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Ethanol in Human Brain by Magnetic Resonance Spectroscopy: Correlation With Blood and Breath Levels, Relaxation, and Magnetization Transfer

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Abstract

Background—Proton magnetic resonance spectroscopy (^1H MRS) allows measurement of alcohol in the human brain after alcohol consumption. However, the quantity of alcohol that can be detected in the brain by ^1H MRS pulse sequences has been controversial, with values ranging from about 24% to 94% of the temporally concordant blood alcohol concentrations. The quantitation of brain alcohol is critically affected by the kinetics of alcohol uptake and elimination, by the relaxation times of the protons that give rise to the brain alcohol signal, and by the specifics of both pulse sequence timing and radio frequency pulse applications.

Methods—We investigated these factors in 20 light-drinking subjects after oral administration of approximately 0.85 g/kg body weight of alcohol by localized ^1H MRS and measurements of blood and breath alcohol concentrations obtained at the same time. Specifically, we measured transverse and longitudinal relaxation times of brain alcohol and its signal saturation on application of on- or off-resonance radio frequency pulses. All ^1H MRS measurements were performed at a time after brain and blood alcohol concentrations had equilibrated.

Results— ^1H MRS measures of brain alcohol were correlated highly with both breath and blood alcohol concentrations after equilibration in brain tissue. The measured ^1H MRS relaxation times of brain alcohol were shorter than given in previous reports that were limited by smaller subject numbers, improper use of ^1H MRS methods, and estimates rather than measurements. The brain alcohol signal decreased by about 30% on application of on- or off-resonance saturation pulses.

Conclusions— ^1H MRS allows direct measurement of brain alcohol, formerly only possible indirectly through inferences from breath alcohol levels. Quantitation of brain alcohol levels need to take into account measured relaxation times and alcohol signal attenuation due to presence and timing of standard radio frequency MRS pulses. Saturation experiments give evidence for the existence of more than one compartment of brain alcohol characterized by different molecular environments. They suggest that a fraction of brain alcohol is invisible to ^1H MRS.

Keywords

Magnetic Resonance Spectroscopy; Brain; Ethanol; T₁; T₂; Magnetization Transfer

The brain is the organ system through which ethanol (EtOH) intoxication, tolerance, and dependence are manifest. Research into the brain systems involved in the acute and chronic effects of EtOH would be facilitated greatly by a methodology for directly measuring EtOH concentrations throughout the brain. In vivo proton magnetic resonance spectroscopy (¹H MRS) can measure brain EtOH. However, ¹H MRS-derived brain EtOH measures have been, in general, significantly below EtOH concentration measured by standard analytical methods in temporally concordant blood samples (Chiu et al., 1994; Hanstock et al., 1988, 1990; Hetherington et al., 1989; Mendelson et al., 1990, 1992; Moxon et al., 1991; Spielman et al., 1993). This is most likely because ¹H MRS signals are affected dramatically by the molecular environment of the molecule under study. Our working model is that brain EtOH exists in at least two different molecular environments. Brain EtOH exists in intra- and extracellular fluid, where its molecular motion is unrestricted, and hence it is visible via ¹H MRS. Brain EtOH also exists in close association with macromolecules in cell membranes. In such an environment, the molecular mobility of EtOH is highly restricted, and it has been postulated that EtOH in this compartment (i.e., associated with cell membranes and proteins) is invisible by ¹H MRS. Although these multiple EtOH compartment (i.e., associated with cell membranes and proteins) is invisible by ¹H MRS. Although these multiple EtOH compartments have been postulated in tissue, only limited experimental data are available that support this compartmentalization.

EtOH intoxication is likely to be mediated through EtOH's nonspecific interactions with brain cellular membranes or via direct interactions with receptor proteins. Both mechanisms require EtOH molecules in close contact with brain macromolecules. Early electron spin resonance and fluorescence studies showed that the degree to which EtOH is associated with macromolecules (its solubility or partition into membranes) is altered in chronic alcoholism, which possibly explains resistance to acute effects of alcohol (Chin and Goldstein, 1977; Rottenberg et al., 1981). In addition, infrared studies have shown that the anesthetic action of alcohol is associated with EtOH's binding to membrane phospholipids (Chiou et al., 1992). These studies support our working model in which brain EtOH exists in different molecular environments.

Studies that combined magnetization transfer and ¹H MRS methods in examining animal brain and brain membrane suspensions strongly support the existence of EtOH in multiple molecular environments (i.e., compartments) (Fein et al., 1995; Govindaraju et al., 1997; Meyerhoff et al., 1996). The different mobility of EtOH in these compartments is the physical basis for different relaxation characteristics and signal linewidths, which in turn give rise to different "visibilities" of these compartments in in vivo ¹H MRS experiments. Freely mobile brain EtOH gives rise to a narrow and readily MRS-visible signal with a relatively long transverse relaxation time, whereas EtOH associated with macromolecules gives rise to a very broad and essentially MRS-invisible signal with an extremely short transverse relaxation time. Thus, it is possible that after alcohol consumption, not all EtOH

in brain is observable by ^1H MRS. Such partial EtOH visibility may help explain ^1H MRS-derived brain EtOH concentrations that are significantly below blood-derived EtOH concentrations (Chiu et al., 1994; Hanstock et al., 1988, 1990; Hetherington et al., 1989; Mendelson et al., 1990, 1992; Moxon et al., 1991; Spielman et al., 1993). In addition, considerable uncertainty exists even about the relaxation characteristics of the MRS-visible portion of brain EtOH. Knowledge of relaxation times is needed both to choose appropriate experimental parameters for ^1H MRS studies of brain EtOH and for subsequent accurate quantitation of visible brain EtOH. Various studies report transverse relaxation times (T_2) that differ by a factor of four (Haseler and Brooks, 1993; Hetherington et al., 1989; Rose et al., 1992; Spielman et al., 1993), and longitudinal relaxation times (T_1) have been inferred but never measured (Haseler and Brooks, 1993; Spielman et al., 1993).

More recently, the visibility of brain EtOH has been postulated to be strongly affected by the development of either chronic (Chiu et al., 1994) or acute alcohol tolerance (Kaufman et al., 1996). The model here is that alcohol tolerance affects the proportion of brain alcohol that is associated with brain macromolecules (MRS invisible) versus the proportion that is in intra- and extracellular fluid (MRS visible). These hypothesized effects have fueled interest in the use of quantitative ^1H MRS measurements of EtOH for research and clinical purposes.

Finally, early ^1H MRS measurements suggested only a weak correlation between brain EtOH levels determined by ^1H MRS and concurrent EtOH levels determined in blood (Mendelson et al., 1990). Here, we describe an EtOH administration and in vivo ^1H MRS protocol for measuring brain EtOH over an extended period of time with simultaneous monitoring of blood and breath alcohol concentrations. The aim of the study was to establish optimal parameters for measuring visible brain EtOH and to test our working model of EtOH compartmentalization. We performed localized ^1H MRS of EtOH at 1.5 T magnetic field strength in different regions of the human brain to determine if there is a correlation between ^1H MRS-derived brain EtOH levels and blood and breath alcohol concentrations; to measure in vivo brain EtOH relaxation times; and to determine if a magnetization transfer effect can be detected in human brain after EtOH consumption, which would suggest different compartments of brain EtOH.

METHODS

Subjects

Twenty subjects (18 men, 2 women; 32 ± 6 years) were studied according to protocols approved by the local Institutional Review Board and after written informed consent was obtained. Subjects were recruited from the community via advertisements and were screened to exclude individuals with a current or past history of medical, neurological, or psychiatric disorders, which included a history of head injury with loss of consciousness, stroke, cerebral infarctions, or other major brain abnormalities on magnetic resonance imaging (MRI) scans. A lifetime drinking (and other drug use) history (timeline follow-back method) was obtained which showed that all subjects were social drinkers and that no subject had a history of alcohol or other substance abuse. Subjects drank for an average of 15.3 ± 6.1 years (range 6–27 years) at a monthly average of 25 ± 14 drinks (range 1–65). One drink was considered an alcoholic beverage that contained approximately 11 g of alcohol. Subjects

had a light lunch about 4 to 6 hr before the study and had no measurable alcohol at the beginning of the study as estimated from breath samples.

Methods

A flexible Teflon catheter was inserted into the antecubital vein for blood sampling. The catheter was prevented from clotting by allowing a constant slow drip of saline into the vein. Subjects consumed within 15 min approximately 0.85 g/kg body weight of EtOH (100%) that was diluted with orange juice to 28% EtOH content. The exact amount of alcohol was calculated for each individual based on total body water derived from anthropometric calculations (Watson et al., 1981) and was intended to produce a theoretical peak whole blood alcohol level of 0.125% at the start of drinking. Six or seven blood samples were taken over the course of the study for enzymatic determination of blood alcohol levels. Blood alcohol concentrations (BAC) reported here were determined in serum, which are approximately 15% higher than whole blood alcohol levels. Breath alcohol concentrations (BrAC) were monitored throughout the study by using a Breathalyzer (Alco-Sensor IV, Intoximeters, Inc., St. Louis, MO). Because the Breathalyzer does not function within the strong magnetic field of the MR scanner, the subject blew through a rubber extension tube into the Breathalyzer that was functioning properly at the end of the magnet bore in the much weaker magnetic fringe field. The length and flexibility of the rubber tube allowed subjects to give breath samples without moving the head inside the head coil. The Breathalyzer takes a 1 ml breath sample for alcohol analysis at the end of a steady flow of end-expired air so that the dead air space in the extension tube (approximately 100 ml) did not affect the BrAC reading. Within the fuel cell of the Breathalyzer, alcohol is oxidized catalytically and the associated electrical current is measured and converted into a BrAC reading. BrAC levels were used primarily for the timing of the experiments (e.g., to determine if the BrAC maximum was achieved). In general, BrAC was approximately 30% below BAC in these experiments and, thus, approximately 15% below the corresponding whole venous blood alcohol concentration. This is consistent with the literature (Jones and Anderson, 1996).

MR Methods—All studies were performed on a 1.5 T Magnetom VISION™ system (Siemens Inc., Iselin, NJ) equipped with a standard quadrature head coil. A padded head holder was used to restrict the subject's head movement. Standard MRI and shimming were performed during the alcohol absorption phase. The MRI protocol consisted of sagittal T₁ weighted localizer scans, oblique axial double spin-echo scans (DSE; TR/TE1/TE2 = 3000/20/80 msec) that used contiguous 3 mm thick slices angulated along a line drawn at -10° from the planum sphenoidale, and three-dimensional magnetization prepared rapid gradient echo acquisition (TR/TI/TE = 10/250/4 msec) perpendicular to the optic nerve. These images were analyzed with home-written tissue segmentation software (MacKay et al. 1996; Tanabe et al. 1997) to yield the amounts of gray matter (GM), white matter (WM), and cerebral spinal fluid (CSF) contained in the spectroscopy volumes of interest (VOI). After selection of the VOI on DSE images, the magnet was shimmed automatically over the entire head and manually over the VOI to a water line widths-at-half-height between 4 and 8 Hz. We examined three different VOIs by using single-volume point resolved spectroscopy (PRESS) (Bottomley, 1987) (see Fig. 1): (1) a VOI centered in the WM of the parietal brain that included approximately 30% cortical GM (range 21–48%) and <5% ventricular cerebral

spinal fluid (CSF) (range 2–6%), with a typical voxel size of 40 mm (left-right) $\times$ 40 mm (anterior-posterior) $\times$ 15 mm (caudocranial); (2) a VOI centered on the basal ganglia that included primarily lenticular nucleus, thalamus, and very little CSF from the insular cortices with a typical voxel size of 30 mm $\times$ 45 mm $\times$ 15 mm; and (3) a VOI centered on the cerebellar vermis that included cerebellar tissue only, cerebellar CSF, and little CSF from the fourth ventricle with a typical voxel size of 40 mm $\times$ 25 mm $\times$ 15 mm. If time allowed, spectra were acquired from all three VOIs in a single session, with the saturated spectrum for a given VOI acquired immediately after acquiring the spectrum without saturating magnetization transfer (MT) pulse.

To minimize signal loss due to J-evolution during the first echo time TE_1 , we used a PRESS pulse sequence with asymmetrical rf-pulse timing (Jung and Lutz 1992; Yablonskiy et al., 1998) with $TE_1 = 15$ msec and $TE_2 = 120$ msec, which yielded a total TE of 135 msec. In all cases TR/TE was 1800/135 msec, the spectral sweep width was 1538 Hz, acquisition size was 512 points, and acquisition time was less than 4 min per spectrum. Proton spectroscopic images (1H MRSI) were acquired by using a 15 mm thick PRESS preselected slice in the centrum semiovale (CSO), which covered central WM and medial GM with a typical size of 80 mm (left-right) $\times$ 120 mm (anterior-posterior) (see Fig. 1). A field of view of 240 $\times$ 240 mm² was selected with circular encoding of a nominal 24 $\times$ 24 grid, which yielded a nominal voxel size of 1.5 ml. Acquisition time was 13 min per MRSI data set with TR/TE = 1800/135 msec. All 1H MRS experiments were started when BrAC was close to or past its maximum (in general about 75 min after the start of drinking when brain EtOH levels had equilibrated) and continued throughout the linear alcohol elimination phase. EtOH signal intensities for SI and relaxation time studies were corrected for elimination by using BAC measures that bracketed the experiments.

MT 1H MRS experiments employed two methods commonly used in the literature for MT MRI, namely off-resonance and pulsed on-resonance saturation. Both methods, including MT pulse power, have been described in detail previously (Meyerhoff et al., 1999a). The power of the MT pulses was as high as possible and was a tradeoff between optimal saturation of short T_2 -components and specific absorption rate limitations given by the application of the entire pulse sequence. Therefore, the maximum MT effect may not have been achieved in these in vivo experiments. Direct saturation effects for either MT pulse on any of the metabolites were determined on T_2 -adjusted phantoms at <3%. Water suppression used three Gaussian-shaped 90° pulses of 25.6 msec length applied at the water resonance and followed by spoiler gradients chemical shift selective suppression (CHESS). This water suppression method was shown to have a negligible MT effect (deGraaf et al., 1999a,b; Röhl et al., 1998), which we confirmed with agar phantoms.

Relaxation times of the methyl group of EtOH and other brain metabolites were determined in the posterior-parietal PRESS VOI. These time-consuming studies were not performed in the basal ganglia or cerebellar VOIs. T_1 relaxation times were measured in seven subjects at TR/TE = 2300/135 msec with a fast inversion recovery sequence by using six inversion recovery delays (TI = 110, 250, 400, 1050, 1300, and 1550 msec). In addition, a saturation recovery method was used in two subjects with TR = 1800, 3000, and 5000 msec at TE = 135 msec. T_2 relaxation times were determined in 10 subjects with TR = 1800 msec and TE

$= 2n/J_{\text{EtOH}} = 272, 544, \text{ and } 816 \text{ msec}$, which are echo times at which the methyl-triplet is fully refocused and positive in-phase detection maximizes signal detection (Jung and Lutz 1992; Meyerhoff, 1998; Yablonskiy et al., 1998).

MRS Analysis—All ^1H MR spectroscopy data were transferred to a SUN workstation. The ^1H MRSI data were zero-filled to a rectangular matrix of $(32 \times 32 \times 1024)$ points, Fourier transformed, and phase-corrected with software developed in house (Maudsley et al., 1992). Two hertz Gaussian line broadening was used in the spectral direction, and mild Gaussian apodization was applied along the spatial directions to reduce Gibbs ringing effects, which resulted in an effective spatial MRSI resolution of $<1.5 \text{ ml}$. Further processing of data used NMRITM software (New Methods Research Inc., Syracuse, NY) by a single experienced rater (D.J.M.). Single voxel data processing included line broadening by 2 Hz by using a Gauss filter, zero-filling to 1024 spectral points, Fourier transformation, zero- and first-order phase correction on the furthest separated resonances, and linear baseline correction by drawing a straight baseline through the center of the noise. Spectra were curve fit by using Gaussian line shapes to yield peak integrals for the alcohol methyl resonance at 1.18 ppm (EtOH), for N-acetyl-containing metabolites (NA, primarily N-acetylaspartate, NAA) at 2 ppm, for choline-containing metabolites (Cho) at 3.2 ppm, and for creatine-containing metabolites (Cr) at 3.05 ppm. In previous assessments of this data processing method, test-retest reliability was excellent with intrarater correlation coefficients for the three natural brain resonances >0.92 . The peak integrals were corrected for receiver gain and coil loading (based on the transmitter amplitude necessary to achieve a 180° flip angle pulse), which allowed comparison of integrals between subjects. The effect of MT on the spectra was measured as a signal attenuation calculated from the signal difference of individual metabolites from spectra obtained with and without saturating MT pulse and then was normalized to the corresponding metabolite signal integral obtained from the spectrum without MT pulse.

Statistical Analysis

Metabolite integrals from spectra obtained with and without applications of MT pulses were tested for differences by using paired Student's *t* tests. Data are reported as mean $\pm$ 1 SD, and results were considered significant at the 0.05 level.

RESULTS

Figure 2 shows the kinetics of EtOH uptake and elimination in blood and breath of 12 of the subjects examined. Mean and standard deviations are shown for BAC values averaged over approximately 10 min intervals. The two more contiguous appearing curves are fits to a simple model of first-order absorption and zero-order (linear) elimination (Martin et al., 1984) for BAC and BrAC data separately. The maximal mean BAC achieved in all 20 subjects was $0.112 \pm 0.022\%$, achieved $92 \pm 28 \text{ min}$ after start of drinking. This maximum was followed by linear alcohol elimination, which resulted in a BAC of $0.087 \pm 0.018\%$ at the end of the MR study $173 \pm 19 \text{ min}$ after the start of drinking. This corresponded to an average EtOH elimination rate of 0.019% per hour, consistent with literature reports. The structural MRI measurements and magnet shimming in preparation for the localized ^1H

MRS studies were performed during the EtOH absorption phase, whereas all relaxation and magnetization transfer measurements were performed during the linear EtOH elimination phase, after brain and venous blood alcohol levels had equilibrated (Goldstein, 1983).

In five subjects, we obtained up to six ^1H MRS measures of the EtOH methyl signal integral in the posterior-parietal WM VOI in a single session for correlation with BAC and BrAC measures. These measures were obtained >60 min after start of drinking to allow full equilibration of EtOH throughout poorly vascularized brain regions included in the VOI. Data from these five subjects are shown in Fig. 3. The data reveal a strong positive correlation of the ratio of EtOH to NAA integrals with both BAC ($r = 0.58$, $p < 0.002$, by Pearson) and BrAC ($r = 0.79$, $p < 0.0001$). In a repeated-measures regression that separated within versus between subject variance, both BAC ($p < 0.006$) and BrAC ($p < 0.0001$) predicted EtOH/NAA.

The relaxation times of EtOH and endogenous brain metabolites measured in posterior-parietal WM are listed in Table 1. The T_1 relaxation time of the methyl group of EtOH was determined by two different methods. Inversion recovery in seven subjects yielded 1047 ± 173 msec, whereas saturation recovery in two subjects yielded a similar T_1 value of 1228 ± 35 msec. The T_2 relaxation time of the EtOH methyl group and other metabolites was measured at echo times at which the methyl-triplet is fully refocused and positive in-phase detection maximizes signal intensity (Jung and Lutz, 1992; Meyerhoff, 1998; Yablonskiy et al., 1998). The EtOH T_2 was 212 ± 40 msec, which was lower than previous estimates of T_2 around 350 msec and 380 msec (Haseler and Brooks, 1993; Hetherington et al., 1989; Rose et al., 1992; Spielman et al., 1993).

In MT ^1H MRS experiments, a signal reduction of the methyl group of free EtOH was observed on either off- or on-resonance saturation. Both methods are commonly used to saturate the bound water compartment in MT MRI pulses sequences and were tested to evaluate their efficiency in diminishing the signal from free EtOH. Figure 4 shows ^1H MR spectra obtained from the posterior-parietal VOI of a 30-year-old man in the presence and absence of an on-resonance MT pulse. The EtOH signal reduction was 40% in this case. The Cr signal in the presence of the MT pulse was lower by about 18%, consistent with previous reports of signal reductions for this resonance under identical experimental conditions (Helms and Frahm, 1999; Meyerhoff et al., 1999a). Table 2 shows the EtOH signal reduction after on- or off-resonance saturation in different brain regions, the number of subjects studied for that particular brain region, and the localization experiment employed.

In general, EtOH methyl signal reductions were around 30% with both saturation methods. Signal reductions were largely similar between regions and with both saturation methods as applied here. In SI studies, signal reductions were between 22% and 25% in frontal brain and 29% in posterior-parietal brain ($p = \text{NS}$ for region). Single-volume studies yielded similar reductions in the posterior-parietal VOI and the cerebellar VOI, with the greatest signal reduction of 39% (range 25–60%) observed in the basal ganglia.

DISCUSSION

The major findings of this study are (a) ^1H MRS-derived human brain EtOH measures are highly correlated with temporally concordant BAC and BrAC once EtOH levels have equilibrated throughout the sampled brain parenchyma; (b) the measured MR relaxation times of human brain EtOH are different from earlier estimates in the literature used to quantitate brain EtOH; and (c) the ^1H MRS signal of human brain EtOH decreases on application of on- or off-resonance saturation pulses, with different brain regions exhibiting similar signal reductions.

Correlation of Brain, Blood, and Breath EtOH Measures

Our study demonstrates that, at least over the measured BAC range of 0.045 to 0.100% (10–22 mM) and after equilibration throughout the brain, the spectroscopically derived brain EtOH measures correlate very strongly with standard alcohol concentrations measured in blood and breath. The existence of a direct proportionality also validates direct quantitative comparisons of ^1H MRS-derived EtOH measures obtained at different alcohol concentrations. The correlation of brain EtOH measures are stronger with BrAC than with BAC, owing possibly to the fact that BrAC more closely reflects arterial rather than venous blood concentrations and, thus, more closely tracks brain EtOH levels. The correlations suggest that ^1H MRS allows direct measurement of transient changes of EtOH levels in the brain, formerly only possible indirectly through inferences from BrAC levels.

Relaxation

The measured T_1 relaxation time of brain EtOH was similar to those of the endogenous brain metabolites Cho, Cr, and NAA, which suggests that the MRS-visible EtOH pool exists in a similar molecular environment. To the best of our knowledge, T_1 relaxation of brain EtOH has not been measured previously in either animals or humans. T_1 estimates, however, that were used previously for absolute quantitation of brain EtOH were significantly longer than our measurements indicated. In one ^1H MRS study, human brain EtOH T_1 was assumed to be similar to that of EtOH in 1% agarose, which was measured at 4 sec (Spielman et al., 1993). Application of such a long T_1 estimate in quantitative studies leads to an overestimation of actual EtOH concentration. In a preliminary report in men, scan repetition times of 5 sec yielded brain EtOH spectra without saturation effects (Haseler and Brooks, 1993). This suggested that the EtOH T_1 is shorter than about 1.3 sec, consistent with our measurements.

The measured T_2 relaxation time of brain EtOH of 212 msec is shorter than those of NAA and Cho, which are around 300 msec. The longer T_2 of the endogenous brain metabolites indicates considerable molecular mobility characteristic for metabolites that freely tumble in intracellular and extracellular space, such as NAA, which is thought to be fully MRS visible (Helms and Frahm, 1999; Meyerhoff et al., 1999). We observed a significant decrease of the T_2 of EtOH in saline solution when the centrifuged solids of a rat brain homogenate were added, which suggests that interaction of EtOH with membranes reduces the T_2 of the MRS-visible EtOH fraction (Govindaraju et al., 1997). The measured EtOH T_2 is comparable with that of Cr, a molecule of roughly the same size as EtOH. The Cr resonance, which consists

of signal from creatine and phosphocreatine, has been shown to diminish by off-resonance saturation in brain (deGraaf et al., 1999b; Dreher et al., 1994; Helms and Frahm, 1999; Meyerhoff et al., 1999a; Röhl et al., 1998), skeletal muscle (Kruiskamp et al., 1997, 1998), and perfused heart (Schneider et al., 1999). These molecules are thought to exist in freely mobile form in cellular fluid and bound to membranes or specific proteins, especially the enzyme creatine kinase, which gives rise to partial MRS visibility of Cr. The similar EtOH behavior described here suggests that also EtOH is compartmentalized and only partially MRS visible.

Early studies at 1.5 T measured human brain EtOH T_2 at 94 msec (Hetherington et al., 1989). This short T_2 explained the very low EtOH visibility determined in those experiments performed at TE = 100 msec. However, later studies generally yielded longer T_2 relaxation times. Dog studies performed at 2.4 T (Rose et al., 1992) gave some evidence from relaxation time studies of two pools of brain EtOH, one that relaxed at a rate of <50 msec, the other at 335 msec. Relaxation time estimates in a small number of volunteers at 1.5 T revealed a single, relatively long T_2 of 380 msec in one study (Haseler and Brooks, 1993) and 350 msec in another study (Spielman et al., 1993). In these studies, T_2 s were estimated from TE values that have been shown to yield signal intensities modulated not only by J-coupling but also by the exact timing of the localization sequence, which causes positive and negative in-phase detection with different overall signal intensity (Jung and Lutz, 1992; Meyerhoff, 1999b; Yablonskiy et al., 1998). In our study, these complications were avoided by measuring at TE values at which the EtOH triplet is fully refocused (TE = $2\pi/J_{\text{EtOH}}$) and positive in-phase detection maximizes EtOH signal intensity. Furthermore, in at least one of the human studies (Haseler and Brooks, 1993), T_2 values were not corrected for EtOH washout during the experiment. Thus, these reported values are likely erroneous. A recent preliminary report of human brain EtOH T_2 measurements at 4 T magnetic field strength yielded a T_2 of <100 msec (Sammi et al., 1999). This corresponds to an extrapolated T_2 at 1.5 T in the 200 msec range (Sammi et al., 1999). Although the 4T study used 16 TE values and suppressed J-modulation, the variance of measured EtOH T_2 values was comparable to that of our more conventional approach.

In all of these studies and in our study, the MR experimental conditions did not allow direct measurement of metabolite components that are associated with macromolecules, such as membrane bilayers and proteins. Indirect evidence for the existence of both macromolecule-associated and free EtOH, however, comes from our MT experiments (see subsequent discussion). Studies have shown that EtOH is likely to be found on the hydrophilic membrane surface or in protein pockets (Barry and Gawrisch, 1994; Chiou et al., 1992; Hitzeman et al., 1986; Klemm, 1998; Moxon et al., 1991; Rottenberg, 1987, 1992) or, less likely, in the core of biological membranes, which show a relatively low partition coefficient for short chain alcohols such as EtOH (Barry and Gawrisch, 1994; Chiou et al., 1992; Klemm, 1998; Metcalfe et al., 1968; Rottenberg, 1992). The amount of macromolecule-associated EtOH in membranes has been described to range from 6% to 90%, depending on the type of membrane investigated (Grenell, 1975; Kelly-Murphy et al., 1984; Nie et al., 1989; Rottenberg et al., 1981; Sarasua et al., 1989), and to be affected by chronic alcohol exposure (Beauge et al., 1985; Chin and Goldstein, 1977; Kelly-Murphy et al., 1984; Littleton and John, 1977; Rottenberg et al., 1981, 1987; Sarasua et al., 1989; Wood et al.,

1987; reviewed in Rottenberg, 1992). The EtOH molecules in any of these molecular environments are restricted motionally, so that their T_2 relaxation times are extremely short (<1 msec). Any volume-localization method, however, requires the application of several radio frequency pulses separated by relatively long time intervals, which precludes the direct detection of short- T_2 components. Our MT experiments, though, give evidence for the existence of such a motionally restricted EtOH pool with short T_2 .

Magnetization Transfer

Alcohol in rat brain (Meyerhoff et al., 1996) and in rat brain membrane suspensions (Govindaraju et al., 1997) shows decreases of MRS signal intensity upon off-resonance saturation at high magnetic fields. The basis for this MT effect was described earlier (Fein et al., 1995). In short, if a membrane-associated EtOH compartment with its short T_2 and broad, MRS-invisible signal exists, selective radio frequency saturation of this signal visibly diminishes the signal from the free EtOH, because of a transfer of saturation consequent to EtOH molecules constantly and rapidly exchanging between the two compartments. Thus, an EtOH signal intensity reduction upon off-resonance saturation is evidence for the postulated existence of more than one compartment of brain alcohol (Fein et al., 1995). On-resonance saturation is effectively equivalent to off-resonance saturation except that its selective saturation of the postulated membrane-associated EtOH compartment is based on the greatly different T_2 of the compartments, not their different signal bandwidths (Meyerhoff et al., 1999a). Our experiments showed that both methods as applied here yielded very similar free EtOH signal reductions. Experimentally, the off-resonance method is easier to implement.

In a preliminary report (Meyerhoff, 1998) and in this study at 1.5 T, we observed a decrease of the signal from free EtOH in human brain upon either on- or off-resonance saturation after EtOH administration. Direct bleedover from the application of the saturation pulses cannot explain the observed EtOH signal attenuation, based on the characteristics of the applied pulses, the comparatively long T_2 of free brain EtOH, and results of phantom experiments (Meyerhoff, 1999a). The EtOH signal decrease, however, can be explained by direct dipolar coupling, which is a through-space exchange of magnetization between EtOH and bound water or other proton-bearing compounds that are associated with lipids and/or proteins. These components have very short T_2 and are saturated by the MT pulses. In this case, for MT effects to be observable, EtOH molecules need to be in contact with macromolecules or with water in the hydration sphere (at the surface or in buried binding sites) of macromolecules for a time long enough to allow for transfer of magnetization; that is, the intermolecular correlation time of the system needs to be sufficiently long for effective dipolar relaxation. Independent of the exact mechanism, the reduced mobility for EtOH in such an environment significantly shortens the T_2 of this pool. The applied saturation pulses thus can directly and selectively saturate this motionally restricted EtOH pool with its broad (and MRS-invisible) resonance. Rapid exchange of magnetization (molecules) between the motionally restricted and mobile EtOH pools therefore can also cause signal attenuation of the observable free EtOH. Both scenarios (dipolar or molecular exchange) presume the existence of EtOH in more than one compartment (molecular environment) and provide a likely explanation for the observed EtOH MT effect. Note,

however, that the two possible mechanisms are not mutually exclusive, that they can be present at the same time, and that both are consistent with the existence of brain EtOH in different molecular environments. The major difference between these two mechanisms is the involvement of water in the exchange process. We have gathered evidence for dipolar magnetic coupling between water and EtOH in bovine serum albumin phantoms (Estilaei et al., 1999), in rat brain (Meyerhoff et al., 1996) and in rat brain suspensions (Govindaraju et al., 1997). These findings suggest a dipolar mechanism for MT with the involvement of “biological water” (Klemm, 1998) in the exchange process.

Further studies are needed to answer questions about specific mechanisms or pool size, such as recently described rat studies that evaluated Cr signal attenuation after off-resonance saturation (deGraaf et al., 1999b). The required time-consuming experiments, however, may limit such investigations to the animal brain. They alone may be able to answer whether the bound EtOH pool is large enough to account, at least in part, for the commonly reported incomplete MRS visibility of brain EtOH, which has been described to range from 24% to 94% in human brain, depending on pulse sequences, relaxation times, and exact quantitation methods used (Chiu et al., 1994; Hanstock et al., 1988, 1990; Hetherington et al., 1989; Mendelson et al., 1990, 1992; Moxon et al., 1991; Petroff et al., 1990; Spielman et al., 1993). Thus, the EtOH signal decreases measured in our on- and off-resonance saturation experiments are interpreted as MT between a free, mobile EtOH pool observable by in vivo ^1H MRS and a membrane-associated EtOH pool that escapes direct detection. These experiments provide evidence for the existence of an MRS-invisible EtOH pool in brain that has only been inferred in the past and that supports our working model of EtOH compartmentalization in brain.

Water Signal Suppression

The saturating effects of commonly used water suppression schemes in in vivo ^1H MRS have been shown to decrease observable Cr magnetization (deGraaf et al., 1999b; Kotitschke et al., 1994) and therefore may affect EtOH signal intensities as well. However, the CHESS water suppression pulses used in our in vivo studies have been shown to have a negligible effect on Cr signal intensity in rat brain (deGraaf et al., 1999b) and in agar phantoms. Nevertheless, any rf-pulses used for volume localization or lipid or water suppression may contribute unintentionally to saturation of the bound EtOH pool, which may lead to some degree of signal attenuation that needs to be carefully considered in quantitative EtOH studies.

In summary, for quantitative in vivo ^1H MRS studies of EtOH that use standard localization sequences such as PRESS and stimulated echoes, investigators need to be aware of the following: (1) The spacing of rf-pulses in the localization sequence in addition to the echo time affects the signal intensity of the J-coupled EtOH signal; (2) EtOH relaxation times need to be measured and the proper echo times need to be chosen for T_2 relaxation time measurements; (3) certain water suppression pulses can reduce EtOH signal intensity due to nonintended MT effects; and (4) EtOH has to have equilibrated throughout the MRS VOI before measurements are started. The studies described here form the experimental groundwork for further studies into the MRS visibility of EtOH in brain of light and heavy

drinkers, into how EtOH visibility relates to the development of chronic tolerance, and whether MRS visibility of brain EtOH can be used to estimate the extent to which tolerance may be determined genetically. In addition, similar ^1H MRS studies together with phosphorus MRS studies of membrane phospholipids may provide further insight into the association of structural membrane changes with acute and chronic alcohol ingestion and with a postulated inherited membrane rigidity that reflects innate tolerance to alcohol in subjects at genetic high risk of alcoholism. Thus, in vivo ^1H and phosphorus MRS may become valuable new tools to illuminate cellular mechanisms that underlie alcohol action on the brain.

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Fig. 1.

MR images that show the typical locations of VOIs for MR spectroscopy. The smaller white boxes represent single-volume PRESS experiments, and the larger gray box represents a PRESS volume selected for MR spectroscopic imaging.

Fig. 2.

Kinetics of EtOH uptake and elimination in blood and breath of 12 subjects. Mean and standard deviations are shown for BAC values only and are averaged over approximately 10 min intervals (triangles). The two more contiguous appearing curves are fits to a simple model of first-order absorption and zero-order (linear) elimination for BAC (circles) and BrAC (squares) data separately.

Fig. 3.

Correlations of normalized EtOH signal intensities (EtOH/NAA) determined by ^1H MRS with blood alcohol concentration (BAC) and with breath alcohol concentration (BrAC). Data were obtained from the posterior parietal volume of interest of five different subjects represented by different symbols. Straight line, regression line; curved line, 95% confidence interval.

Fig. 4.

^1H MR spectra (TR/TE = 1800/135 msec) from posterior parietal brain obtained with (top spectrum labeled "MT") and without (bottom spectrum labeled "no MT") the application of an on-resonance saturation pulse. Resonances are from the alcohol methyl group at 1.18 ppm (EtOH). N-acetyl-containing metabolites (mostly N-acetyl-aspartate) at 2 ppm (NAA), creatine-containing metabolites at 3.05 ppm (Cr), and choline-containing metabolites at 3.2 ppm (Cho). MT, magnetization transfer.

Table 1

EtOH and Metabolite Relaxation Times (ms) Obtained With Different Methods

Metabolite	T ₁ by inversion recovery (n = 7)	T ₁ by saturation recovery (n = 2)	T ₂ (n = 10)
EtOH	1047 ± 173	1228 ± 35	212 ± 40
NAA	860 ± 72	788 ± 64	321 ± 26
Cr	1123 ± 135	910 ± 144	206 ± 35
Cho	1196 ± 208	960 ± 265	287 ± 42

Table 2

Percentage EtOH Signal Reduction After On-Resonance or Off-Resonance Saturation

Brain region	% Signal reduction after on-resonance saturation		% Signal reduction after off-resonance saturation		<i>n</i>	Experiment
	saturation	<i>n</i>	saturation	<i>n</i>		
Posterior-parietal	33 ± 10	16	29 ± 12	13	13	Single vol.
Basal ganglia	39 ± 12	11	28 ± 9	7	7	Single vol.
Cerebellum	30 ± 13	6	33 ± 15	5	5	Single vol.
WM—posterior parietal	29 ± 8	10	—	—	—	SI
WM—frontal	25 ± 9	10	—	—	—	SI
GM—posterior parietal	29 ± 8	10	—	—	—	SI
GM—frontal	22 ± 10	10	—	—	—	SI