Magnetic Resonance Spectroscopy in Schizophrenia: Methodological Issues and Findings–Part I

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Our knowledge of the biological basis of schizophrenia has significantly increased with the contribution of in vivo proton and phosphorus magnetic resonance spectroscopy (MRS), a noninvasive tool that can assess the biochemistry from a localized region in the human body. Studies thus far suggest altered membrane phospholipid metabolism at the early stage of illness and reduced N-*acetylaspartate, a measure of neuronal volume/viability in chronic schizophrenia. Inconsistencies remain in the literature, in part due to the complexities in the MRS methodology. These complexities of in vivo spectroscopy make it important to understand the issues surrounding the design of spectroscopy protocols to best address hypotheses of interest. This review addresses these issues, including 1) understanding biochemistry and the physiologic significance of metabolites; 2) the influence of acquisition parameters combined with spin–spin and spin–lattice relaxation effects on the MRS signal; 3) the composition of spectral peaks and the degree of overlapping peaks, including the broader underlying peaks; 4) factors affecting the signal-to-noise ratio; 5) the various types of localization schemes; and 6) the objectives to produce accurate and reproducible quantification results. The ability to fully exploit the potentials of in vivo spectroscopy should lead to a protocol best optimized to address the hypotheses of interest.* Biol Psychiatry 2000;48:357–368 © *2000 Society of Biological Psychiatry*

Key Words: in vivo, proton, phosphorus, brain, spectroscopy, quantification

Introduction

Over the past decade, in vivo proton (^{1}H) and phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) have found increasing application in schizophrenia research. Phosphorus MRS studies have observed altered levels of membrane phospholipid metabolites in the prefrontal region of first-episode schizophrenia subjects, supporting the hypothesis of a neurodevelopmental abnormality in this illness (Pettegrew et al 1991; Stanley et al 1995b). Proton MRS studies have observed reduced *N*-acetylaspartate (NAA), a metabolite reflecting the volume and/or viability of neurons in the prefrontal and temporal lobes. Although these observations offer insightful information about the the biological basis of schizophrenia, inconsistencies remain in the literature. At least in part, this may be due to the complexities of the rapidly changing MRS methodology.

Magnetic resonance spectroscopy, like magnetic resonance imaging (MRI), is based on the principle of nuclear magnetic resonance (MR) (Farrar and Becker 1971; Fukushima and Roeder 1981; Gadian 1995); however, unlike MRI, which provides cross-sectional, anatomic images based on the tissue water environment, MRS provides a "frequency-signal intensity" spectrum of multiple peaks that reflect the biochemical composition of a localized region in the body. Each biochemical or metabolite is identified by its unique position or chemical shift along the frequency axis of a spectrum, and the peak intensity or the area under the peak is proportional to the concentration of that assigned metabolite. Magnetic resonance spectroscopy is a challenging and complex noninvasive tool, which can be applied using numerous approaches. The investigator has the choice of selecting the nuclei of interest (e.g., ^{1}H or ^{31}P), the magnetic field strength of the MR system, the transmit/receive coil configuration, the method used to localize the region of interest, and the method to postprocess and quantify the MRS data. As a result, the observed data can represent biochemical information from a single localized three-dimensional volume, or from multiple voxels in a single cross-sectional slice or multiple slices—also known as spectroscopic imaging (MRSI) or chemical shift imaging (CSI). Depending on the magnetic field strength of the MR system, sensitivity of the nuclei, coil configuration, localization scheme, and acquisition parameters, the localized brain tissue volume size can range from a fraction of 1 cm^3 up to approximately 60 cm^3. The metabolite information obtained by quantifying the peak areas can pertain to the metabolism of membrane phospholipids, high-energy phosphates, and

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neuronal processes. Due to the above different approaches available to address a particular biochemical question, it is important to have a comprehensive understanding of MRS methodology. Such understanding is critical when designing an in vivo MRS protocol best optimized to test the hypotheses of interest in psychiatric research. In this review we discuss the issues surrounding the process of designing and optimizing an in vivo ^{1}H and ^{31}P MRS protocol to study the human brain, including 1) the biochemical information, 2) factors influencing the signal-to-noise ratio (S/N), 3) acquisition schemes, and 4) post-processing and quantification methods.

Understanding the Biochemistry

^{1}H Metabolites

A typical in vivo ^{1}H brain spectrum includes singlet resonances from NAA, phosphocreatine plus creatine (PCr+Cr), and trimethylamines (TMAs) at 2.01, 3.02, and 3.20 ppm, respectively (Figure 1). Multiple spectral peaks or multiplets from glutamate, glutamine, *myo*-inositol, γ-aminobutyric acid (GABA), aspartate, *N*-acetylaspartylglutamate (NAAG), taurine, glucose, and *scyllo*-inositol are also present in a typical in vivo ^{1}H brain spectrum. These multiplets (e.g., doublets or triplets) are the result of through-bond interactions between protons of CH, CH_2, CH_3 groups, etc. (and, likewise in the ^{31}P mode, the phosphorus of PO_3 groups) from the same molecule and the strength of this interaction is characterized by a scalar J-coupling constant. The phase of the spectral peaks within a multiplet changes or modulates as a function of the acquisition parameter (e.g., the spin–echo time [TE] of a 90°-TE/2-180°-TE/2 spin–echo pulse sequence; for further details, see Gadian 1995) and the scalar J-coupling constant (Ernst and Hennig 1991). Therefore, these latter metabolites are best observed by minimizing the modulation (i.e., by minimizing the TE) or by choosing a TE/2 interval that is a multiple of 1/2 J (Table 1).

N-Acetylaspartate, which is second to glutamate in terms of total concentration of free amino acids, accounts for approximately 85–90% of the proton signal of the *N*-acetyl group, and NAAG primarily accounts for the remaining 10–15%, depending on the localized brain region (Frahm et al 1991; Pouwels and Frahm 1997). Both NAA and NAAG are localized exclusively in mature neurons and neuronal processes, and not in glia (Urenjak et al 1993). *N*-Acetylaspartate is formed in mitochondria from acetyl-CoA and aspartate by the membrane-bound enzyme L-aspartate *N*-acetyltransferase, an enzyme selectively found in the brain (Truckenmiller et al 1985). *N*-Acetylaspartate is considered a putative marker of the number or volume of neurons (Arnold et al 1990). Additionally, decreases in NAA have been shown to be reversible (De Stefano et al 1995), and therefore the levels of NAA detected by in vivo ^{1}H MRS depend not only on factors that change the neuronal composition of the tissue sampled, but also on factors affecting the general viability of neurons.

The observed ^{1}H PCr+Cr resonance is not a preferred measure to assess the high-energy phosphate metabolism, since the *N*-methyl resonance of PCr and Cr are indistinguishable and both PCr and Cr are reactants in the creatine kinase high-energy phosphate reaction. The TMA resonance observed in a ^{1}H brain spectrum is primarily comprised of freely mobile metabolites, including glycerolphosphocholine (GPC), phosphorylcholine (PC), and choline; however, the choline concentration is below the limit of detection, being approximately 1/50th of the combined observed in vivo PC and GPC concentration (Miller 1991).

Glutamate is the most abundant amino acid in the brain and plays a major role as an excitatory neurotransmitter in the cerebral cortex (Erecinska and Silver 1990), which is rich in glutamatergic neurons (Shepherd 1994). Following the release of glutamate from the presynaptic terminal to the synaptic cleft, excess glutamate is taken up by surrounding glia and is subsequently converted predominantly to glutamine by glutamate synthase. Glutamine is then released, taken up by the presynaptic neuron, and converted into glutamate by mitochondrial glutaminase. The synthesis of glutamine is primarily derived from this glutamate–glutamine cycling, which increases with brain activity and is directly coupled with glucose utilization and lactate production in glia (Magistretti and Pellerin 1999; Rothman et al 1999). The above and the fact that the in vivo concentration of glutamate is greater than glutamine (Pouwels and Frahm 1998) suggest that part of the glutamate observed using in vivo ^{1}H MRS is not directly associated with the glutamate–glutamine cycle and may be part of the metabolic glutamate pool (Erecinska and Silver 1990; van den Berg and Garfinkel 1971). Thus, in the context of in vivo ^{1}H MRS, glutamine may prove to be more sensitive than glutamate as an indicator of glutamatergic neurotransmission (Bartha et al 1997; Stanley et al 1996).

^{31}P Metabolites

In vivo ^{31}P MRS provides direct assessment of brain membrane phospholipid and high-energy metabolism by quantifying the ^{31}P resonances of phosphomonoesters (PMEs), phosphodiesters (PDEs), PCr, inorganic orthophosphate (Pi), and nuceloside di- and triphosphate (NDP and NTP). The PME resonance is composed of predominantly PC and phosphorylethanolamine (PE), which are freely mobile precursors of membrane phospho-

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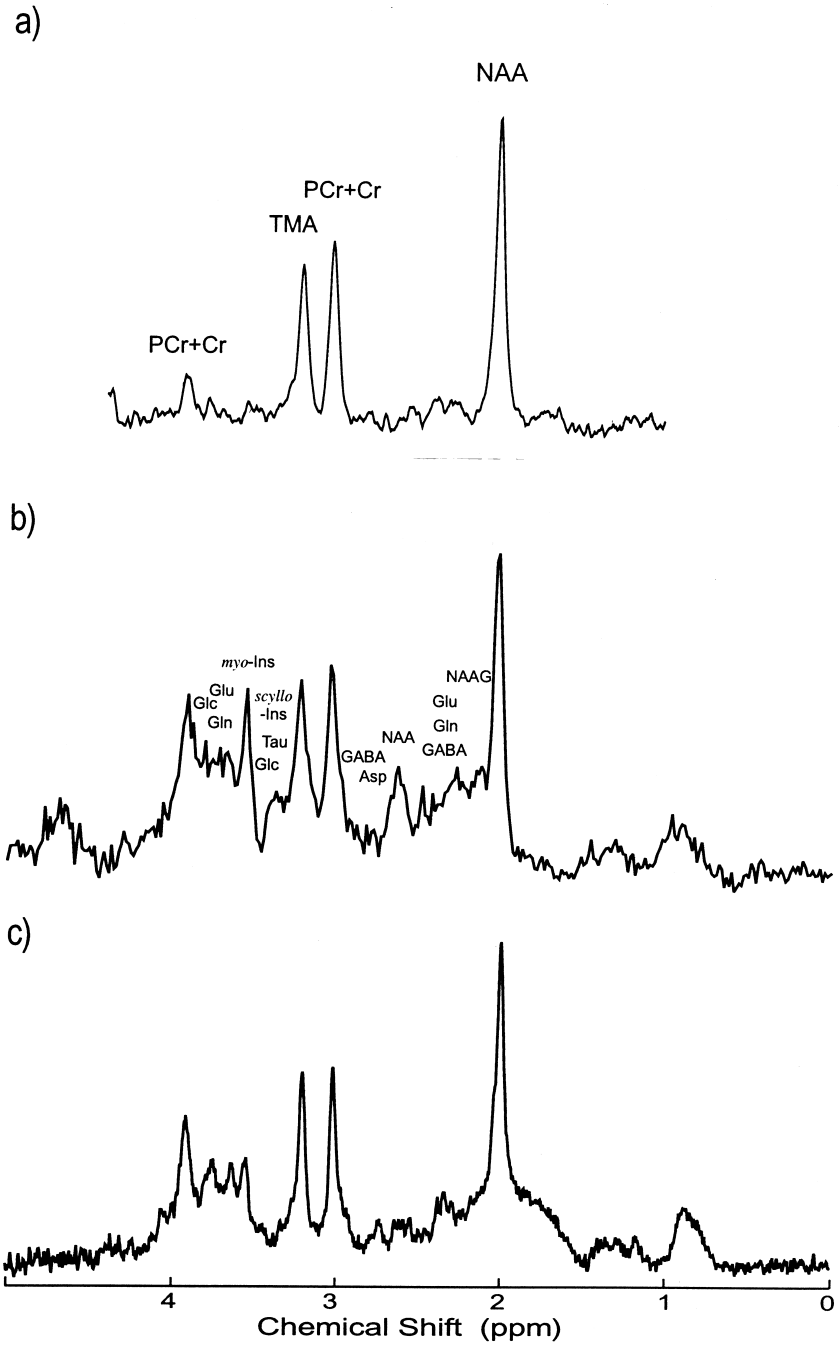

Figure 1. Single-voxel in vivo ^{1}H spectra of the left prefrontal region (8 cm^{3}) acquired with the stimulated acquisition mode (STEAM) sequence, and with different echo times (TEs) and field strengths: **(a)** TE = 135 msec at 1.5 T, **(b)** TE = 20 msec at 1.5 T, and **(c)** TE = 20 msec at 4.0 T. The metabolite assignment in **b** approximates the position of their multiplets. PCr, phosphocreatine; Cr, creatine; TMA, trimethylamine; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; Glc, glucose; Glu, glutamate; Gln, glutamine; *myo*-Ins, *myo*-inositol; *scyllo*-Ins, *scyllo*-inositol; Tau, taurine; GABA, γ-aminobutyric acid; Asp, aspartate. The spectrum **(c)** is courtesy of Peter C. Williamson, Laboratory for Functional Magnetic Resonance Imaging, Robarts Research Institute, London, Canada.

lipids (Dawson 1985; Pettegrew et al 1987). Likewise, GPC and glycerolphosphoethanolamine (GPE), which are freely mobile breakdown products of membrane phospholipids, predominantly constitute the PDE resonance (Dawson 1985; Pettegrew et al 1987). Underlying these two resonances are less mobile molecules with PME and PDE moieties (Figure 2), such as phosphorylated proteins, vesicles, micelles, and the phospholipid bilayer of cell membranes (Burnell et al 1980; de Kruijff et al 1980; Kilby et al 1991; McNamara et al 1994; Pettegrew et al 1994).

Measurements of the freely mobile PME and PDE metabolites are sensitive to the anabolic and catabolic activity of membrane phospholipids that occur during neurodevelopment. Early in postnatal brain development, levels of membrane phospholipid precursors (PE and, to a lesser degree, PC) are high and those of breakdown products GPE and GPC are low; this is followed by

Table 1. The "Preferred" Experimental Condition for the Metabolites of Interest

	The "preferred" condition				
Metabolites of interest	Field strength	Coil configuration	Localization type	Acquisition sequence	Acquisition parameters
^{1}H metabolites					
NAA, PCr+Cr, and TMA	Not critical	Volume or surface coil	Single voxel or MRSI	Not critical	Long TE (135 or 272 msec)
Glutamate, glutamine, and *myo*-inositol	≥3 T, if available	Volume or surface coil	Single voxel or MRSI	Not critical; however, STEAM can provide a shorter TE	Short TE (≤30 msec)
GABA and gluthathione	Not critical	Surface coil to optimize the S/N	Single voxel	Spectral editing type sequence	
^{31}P metabolites					
PCr, Pi, and ATP	Not critical	Volume or surface coil	Single voxel or MRSI	Not critical	Not critical
PME and PDE	>2 T to minimize the long-τ_c species	Volume or surface coil	Single voxel or MRSI	Not critical	Short preacquisition delay or echo time
PE, PC, GPE, and GPC	Not critical	Volume or surface coil	MRSI	^{1}H decoupling	Short preacquisition delay or echo time
Long-τ_c species	≤2 T	Surface coil	Single voxel	Single, nonlocalized RF pulse or ISIS	Very short preacquisition delay time (~100 μsec or less)

^{1}H, proton; NAA, *N*-acetylaspartate; PCr, phosphocreatine; Cr, creatine; TMA, trimetylamine; MRSI, magnetic resonance spectroscopic imaging; TE, echo time; STEAM, stimulated acquisition mode; GABA, γ-aminobutyric acid; S/N, signal-to-noise ratio; ^{31}P, phosphorus; Pi, inorganic orthophosphate; ATP, adenosine triphosphate; PME, phosphomonoester; PDE, phosphodiester; τ_c, correlation time; PE, phosphorylethanolamine; PC, phosphorylcholine; GPE, glycerolphosphoethanolamine; GPC, glycerolphosphocholine; RF, radio frequency; ISIS, image-selected in vivo spectroscopy.

dramatic decreases in precursor levels and increases in breakdown products that then plateau, as the brain reaches maturation (Bluml et al 1999; Buchli et al 1994c; Burri et al 1988; Hanaoka et al 1998; Pettegrew et al 1990, 1997). The interpretation of the in vivo PME and PDE measurements depends on the ability to quantify the freely mobile metabolites with minimal contamination of the broader, underlying resonances, which will be discussed later.

The ^{31}P resonances of PCr, Pi, NDP, and NTP provide information about high-energy phosphate metabolism (Figure 2). Adenosine diphosphate (ADP) and adenosine triphosphate (ATP) predominantly constitute the NDP and NTP resonances. Since the β- and α-ADP reside on the shoulders of the γ- and α-ATP, respectively (Figure 2a), the β-ATP becomes the simplest and the preferred resonance in assessing ATP. Adenosine triphosphate serves as the high-energy reservoir in the metabolism of carbohydrates, lipids, and proteins, via the Krebs' cycle. High-energy PCr, synthesized by creatine kinase, serves as an energy buffer and as an energy shuttle from subcellular sites of energy production to sites of energy consumption, which in both cases help brain ATP levels to remain constant (Bessman and Geiger 1981; Wallimann et al 1992). Under the condition of increased energy utilization and/or depletion of PCr production, the PCr level decreases and Pi increases.

Phosphomonoester and PDE levels are greater and PCr levels are lower in white matter than in gray matter (Buchli et al 1994a; Mason et al 1998). In white matter, which has a greater content of sphingomyelin, PC and PE act as both anabolic and catabolic products. It is unclear if the above increase in PMEs and PDEs is due to the freely mobile metabolites or to the less mobile metabolites; however, under ex vivo conditions the phospholipid bilayer signal is significantly greater in white matter (Buchli et al 1994a; Burt et al 1989). This may be due to the vastly different cellular architecture: long axonal fibers surrounded by myelin sheaths dominate the white matter, whereas the gray matter is composed of the cell bodies and of neuronal and glia processes.

Understanding the MR Signal

In any cross-sectional study, it is the "effect size" that determines if a finding is significant between groups. A way to increase the effect size is to reduce the variance of the measured variable, assuming that the variance is not dominated by intersubject variability. To a MR spectroscopist, this is the most critical factor, since variance is directly related to the S/N. When one designs an MRS protocol, there are many factors that control the S/N. For example, the sensitivity of the ^{1}H nuclei is 15 times that of the ^{31}P nuclei. Thus, the tendency is to reduce the size of the localized volume or voxel in the ^{1}H mode (e.g., approximately 0.1–8.0 cm^3). The smaller voxel potentially increases the specificity by minimizing the partial volume

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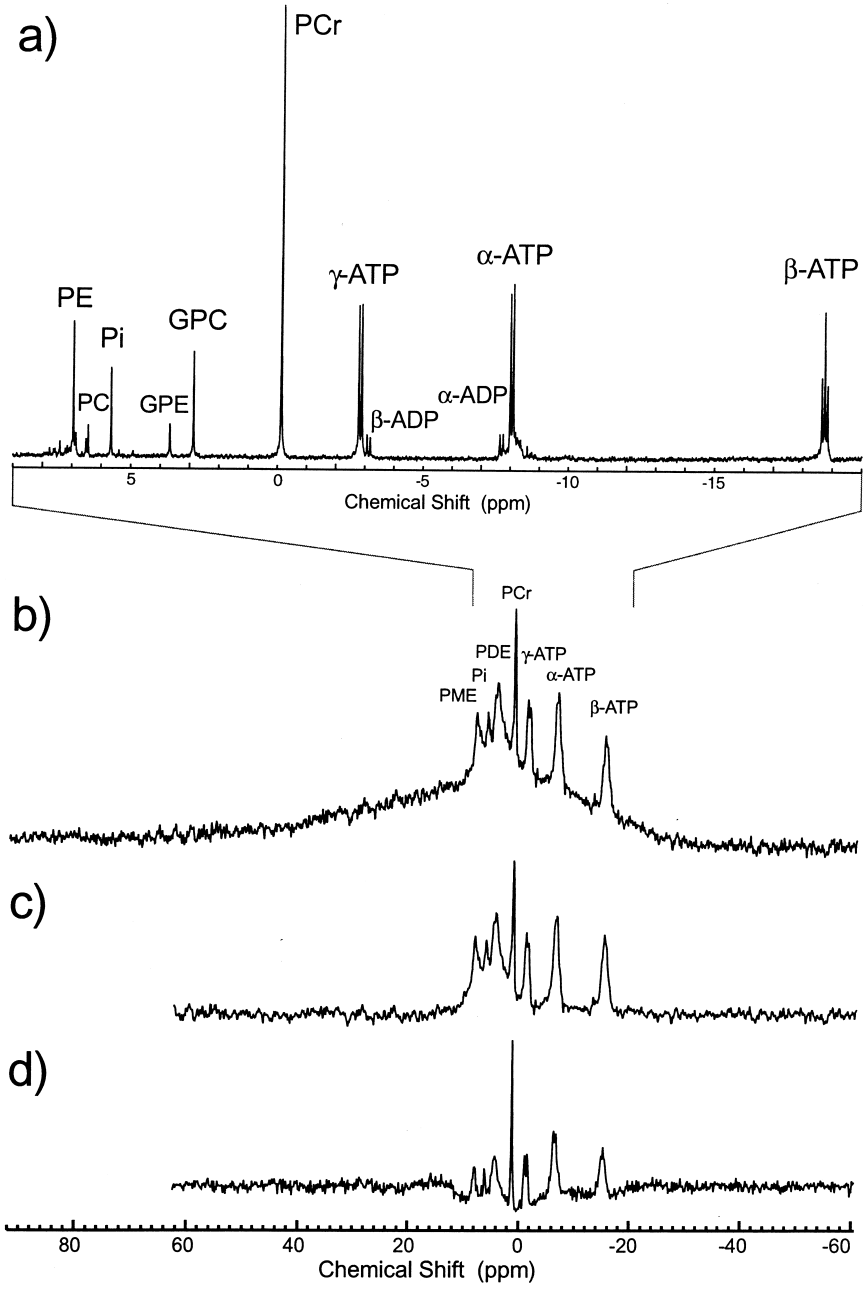

Figure 2. **(a)** Phosphorus (^{31}P) spectrum of a perchloric acid extract of a freeze-clamped rat brain acquired at 11.7 T. In vivo ^{31}P spectra of the human brain acquired with a single radio frequency pulse and different preacquisition delay times—**(b)** 150 μsec, **(c)** 500 μsec, and **(d)** 2 msec—on a whole-body 1.5-T magnetic resonance system. PE, phosphorylethanolamine; PC, phosphorylcholine; Pi, inorganic orthophosphate; GPE, glycerolphosphoethanolamine; GPC, glycerolphosphocholine; PCr, phosphocreatine; ATP, adenosine triphosphate; ADP, adenosine diphosphate. (Parts **b** and **c** reproduced with permission from Stanley et al 1994.)

of gray and white matter and cerebrospinal fluid (CSF) at the expense of reducing the S/N. Therefore, in general the localization of ^{1}H MRS enables examination of small anatomic structures, whereas much larger voxels and longer scan times are required for adequate S/N in ^{31}P MRS.

The observed human brain concentration of NAA (which constitutes nine protons) is approximately similar to that of the glutamate concentration (which constitutes eight protons); however, the reliability/sensitivity in the quantification of these two in vivo ^{1}H metabolites differs greatly (de Graaf and Bovee 1990; Provencher 1993; Stanley et al 1995a), due mainly to the differences in the number of protons that resonate at a particular chemical shift. The CH_3 of NAA (three protons) gives rise to an uncoupled singlet with a relatively high S/N at 2.01 ppm. In contrast, the α- and β-CH_2 groups of glutamate (two protons each) are strongly coupled at low fields (e.g., 1.5 T), leading to complex multiplets with poorer S/N and, hence, less reliable measurements. The quantification of glutamate is further impaired by other metabolites with a similar chemical structure and, hence, similar chemical

shifts and multiplets that overlap each other, such as glutamine and GABA (Figure 1).

In addition to a change in metabolite concentration, signal amplitude in the spectral peak area also can change due to alteration in the spin–lattice (T_1) relaxation time constant of the metabolite. The T_1 relaxation determines the time required to fully recover the MR signal following the radio frequency (RF) pulses of a localization sequence (Gadian 1995). Typically, T_1 relaxation times of ^{1}H metabolites NAA, PCr+Cr, and TMAs at 1.5 T are approximately between 1.0 and 1.5 sec (Kreis et al 1993), which differ depending on brain region, tissue type, and field strength. To fully recover the MR signal, the pulse repetition time (TR) must be at least $5 \times T_1$. If this condition is not met, then the observed MR signal will have a signal amplitude less than the fully relaxed MR signal; this is referred to as *partial saturation.* The T_1 of metabolites may differ between subject groups; therefore, significant differences in signal amplitudes could be due to a partial saturation effect and not to a change in metabolite concentration. For example, if the T_1 changes from 1 sec to 1.5 sec, the signal amplitude decreases by 19%, using a TR of 1.5 sec. To avoid this issue of possible T_1 saturation, the protocol should have a T_1 that is at least five times the longest T_1 of the measured metabolites.

The last concept to introduce deals with the motional and/or rotational mobility of the observed metabolites, which is characterized by a correlation time, τ_c, and is inversely proportional to the spin–spin relaxation time constant (T_2). The T_2 is an exponential time constant that characterizes the decay of the MR signal or free-induction decay (FID), including the decay of a spin–echo signal (Gadian 1995). This implies that freely mobile molecules with a short τ_c will have a longer observed FID signal (or longer T_2), whereas less mobile and/or larger molecules with a longer τ_c will have a much shorter observed FID signal (or shorter T_2). If the preacquisition delay or TE of the acquisition scheme is short, the resulting FID or spectral information not only includes narrow line shapes, but also relatively broader line shapes underlying the narrower peaks (i.e., long τ_c species with short short T_2s). In the case of a short TE ^{1}H acquisition, these broader peaks are distributed throughout the quantified spectral region and are due to macromolecules (Behar et al 1994; Kauppinen et al 1992). This should be taken into account when quantifying short TE ^{1}H MRS data (Bartha et al 1999a; Pfeuffer et al 1999; Provencher 1993; Stanley et al 1995a). In ^{31}P MRS with a short preacquisition delay time, the PME and PDE line shapes may include not only the freely mobile metabolites, but also less mobile molecules with PME and PDE moieties (i.e., vesicles, micelles, membrane proteins, and membrane phospholipids), as noted earlier (Figure 2). As a result, having the contribution of both short and longer τ_c metabolites as part of the quantified PME and PDE levels complicates the interpretation of any PME or PDE changes in a study.

Acquisition Protocol

^{1}H MRS

In designing an MRS protocol, it is important to define the metabolite(s) of interest to best address the hypothesis (Table 1). If NAA is central to the hypothesis, then the critical parameter is echo time. Use of a longer TE (e.g., 272 msec) results in well-separated and easily quantifiable singlets, including NAA, PCr+Cr, and TMAs (Figure 1a). In general, the larger the voxel, the more difficult it is to optimize the homogeneity of B_0 over that region. Consequently, since the spectral resolution is not as critical to resolve the singlets for a long TE sequence, NAA, PCr+Cr, and TMAs are frequently measured using an MRSI-type acquisition scheme as opposed to a long TE single-voxel technique. In general, MRSI techniques tend to have greater spatial resolution (approximately less than 1 cm^3, compared with several cm^3 for a single-voxel technique). The advantage of greater spatial resolution reduces the partial volume of unwanted tissue.

If glutamate, glutamine, and/or *myo*-inositol are the metabolites of interest, a short TE should be used, as noted earlier (Table 1). In this case, maximizing the B_0 homogeneity over the voxel (i.e., by "shimming") is crucial to best resolve these overlapping peaks (Gruetter 1993; Hu et al 1995; Shen et al 1997). Therefore, a short TE sequence is frequently done with a single-voxel study, with a few exceptions (Mason et al 1994; Pioro et al 1999; Posse et al 1993; Soher et al 1998). This is the trade-off between the long and short TE acquisition schemes. Using a shorter TE provides a longer list of measurable metabolites, at the expense of acquiring information from a single voxel per measurement. Importantly, the T2 weighting on the metabolite resonance amplitudes is greater for the longer TE acquisition, and significant differences in metabolite levels or ratios between subject groups may reflect T2 differences and not true concentration differences. In vivo short TE ^{1}H spectroscopy at a higher B_0 field (Mason et al 1994; Posse et al 1995) has the advantage of weakening the apparent coupling by the increased chemical shift dispersion, which in turn reduces the degree of overlap between the multiplets (Figure 1) and improves the accuracy of quantifying the data (Bartha et al 1999b).

The in vivo concentration of GABA in the human brain is less than half that of glutamine and has complex multiple ^{1}H peaks within the 2.5 to 2.0 ppm spectral region and a triplet approximately at the PCr+Cr resonance. Consequently, in vivo GABA quantification has poor reliability unless a spectral editing type sequence or

two-dimensional J-coupling–resolved spectroscopy can be used to isolate a particular resonance of GABA (Keltner et al 1997; Rothman et al 1993; Wilman and Allen 1995; Table 1). A similar approach has been used to quantify other, less prominent metabolites including glutathione (Trabesinger et al 1999), which is of interest in schizophrenia research (Do et al 1999).

Briefly, other issues to consider in designing an acquisition protocol include 1) testing the localization and reproducibility of the sequence with known solution phantoms, including measuring the effective size of the localized voxels (Bovee et al 1995; Leach et al 1995; Podo et al 1998); 2) optimizing the correct order of the slice-selective RF pulses in the stimulated acquisition mode (STEAM) or point-resolved spectroscopy (PRESS) sequence to minimize the eddy current effects and susceptibility effects that distort the homogeneity of the B0 field due to strong field gradients generated at the interface between air and water (e.g., in the sinuses and mouth; Ernst and Chang 1996; Seeger et al 1999); 3) incorporating outer volume suppression pulses to minimize any susceptibility artifacts (Seeger et al 1999); 4) obtaining absolute metabolite concentration levels (discussed in the next section); and 5) incorporating a three-dimensional imaging sequence or a series of fully relaxed unsuppressed water acquisitions at different TEs to obtain gray and white matter and CSF composition in each voxel (Doyle et al 1995; Hetherington et al 1996; Kreis et al 1993; Lim and Spielman 1997; Lundbom et al 1999).

^{31}P MRS

High-energy phosphate metabolism is best assessed using ^{31}P spectroscopy (Table 1). Unlike in the ^{1}H mode, where the Cr and PCr resonances are indistinguishable, an in vivo ^{31}P brain spectrum is composed of PCr, Pi, and ATP, which are reliably quantifiable. Choices for localization sequences include the image-selected in vivo spectroscopy applied as a single- or multiple-voxel technique (Burger et al 1992; Ordidge et al 1986) and spin–echo (Lim et al 1994; Merboldt et al 1990) and CSI sequences (Brown 1992). The surface coil provides the most sensitivity, followed by the Helmholtz coil and the volume coil.

If membrane phospholipid metabolism is of interest in the hypothesis, the preferred metabolites are the PMEs and PDEs (Table 1). The TMA resonance in ^{1}H MRS is not a preferred candidate to assess the membrane phospholipid metabolism because this resonance is composed of both the precursor and breakdown products; however, the difficulty with ^{31}P MRS is the quantification of the PME and PDE spectral region. Depending on the preacquisition delay or TE used to acquire the data, an in vivo ^{31}P brain spectrum may contain not only the freely mobile metabolites, but also less mobile molecules with PME and PDE moieties, as discussed earlier. This also includes the membrane phospholipid bilayers if the preacquisition delay time is very short (Figure 2). The membrane phospholipid bilayer contribution can be minimized by applying an off-resonance saturation prepulse (McNamara et al 1994) or by using a high B_0 field strength of $\gg$2 T (Bates et al 1989). Postprocessing methods, discussed later, also can be used to either eliminate or quantify the relatively broader underlying peak.

Major advances in hardware/software over the years have enabled the use of ^{1}H decoupling with in vivo ^{31}P MRS. This method removes spectral broadening due to the ^{1}H–^{31}P interaction, and as a result, the individual PC and PE and GPC and GPE resonances are resolvable (Luyten et al 1989; Murphy-Boesch et al 1993). Additionally, S/N can be further enhanced by using the nuclear Overhauser effect (NOE; Li et al 1996). If the decoupled nuclei have a greater sensitivity than the observed nuclei (e.g., ^{1}H $>$ ^{31}P) and these nuclei are coupled through space (via dipolar coupling), then their increased signal arises from the transfer of signal from the decoupled nuclei to the observed nuclei. For the ^{1}H decoupling ^{31}P observed condition, the maximum theoretical enhancement is 24%, which assumes that the ^{1}H–^{31}P interaction is purely dipolar and there is no T_1 saturation effect occuring on the transferred ^{1}H signal. The ability to sustain a constant ^{1}H decoupling across the sample without exceeding the RF absorption limit for the body and to ensure constant magnitude of NOE enhancement between subjects is critical for this technique; this may prove to be problematic if not carefully tested and monitored. The importance of quantifying the broader underlying resonance is demonstrated in a ^{1}H decoupled ^{31}P MRS study by observations of increased PDE levels in chronic medicated schizophrenia subjects being due to the broad underlying peak, and not to the freely mobile breakdown products of membrane phospholipids (Potwarka et al 1999).

Postprocessing and Quantification

In recent years the quantification procedures (de Beer and van Ormondt 1992; Provencher 1993) have dramatically improved to produce accurate and reproducible results (Bartha et al 1999a; Brooks et al 1999a, 1999b; Keevil et al 1998; Stanley et al 1995a). Advances in quantification include:

1. *Fully automated postprocessing and quantification steps to eliminate operator bias.* Modeling the in vivo MRS data in the time domain with a minimization fitting routine (e.g., Levenberg-Marquardt, VAPRO, AMARES [de Beer and van Ormondt

1992; Vanhamme et al 1997]), the domain in which the raw data is collected, provides greater flexibility in choosing the correct model function (e.g., Lorentzian, Gaussian or Voigt), and the phase and preacquisition delay time parameters are optimized in the fitting routine. The latter eliminates manual phasing by an operator and any subjective baseline correction in the frequency domain due to the preacquisition delay time. Additionally, the model functions used in the frequency domain are not the exact time domain counterpart functions (de Beer and van Ormondt 1992). Methods that integrate spectral peaks by placing two boundaries on either side of the peak are unreliable, since the integrated area does not account for the degree of overlap, especially when applied to quantifying the PCr+Cr and TMAs, in which these resonances could overlap.

2. *Minimal manipulation of the raw data prior to quantification in order to best preserve the signal amplitudes.* Resolution enhancement methods such as the Lorentzian-to-Gaussian transformation (Ferrige and Lindon 1978) should be avoided, as they do not preserve the true metabolite amplitudes. The degree of enhancement depends on the spectral line width, which may vary from subject to subject and, hence, vary the enhancement amount between subjects. Quantifying magnitude spectra, which are created from the real and imaginary parts of the signal, is also problematic. Negative signals become positive, which artificially increases the amplitudes of the measured metabolites, and as the S/N decreases the noise tends to dominate the measured amplitudes (e.g., in regions near or in ventricles). Additionally, choosing Lorentzian or Gaussian as the model function would not accurately represent the line shape of the spectral peaks in a magnitude spectrum. The convolution difference method (Ackerman et al 1980) is very effective in removing relatively broad underlying peaks, but this method also should be avoided because the appropriate line-broadening factor is subjective and the amount of signal subtracted also is dependent on the spectral line width, which may vary between subjects. Alternatively, one can quantify the data in the time domain by omitting the first several data points of the FID (i.e., the signal from the rapidly decaying component attributing to the broader underlying peaks; Stanley et al 1997). As a result, the fitting will have no influence from the broad underlying signal, which can also be quantified with this method.
3. *Incorporating* a priori *knowledge to improve the specificity of resolving overlapping resonances.* In quantifying the ATP resonances in a ^{31}P spectrum, typically the model includes two doublets and one triplet. Likewise, *a priori* knowledge of multiplets for each metabolite should be incorporated in the quantification approach, especially for short-TE ^{1}H data, to accurately model the data (Bartha et al 1999a; de Graaf and Bovee 1990; Provencher 1993; Stanley et al 1995a). Recent short-TE in vivo ^{1}H MRS studies also have shown that incorporating spectral resonances of long-τ_c species from macromolecules can significantly improve the accuracy and precision of the quantified in vivo metabolites (Bartha et al 1999a; Behar et al 1994; Stanley et al 1995a).
4. *Obtaining absolute metabolite concentration levels instead of expressing results as metabolite ratios.* It is always assumed that the denominator of a metabolite ratio remains constant when values are compared between subject groups. Observations of changes in the metabolite level of the denominator such as PCr+Cr (Connelly et al 1994; Gadian et al 1994) stress the importance of absolute quantification. Several studies have compared different absolute quantification methods (Barantin and Akoka 1997; Buchli et al 1994b; Danielsen et al 1995; Keevil et al 1998), including the unsuppressed water signal with no T_1 saturation effect as an internal standard; collecting the signal of an external reference standard; using the flip angle of a modified water suppression RF pulse; a phantom replacement method; and using an externally generated, calibrated, modulated RF signal that the transmit coil is able to receive. Using the unsuppressed water signal as an internal reference is a quick and easy method to apply; however, this method assumes that all of the tissue water signal is MR visible and that the apparent T_2 (and T_1, if the unsuppressed water signal is not fully relaxed) of the water signal is similar between subject groups. This may not be the case (e.g., in schizophrenia; Williamson et al 1992). Overall, it is unclear which method provides the most accuracy and reliability.

Reporting Parameters Describing the MRS Protocol

As noted throughout this text, in vivo spectroscopy can be applied in numerous ways for various reasons. Consequently, inconsistencies in schizophrenia research using in vivo MRS may involve such factors as 1) brain region; 2) voxel size and the amount of partial volume of unwanted tissue; 3) amount of T_2 attenuation, which is determined by the echo time or the preacquisition delay time; 4) T_1

Table 2. Standard Parameters and Issues That Should Be Included as a Minimum in an MRS-related Publication

Acquisition protocol
Magnetic field strength of the MR system (e.g., 1.5 T)
Nuclei (e.g., proton [^{1}H])
Coil configuration (e.g., quadrature head coil)
Localization scheme (e.g., MRSI or single voxel)
Localization sequence (e.g., STEAM or PRESS)
Acquisition parameters (e.g., TR, TE, TM, TI, digitized points, spectral bandwidth, preacquisition delay time, RF flip angle, number of averages, phase cycling, MRSI matrix, water suppression, lipid suppression, outer volume suppression)
Localized brain region(s), nominal/effective voxel size, and the amount of partial volume
Absolute quantification approach (e.g., internal water signal)
Postprocessing procedure
k-space filtering and resulting effective voxel size
Apodization/resolution enhancement (e.g., exponential line broadening)
First- and second-order phasing/baseline correction
Removal of unwanted water and/or lipid signal
Processing steps that are operator dependent (e.g., manual phasing)
Quantification method
Modeling method (e.g., VAPRO or LC model)
Model function (e.g., exponentially damped sinusoids or Lorentzian)
A priori knowledge (e.g., doublets and triplets)
List of measured metabolites
Include an example of modeling a spectrum, which also demonstrates the quality of data
Units of metabolite levels and what are the assumptions made in the calculation (i.e., T_1, T_2, and CSF corrections)

MRS, magnetic resonance spectroscopy; MRSI, MRS imaging; STEAM, stimulated acquisition mode; PRESS, point-resolved spectroscopy; TR, repetition time; TE, echo time; TM, mixing time; TI, inversion time; RF, radio frequency; CSF, cerebrospinal fluid.

saturation effects; 5) manipulation of the raw data via apodization, resolution enhancement, baseline correction, and the removal of unwanted water and/or lipid signal; and 6) assumptions that are made in the chosen method to express the quantified metabolite levels (e.g., ratios and T_1, T_2, and CSF correction factors). Therefore, to better understand the inconsistency between studies documentation of key acquisition, postprocessing, and quantification parameters/issues and assumptions in the determination of the metabolite levels is critical. Summarized in Table 2 are standard parameters and issues that should be included as a minimum in MRS-related publications in schizophrenia.

Conclusions

Because of the many different aspects of in vivo spectroscopy, the application of this technique in the research environment can be demanding, and the variability in findings thus far in MRS research in schizophrenia may be related in part to the use of diverse approaches (Keshavan et al 2000). Consequently, a considerable amount of knowledge is required to design and develope the appropriate in vivo spectroscopy protocol to address hypotheses of interest. In this review we addressed these issues, including understanding 1) the biochemistry and the physiologic significance of metabolites; 2) the influence of acquisition parameters combined with T_1 and T_2 effects on the MRS signal; 3) the composition of spectral peaks and the degree of overlapping peaks, including the broader underlying peaks; 4) the factors influencing the S/N; 5) the various types of localization schemes, and 6) the objectives of quantification to produce accurate and reproducible results. This ability to fully exploit the potentials of in vivo spectroscopy should lead to a protocol that is best optimized for hypothesis-driven schizophrenia research.

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