resonance-to improve the registration of metabolite and H_2O VOIs. Balanced crusher gradients were used, in combination with the binomial refocus pulse, to improve water suppression. The VOI was shimmed using the unsuppressed H_2O resonance acquired with the same pulse sequence. The full-width-half-height line width of the NA resonance typically was less than 15 Hz. Echo data were collected with a 4000 Hz spectral width and 2048 complex points. R_1 was measured by running the sequence in a progressive saturation mode with recycle times that ranged from 0.75 s to 20 s and an echo time of 0.136 s, and 48 signal averages were collected after six dummy scans. R_2 was measured using echo times that ranged from 0.068 to 0.544 s with 60 signal averages and a TR of 2 s. The intervals between the refocusing pulses was fixed at 0.013 s, and echo times were varied by incrementing τ . To facilitate metabolite quantitation, H_2O signal was acquired using the modified PRESS sequence with the spectrometer frequency shifted 810 Hz so that the H_2O resonance was collected with otherwise identical parameters as was the NA resonance.

Nine additional animals were studied using a simple slice-selective pulse sequence to acquire longitudinal and transverse relaxation data sets. Water suppression was achieved with a single Gaussian-shaped pulse of bandwidth 80 Hz applied before the slice-selective excitation pulse. The binomial refocus pulse was combined with crusher gradients to provide additional water suppression. A 5-mm-thick slice transverse to the long axis of the brain was selected using a 10-mT/m gradient. The longitudinal data were acquired by running the sequence in a progressive saturation mode with an echo time of 0.272 s and pulse recycle times that ranged from 0.4 s to 20 s with 24 signal averages. The transverse data were collected with echo times, varied by incrementing τ , that ranged from 0.136 s to 0.544 s with a recycle time of 2 s and 60 signal averages.

Data Processing

Metabolite time domain data were apodized using a symmetric Gaussian function that produced an effective frequency broadening of 10 Hz. For quantitative analysis, metabolite signal intensities were obtained by numerical integration of magnitude spectra over a 60-Hz window centered on the peak of interest. For the slice-selective pulse sequence, NA signal amplitudes were extracted from peak height measurements. H₂O relaxation data were extracted from echo heights in the time domain. Peak regions centered at 3.25, 3.04, 2.36, 2.03, and 1.33 ppm are referred to as Cho, Cr, Glu, NA, and Lac, respectively. It is recognized that intensity from each of these spectral regions arises from several chemical moieties. However, these labels reflect the dominant source of signal intensity from normal brain and are used for brevity.

R_1 values were extracted using a single exponential function. All fits were optimized using a Levenburg-Marquardt algorithm. Fits to group relaxation data sets were done by weighting residuals by the inverse variance of each data point. Fits to individual data sets were optimized with a function that weighted residuals by a factor that was inversely proportional to the signal integral. This was done so that the high intensity points did not dominate the fitting behavior.

Volume localization requires gradient switching that can increase apparent R_2 . To validate methods used in this study, the PRESS and modified PRESS techniques were compared with a nonselective Hahn spin echo sequence that employed rectangular excitation and refocus pulses. The PRESS and modified PRESS techniques were used to select a $5 \times 5 \times 5$ -mm volume from a 6-mm inner diameter spherical phantom containing CuSO₄ doped H₂O and compared with a nonselective Hahn spin echo for determining R_2 .

Metabolite Quantitation

Absolute signal intensities, returned from the fittings of variable TE and TR data sets, were used for metabolite quantification using tissue water as a reference (14). The following equation was used to estimate metabolite concentration in units of mmol/kg tissue.

$$[\text{met}] = \frac{n_{\text{H}_2\text{O}}}{2n_{\text{met}}} \left\{ \frac{S_{0,\text{met}}^T (1 - e^{-R_{1,\text{H}_2\text{O}} TR_{\text{H}_2\text{O}}})}{S_{0,\text{H}_2\text{O}}^T (1 - e^{-R_{1,\text{met}} TR_{\text{met}}})} + \frac{S_{0,\text{met}}^L e^{-R_{2,\text{H}_2\text{O}} TE_{\text{H}_2\text{O}}}}{S_{0,\text{H}_2\text{O}}^L e^{-R_{2,\text{met}} TE_{\text{met}}}} \right\} \cdot 10^{\Delta G n / 20} p(\nu) \cdot c_{\text{H}_2\text{O}} \cdot wf \quad (1)$$

where S_0 is the signal amplitude extrapolated to either $TR = \infty$ or $TE = 0$ for superscript L (longitudinal) or superscript T (transverse); n_{met} is the number of equivalent protons that give rise to the resonance, $n_{\text{H}_2\text{O}}$ is equal to 2; $\Delta G n$ is the difference in receiver gain used to collect metabolite and H₂O data (typically -40 dB); $p(\nu)$ is a frequency dependent factor to correct the binomial pulse profile (correction factors of 3.6 for Cho, 2.2 for Cr, 1.0 for NA and 2.6 for Lac); $c_{\text{H}_2\text{O}}$ is the molar density of pure water (55 mol/kg); and wf is the water fraction of brain

determined from *ex vivo* measurements. Water and all metabolites were assumed to be completely NMR-visible.

A peak centered at 1.33 ppm in spectra obtained with long echo times was evident in most hyponatremic animals. We assign this peak to the methyl protons of Lac. The intensity of this peak increased during hyponatremia in five of the eight animals studied quantitatively. Because the R_2 of Lac could not be reliably extracted, we report signal intensity values obtained from TE 0.272 s spectra in arbitrary units normalized to controls.

Statistical analysis was performed using an unpaired (paired for proton concentration data) two-sided t test corrected for multiple comparisons using the method of Bonferroni (15).

RESULTS

There were no significant differences between H_2O R_2 values extracted from signal collected by the three sequences used for methods validation. The $CuSO_4$ doped H_2O R_2 values obtained were $8.1 \pm 0.1 \text{ s}^{-1}$ for the Hahn spin echo sequence, $8.3 \pm 0.1 \text{ s}^{-1}$ for the PRESS sequence, and $8.3 \pm 0.1 \text{ s}^{-1}$ for the modified PRESS sequence. This indicates that the localization gradients did not significantly increase R_2 .

Representative MRI obtained from normal and hyponatremic brain with the VOI indicated in white are presented in Figure 1. The major blood vessels (see arrows) appear as dark regions in the control T_2 -weighted image and are isointense with surrounding brain in the image from the hyponatremic brain. This could indicate reduced flow because of brain swelling.

Representative spectra from fully localized longitudinal and transverse data sets of normal and hyponatremic rat brain are displayed in Figure 2 and Figure 3, respectively. The signal-to-noise, spectral resolution, and water suppression are typical for data acquired in this study. Spectra summed from the eight volumetric studies for normal and hyponatremic brains are plotted in Figure 3g and Figure 3h, respectively. A difference spectrum (3h-3g) is plotted in Figure 3i. The difference spectrum clearly shows an increase in signal intensity at 1.33 ppm, and reductions at 2.02 ppm and 2.36 ppm.

Relaxation decay curves for NMR signals found to have different relaxation rate constants between control and hyponatremic states are displayed in Figure 4 and Figure 5. Each point of these plots represents a mean value determined from normalized individual data sets with standard errors indicated by vertical bars.

Physiological data obtained from normal and hyponatremic states is presented in Table 1a. Mean arterial blood pressure was reduced, and brain water content was increased in hyponatremic animals compared with controls.

Results of the longitudinal relaxation studies are summarized in Table 1b. Mean values were determined from parameters extracted from fits to individual data sets. The R_1 of water protons was reduced by 13% in hyponatremic animals compared with controls. The R_1 of the Cr resonance was reduced by 26% in hyponatremic animals compared with controls. In five of the studies, the intensity of the Lac increased substantially in hyponatremia and allowed for estimates of R_1 .

Transverse relaxation properties are summarized in Table 1c. There was a 6% reduction in R_2 of water protons in the hyponatremic group relative to the control group. R_2 of the NA resonance was increased by 25% in hyponatremic animals compared with controls. There was no difference between NA R_1 values determined with the modified PRESS technique ($5.1 \pm 0.2 \text{ s}^{-1}$, $n = 8$) and the slice-selective technique ($5.4 \pm 0.2 \text{ s}^{-1}$, $n = 6$) for controls.

Quantitative measurements of H₂O and four major brain metabolites are presented in Table 1d. The value for H₂O is reported in arbitrary units normalized to the mean value obtained for the control group. The total concentration of [Cho + Cr + NA] was reduced by 14%, and Lac signal intensity was increased 200% for the hyponatremic group compared with controls.

DISCUSSION

The physiological changes that we observe after intraperitoneal water injection include decreased serum sodium, increased serum potassium, increased brain water, and reduced MABP. The first three findings, which agree with those reported by Melton and Nattie (11), are consistent with DH. These authors have shown that in this model of DH, total brain water increased, the intracellular space increased, and the extracellular space decreased. These findings indicate a condition of cytotoxic edema, as has been consistently reported in models of water intoxication (16,17).

The major NMR-related findings of this study were that in cytotoxic brain edema, (1) both R_1 and R_2 of brain H₂O protons were reduced, (2) the R_1 of Cr was decreased, (3) the R_2 of the NA resonance was increased, and (4) the total concentration of [Cho + Cr + NA] was decreased and Lac signal intensity increased.

Changes in H₂O Spin Relaxation

The finding of a decrease in the longitudinal and transverse relaxation rate constants of H₂O protons with increased brain water is in agreement with previous studies (18–20). Although H₂O R_2 is typically more sensitive to changes in tissue hydration than R_1 (18–20), this is contrary to our findings. The discrepancy is likely because of differences in methods used to acquire the relaxation data. Reports that demonstrate greater sensitivity of H₂O R_2 compared with R_1 with brain water fraction used a Carr-Purcell-Meiboom-Gill-type sequence to acquire the data (18–20). In contrast, we used a Hahn spin-echo technique to generate the relaxation data of this report. It is well known that when extremely short-pulse repetition times are used, true transverse relaxation can be determined, and when long repetition times are used, the values measured, particularly for tissue, are dominated by diffusion effects (21). This suggests that diffusion effects reduce H₂O R_2 sensitivity for detecting increased water content.

Changes in Metabolite Spin Relaxation

The R_1 of the Cr resonance decreased by 26% in hyponatremic brains compared with controls. The major constituents of this resonance are methyl protons of creatine (Cr) and phosphocreatine (PCr) (22), and a minor contribution comes from the γ protons of γ -aminobutyric acid (23). The finding of a decrease in the R_1 of the Cr resonance could be related to changes of brain energy status. Specifically, increased Cr/(Cr + PCr) might be expected because Lac increased (see below). The difference in R_1 that we observe could be because of intrinsic relaxation differences between Cr and PCr and an increase in Cr/(Cr + PCr) during DH. Under physiological conditions, PCr has a larger net negative charge than Cr and could have a larger R_1 because of increased interaction with macromolecules. Alternatively, a reduction in enzyme-mediated exchange between PCr and Cr, through a site efficient in inducing longitudinal spin relaxation, could be responsible for the observed decrease in the R_1 of Cr. A decrease in the R_2 of Cr has been reported in postmortem rat brain at 4.7 T (24) and human cerebral infarcts at 2.0 T (25), with speculation that increased Cr/(Cr + PCr) was responsible for the decrease. In contrast, we do not observe a convincing decrease in Cr R_2 in DH. However, the sensitivity to detect change in metabolite R_2 could be field dependent.

The R_2 of the NA resonance was increased by 25% in hyponatremic animals compared with controls. The mechanism for the increase was not determined, and we can only speculate as to

the cause. There was no difference in the NA R_1 between the control and hyponatremic groups, suggesting a secular component for the increase in R_2 . Because *in vivo* R_2 measurements are dominated by diffusion effects (21), a mechanism that increases the diffusibility of NA or the severity of microscopic field gradients could explain the increased R_2 . The distribution of microscopic field gradients could be different if blood oxygen levels were significantly reduced in DH, as could occur in a hypoperfused state. Reduced cerebral blood flow has been reported in severe water intoxication (26). However, this mechanism would be expected to affect spins nonspecifically. Although a net increase in R_2 of Cho, Cr, and H_2O was not observed, an increased diffusional contribution could partially or totally negate a decrease in R_2 due to other interactions. Reductions in Cr R_2 have been reported at lower fields (24,25), and others have measured much larger reductions in H_2O R_2 for a similar increase in water content (18–20) when techniques less sensitive to diffusion were used. It is worth noting that the binomial refocus pulse reduced signal intensity in spectral regions removed from the NA resonance. Although we have corrected for the pulse profile in quantitative estimates of metabolite concentration, the loss of signal intensity for regions other than NA reduces the power to detect change in Cho, Cr, and Lac relaxation properties. Alternatively, a constituent other than NA, possibly methyl protons of N-acetylaspartyl glutamate or methylene protons of Glu or glutamine, could be altered in either its contribution to NA peak intensity or spin relaxation properties.

Changes in metabolite relaxation rate constants that are associated with pathology can have serious implications concerning interpretation of qualitative or semiquantitative spectroscopy data. For example, the 25% increase in NA R_2 from 5.3 s^{-1} to 6.6 s^{-1} we observe would lead to a 30% reduction in relative signal intensity for an echo time of 0.272 s, without any change in metabolite concentration. Similarly, a decrease in the R_1 of Cr peak from 0.63 s^{-1} to 0.50 s^{-1} would lead to a 19% reduction in signal intensity for this peak for a recycle time of 1.0 s. In this example, the changes in the ratio of NA/Cr would be mitigated, and if this index were used, sensitivity in detecting pathology would be reduced. An increase in the NA R_2 during the acute phase of edema could also be relevant to recent reports of reversible NA loss (27, 28).

If metabolite concentrations are desired, acquisition schemes insensitive to changes in relaxation rate constants of the magnitude reported here could be implemented. Collection of echo data at 0.032 s would reduce changes in signal intensity because of NA R_2 differences observed in this report to <4%. However, a reduction in echo time, although offering the additional advantage of increased signal amplitude and information content, places increased technical demands on hardware performance, water and lipid suppression, and parameter extraction algorithms. Sensitivity to differences in R_1 could be eliminated by sacrificing data collection efficiency. However, sacrifices of this type are difficult to make, given the inherently low signal-to-noise conditions associated with *in vivo* MR spectroscopy.

Quantitation of NMR Signals

Metabolite concentrations that we estimated from the NMR signals of Cho, Cr, and NA resonances are in agreement with the literature on NMR values from mammalian brain (29, 30), but all values were systematically greater than values determined from rat brain extracts using standard biochemical analyses (31). However, it should be emphasized that quantitative values reported here are for spectral regions and do not directly reflect the concentration of individual metabolites. Extraction of absolute levels of individual metabolites requires an approach that uses full prior knowledge; one such approach has recently been reported by Provencher (32), in which the spectral patterns of expected cerebral metabolites serve as a basis set for spectral curve fitting. A limitation in using H_2O as an internal reference is related to the observation that roughly 15% of brain water relaxes with a large R_2 (33). This could lead to

overestimates in metabolite concentration if H₂O NMR signals are collected with echo times that are longer than $1/R_2$ of this fast relaxing water component. However, Barker and coworkers (22) found good agreement between cerebral metabolite concentrations estimated from *in vivo* proton spectra using internal H₂O referencing to values obtained from extract studies, thus providing validation of this method.

Mean signal intensity centered at 1.3 ppm in long echo time spectra ($TE > 0.272$ s) was greater in the hyponatremic compared with the control group and was significantly greater than baseline in both groups. The R_1 of this peak, estimated from five hyponatremic animals, was consistent with that of a small molecule. Spectroscopic imaging in acute hyponatremia has demonstrated a uniform distribution of this spectral region throughout the brain (data not shown), consistent with Lac and a global secondary ischemic condition accompanying hyponatremia. Although MABP was reduced from 90 mmHg in controls to 70 mmHg in hyponatremic animals in this study, it is unlikely that this alone was responsible for increased Lac, because an MABP of 60 mmHg did not result in increased Lac in otherwise normal rats (34). The increased Lac observed for the hyponatremic group could be due to a reduction in cerebral perfusion pressure (CPP). It has been reported that intracranial pressure (ICP) is increased in this model of DH (35). The combination of increased ICP and reduced MABP could lead to a reduction in CPP ($CPP = MABP - ICP$) below 40 mmHg, the threshold for increased brain Lac production (34). A reduced flow condition accompanying DH is suggested by qualitative MRI differences between the groups. Major blood vessels in images from control show regions of hypointensity, with flow artifacts apparent surrounding the sagittal sinus in Figure 1a. The corresponding regions in the images from hyponatremic brain are isointense, and no flow artifacts are present. We interpret this to indicate a low flow condition, with the potential for secondary ischemia accompanying cytotoxic edema. Interestingly, ^{31}P spectroscopy of rat brain 48 h after induction of hyponatremia showed a normal PCr level and no indication of ischemia (36). Likewise, gradual onset of hyponatremia does not affect the cerebral energy status of rabbit brain as determined using ^{31}P NMR (37). These discrepancies are likely because of differences in the rate of onset and physiological adaptation to sustained hyponatremia.

We found a 14% reduction in the combined concentrations of Cho and Cr and NA 4 h after induction of DH. The largest changes are reductions in Cho and NA. Reductions in soluble, low-molecular-weight organic compounds have been reported at 24 h after severe hyponatremia (31). These workers found a 20% decrease in NA, a 40% reduction in Glu, and a 33% decrease in Cr. A twofold increase in Cho has been reported in salt-loaded rat brain (38), indicating that the concentration of this compound is affected by differences in brain hydration. It has been postulated that cellular concentrations of these low-molecular-weight metabolites respond to buffer changes in water transport between the plasma and intracellular environments during osmotic stress in an attempt to regulate intracellular volume(38).

In summary, we have presented evidence that the R_1 of the Cr resonance is reduced, and the R_2 of the NA resonance is increased in cytotoxic edema and secondary ischemia in rat brain at 7 T. Further study is required to elucidate mechanistic details for the relaxation changes observed. In addition, we find a reduction in the concentration of Cho + Cr + NA in water-loaded rat brain 4 h after DH was induced. Knowledge of spin relaxation and concentration alterations that accompany pathology, as we have demonstrated in this report, could help guide selection of sequence parameters to improve discrimination of diseased tissue using spectroscopy.

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control

hyponatremic

FIG. 1.

MR images of normal and hyponatremic rat brain. The proton density images displayed in the top row, (a) control and (c) hyponatremic, were acquired with $TE = 13$ msec and $TR = 2000$ ms. The T_2 -weighted images in the bottom row, (b) control and (d) hyponatremic, were acquired with a $TE = 65$ ms and $TR = 1000$ ms. The white square in panel (a) represents the spectroscopy VOI.

FIG. 2.

Representative spectra from the longitudinal relaxation series with $TE = 0.136$ s. Control $TR = 0.75$ s (a), control $TR = 2$ s (b), control $TR = 20$ s (c), hyponatremic $TR = 0.75$ s (d), hyponatremic $TR = 2$ s (e), and hyponatremic $TR = 20$ s (f).

FIG. 3.

Representative spectra from the transverse relaxation series with $TR = 2$ s. Control $TE = 0.068$ s (a), control $TE = 0.136$ s (b), control $TE = 0.272$ s (c), and hyponatremic $TE = 0.068$ s (d), hyponatremic 0.136 s (e), and hyponatremic 0.272 s (f), summed control $TE = 0.272$ s (g), summed hyponatremic $TE = 0.272$ s (h), and difference spectrum (i) (h-g).

FIG. 4.

Longitudinal relaxation behavior. Filled circles represent mean data from normals, and open circles represent mean data from hyponatremic animals. Lines represent nonlinear fits to control (solid) and hyponatremic (dotted) group data. H₂O protons, R_1 s of 0.60 s^{-1} for control and 0.54 s^{-1} for hyponatremia (a), and Cr resonance, R_1 s of 0.67 s^{-1} for control and 0.44 s^{-1} for hyponatremia (b). The ordinate axes are normalized, $S(TR = 20 \text{ s}) - S(TR) / (S(TR = 20 \text{ s}) - S(TR = 0.05 \text{ s}))$ for panel (a) and $S(TR = 20 \text{ s}) - S(TR) / (S(TR = 20 \text{ s}) - S(TR = 0.75 \text{ s}))$ for panel (b).

FIG. 5.

Transverse relaxation behavior. Filled circles represent mean data from normals, and open circles represent mean data from hyponatremic animals. Lines represent nonlinear fits to control (solid) and hyponatremic (dotted) group data. Water protons, R_2 s of 21.2 s^{-1} for control and 20.1 s^{-1} for hyponatremia (a), and NA resonance, R_2 s of 5.1 s^{-1} for control and 6.2 s^{-1} for hyponatremia (b). The ordinate axes are normalized, $S(TE)/S(TE = 0.02 \text{ s})$ for panel (a) and $S(TE)/S(TE = 0.068 \text{ s})$ for panel (b).

Table 1
Summary of Measurements

Variable	<i>n</i>	Control mean $\pm$ SE	Hyponatremic mean $\pm$ SE	<i>P</i>
a. Physiology				
Water fraction (%)	14	78.6 $\pm$ 0.2	80.9 $\pm$ 0.3	<0.001
MABP (mm Hg)	14	91 $\pm$ 4	67 $\pm$ 6	<0.021
Serum sodium (mmol/L)	4	145 $\pm$ 1	119 $\pm$ 3	<0.005
Serum potassium (mmol/L)	4	4.7 $\pm$ 0.2	8.1 $\pm$ 0.3	<0.003
b. Longitudinal relaxation rate constant (s^{-1})				
H ₂ O	14	0.61 $\pm$ 0.01	0.54 $\pm$ 0.01	<0.001
Cho	8	0.71 $\pm$ 0.04	0.67 $\pm$ 0.05	NS
Cr	8	0.63 $\pm$ 0.02	0.50 $\pm$ 0.03	<0.018
Glu	8	0.60 $\pm$ 0.04	0.60 $\pm$ 0.06	NS
NA	14	0.53 $\pm$ 0.03	0.54 $\pm$ 0.02	NS
Lac	5		0.57 $\pm$ 0.05	—
c. Transverse relaxation rate constant (s^{-1})				
H ₂ O	14	21.1 $\pm$ 0.2	19.8 $\pm$ 0.3	<0.045
Cho	8	5.2 $\pm$ 0.2	4.6 $\pm$ 0.2	NS
Cr	8	7.5 $\pm$ 0.2	6.8 $\pm$ 0.3	NS
NA	14	5.3 $\pm$ 0.2	6.6 $\pm$ 0.3	<0.013
d. Concentration				
H ₂ O (arbitrary units, normalized)	14	100 $\pm$ 4	103 $\pm$ 4	NS
(Cho + Cr + NA) (arbitrary units, normalized)	8	100 $\pm$ 5	86 $\pm$ 6	<0.042
[Cho] (mmol/kg)	8	2.0 $\pm$ 0.2	1.4 $\pm$ 0.2	<0.143
[Cr] (mmol/kg)	8	11.1 $\pm$ 0.8	9.8 $\pm$ 0.9	NS
[NA] (mmol/kg)	8	10.5 $\pm$ 0.8	8.4 $\pm$ 0.9	<0.175
Lac (arbitrary units, normalized)	8	100 $\pm$ 20	300 $\pm$ 70	<0.025

NS indicates $P > 0.2$. Probabilities multiplied by 19 to correct for multiple comparisons.