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Variations in the in Vivo P-31 MR Spectra of the Developing Human Brain during Postnatal Life

Work in Progress[1]

With use of a modified surface coil technique, the authors recorded phosphorus-31 magnetic resonance (MR) spectra of the brains of 40 neonates and infants (48 examinations) ranging from 33 weeks postconceptional age to 6 years of age. Signals of phosphorus metabolites were collected in the frontotemporal region of the brain, and various P-31 MR spectral variables were compared at different times during postnatal life. The ratio of the phosphomonoester signal to the phosphodiester signal, which is related to phospholipid synthesis, decreases within the first 6 months of life; during the same time period, the ratio of the phosphocreatine (PCr) signal to the β-adenosine triphosphate (ATP) signal increases. In addition, a difference was observed between the areas under the α- and β-ATP peaks. This difference increases with age and correlates with the PCr/β-ATP signal ratio. The variation of the α-ATP peak with age might be explained by overlap of the signals of nicotinamide adenine dinucleotide (NAD) and α-ATP.

Index terms: Brain, growth and development, 10.99 • Brain, MR studies, 10.1214 • Infants, newborn, central nervous system, 10.99 • Magnetic resonance (MR), in infants and children, 10.1214 • Magnetic resonance (MR), spectroscopy, 10.1214

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THE potential of magnetic resonance (MR) spectroscopy in analyzing the metabolism of the living brain noninvasively is of great interest, especially in pediatric medicine. Investigations in humans (1–6) and in animals (7,8) have revealed significant differences among the phosphorus-31 spectra of brains of different ages, predominantly in the phosphomonoester (PME) region (see Fig 1 for resonance assignments). This finding suggests two conclusions: (*a*) When interpreting the clinical spectra of neonates, one should apply standards that take into account the natural development of the brain. (*b*) The maturation score (9) based on the progression of myelination as seen with MR imaging (10,11) might be supplemented by a spectroscopic maturation index of the developing brain describing "ripeness" of two different metabolic domains.

Phosphorylethanolamine has been identified as the major constituent of the PME signal (12), whereas mobile brain phospholipids (glycerophosphorylcholine/ethanolamine) contribute to the resonances in the phosphodiester (PDE) region (13). Variations of the PME/PDE ratio might thus reflect metabolic turnover in synthesis of the neuronal cell membrane. In addition, long-term changes of the phosphocreatine (PCr), inorganic phosphate (Pi), and adenosine triphosphate (ATP) resonances might reflect the developing energy status of the human brain.

In this study, we examined in vivo the changing patterns in human brain P-31 MR spectra during postnatal life.

PATIENTS AND METHODS

Forty neonates, infants, and children ranging from 33 weeks postconceptional age to 6 years of age (median, 43 weeks postconceptional age) were included in the study, after parental consent had been obtained. Neonates were examined during postprandial sleep. Infants and children had to be sedated with chloral hydrate (Rectiole; Dentinox, Berlin), 75–100 mg/kg of body weight (14).

The 48 examinations of the 40 children can be divided into two groups. Group A consisted of 12 examinations of either healthy children or patients who were expected to have undisturbed spectra for amnestic and clinical reasons. Group B was 36 studies of patients with a potentially abnormal P-31 spectrum (including patients recovering after infections and asphyxia at birth, patients with seizures treated with success, and patients with mild disorders of neurologic development).

P-31 MR spectroscopy was performed on a 2.35-T combined imaging-spectroscopy system (Medspec 24/40; Bruker/Spectrospin, Fallanden, Switzerland) (14). A modified surface coil technique (diameter, 5.5 cm) was used to suppress possible signals from superficial tissue (15). Spectra were obtained from about 30 cm^3 of the frontotemporal regions, bilaterally whenever possible. Two hundred fifty-six transients were recorded in 11 minutes with a repetition time of 2,600 msec, a sweep width of 6,100 Hz (150 ppm), and a single pulse duration of 115 μsec. Reproducible pulse flip angles were ensured with use of an external standard reference attached to the center of the surface coil (signal minimized according to a 180° flip angle at the center of the coil). Trapezoidal multiplication (0, 2, 50, 50 msec) of the free induction decay was ap-

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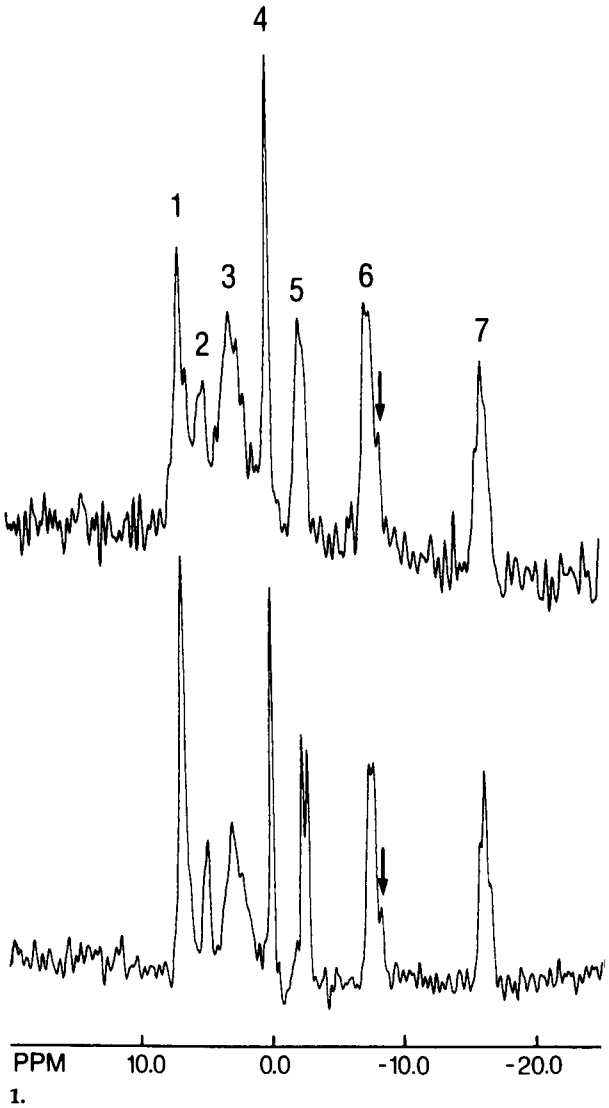
Abbreviations: ATP = adenosine triphosphate, NAD = nicotinamide adenine dinucleotide, PCr = phosphocreatine, PDE = phosphodiester, Pi = inorganic phosphate, PME = phosphomonoester.



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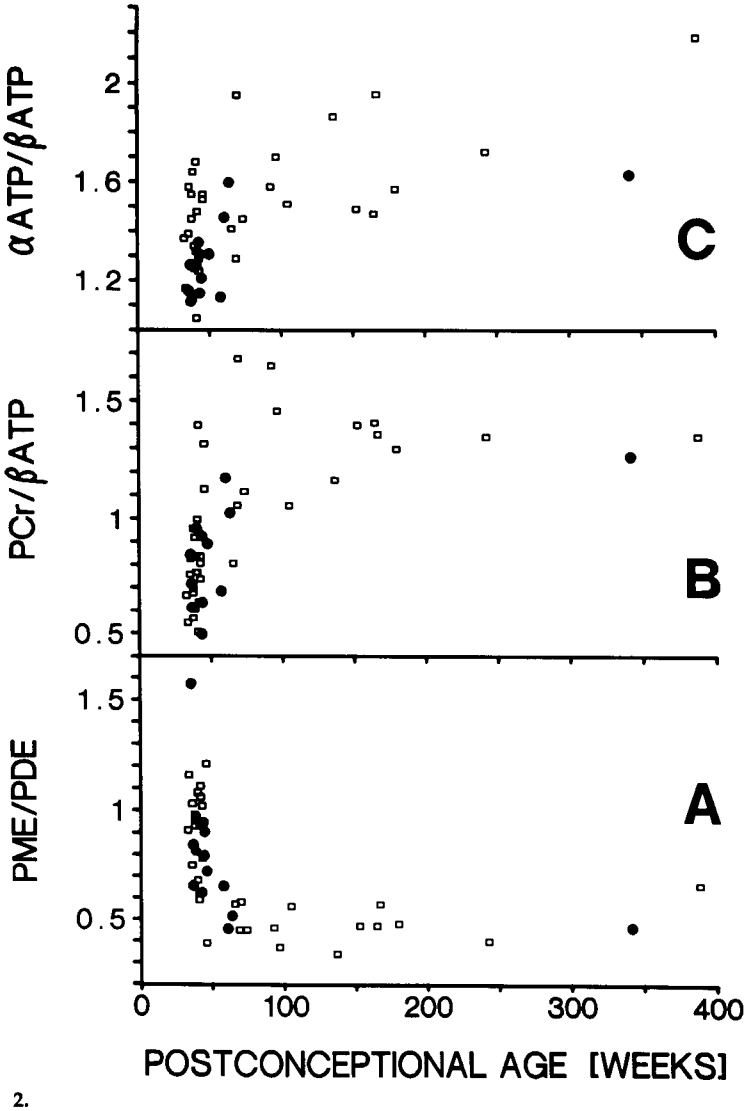

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Figures 1, 2. **(1)** MR spectra, obtained with a surface coil, of two children at 43 weeks (lower spectrum) and 98 weeks (upper spectrum) postconceptional age. Both children were born at term and were receiving anticonvulsant therapy (group B). *1* = PME (6.8 ppm), *2* = Pi (4.9 ppm), *3* = PDE (2.9 ppm), *4* = PCr (set to 0 ppm), *5* = γ-ATP (−2.5 ppm), *6* = α-ATP (−7.6 ppm), *7* = β-ATP (−16.2 ppm). The PME signal intensity is decreased with age, whereas the PDE and PCr resonances are increased. A shoulder at the α-ATP signal can frequently be resolved at −8.3 ppm (arrows), indicating that another resonance, possibly derived from nicotinamide adenine dinucleotide (NAD), adds to the integral. **(2)** Ratios of P-31 signal intensities as a function of postconceptional age. ● = group A children, □ = group B patients. (*A*) PME/PDE ratio. (*B*) PCr/β-ATP ratio. (*C*) Ratio of the area of the peak at −7.6 ppm (indicated by α-ATP) to the area of the β-ATP peak. The distinctly higher values of the α-ATP signal could be explained by overlap with an additional resonance at −8.3 ppm (Fig 1), which was tentatively assigned to NAD.

plied before Fourier transformation. This ensured elimination of the broad hump without a substantial influence on the integrals of the much smaller metabolite resonances. The chemical shift of PCr was set to 0 ppm and used as a reference. Areas under peaks were determined by integration of the signals with use of fixed limits.

RESULTS

Figure 1 illustrates the experimental basis for our work, with a display of two P-31 MR spectra from children of 42 and 98 weeks postconceptional age.

The plots shown in Figures 2 and 3, which relate the intensities of different individual peaks in these spectra, were obtained from the 48 P-31 examinations from the 40 children. The PME/PDE signal ratio showed a steep initial decrease, reaching a plateau at approximately 70 weeks postconceptional age (Fig 2a). The chemical shift of the PME peak did not vary with age (6.74 ppm ± 0.04). An obvious increase in the PCr/β-ATP signal ratio was noted in group A children, whereas the data for the group B patients were widely scattered (Fig 2b). The ratio of the signal intensity of the α-ATP resonance at −7.6 ppm (integral limits, −6.5 to −9.5 ppm) to that of the β-ATP resonance was always considerably greater than 1 and increased with age (Fig 2c). Correlation of the presumed α-ATP resonance at −7.6 ppm with the PCr peak showed a mutual dependence of these signals (Fig 3). A possible explanation for the increased area of the peak at the position of the α-ATP resonance is presented in Figure 1: Frequently, a shoulder on this resonance at −8.3 ppm could be resolved, originating from an additional resonance at this position.

The intracellular pH, estimated by the chemical shift difference between Pi and PCr (16), was 7.12 (standard deviation, 0.15; 47 examinations) for all children studied and 7.08 (standard deviation, 0.10; 12 ex-

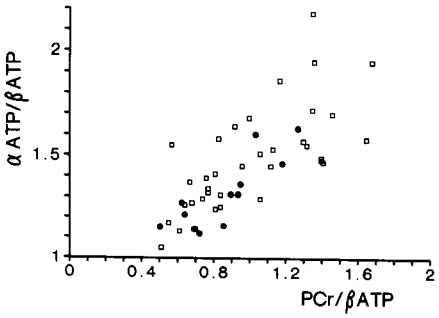

Figure 3. Correlation between the standardized signal intensities of the resonance at −7.6 ppm (indicated by α-ATP) and the PCr resonance.

aminations) for the healthy children (group A).

DISCUSSION

The observed decrease in the PME/PDE signal ratio with postconceptional age (Fig 2a) and the concomitant increase of the PCr resonance (Fig 2b) are in agreement with previous observations (2,4,7,8). Since the PME level remains detectable even several hours after death (3), it seems not to be affected within hours by acute illness. Thus, the changes in PME level over the first 20 months of life seem to reflect lipid metabolism and seem suitable for use as a maturation index for the developing human brain. By contrast, changes in the PCr level can occur within minutes; thus, correlation with age is difficult to assess because short-term physiologic or pathologic alterations may interfere. Especially in group B patients, an influence on the high-energy phosphate level cannot be excluded. As a consequence, our data for PCr scatter much more than those for PME and PDE.

From studies of acidic brain extracts (12,13), the α-ATP peak is known to contain signals from dinucleotides, predominantly NAD. The consistent elevation of the signal at −7.6 ppm relative to the β-ATP signal could be explained by an additional resonance at this position, which is supported by the frequent observation of a resonance shoulder at −8.3 ppm (Fig 1). It thus seems reasonable to consider the increased intensity of the signal integral between −6.5 and −9.5 ppm with postnatal life as an indicator for an age-dependent increase of the dinucleotide signal (eg, NAD). In the future, more precise statements will have to rely on conversions of the MR signal intensities to metabolite concentrations. In such interpretations, much care must be exercised to prevent systematic errors, for example, the contamination of brain spectra by high-energy phosphates from muscle. In our study, this contribution of unwanted signals was diminished with use of a ferromagnetically dotted sheet (15) to broaden signals from surface tissue. In contrast to MR imaging, in which voxel sizes suitable for separation of gray and white matter are used, all P-31 MR spectroscopy studies require much larger volumes, independent of the specific technique used for volume selection. This leads to a certain inability to fully separate gray and white matter in the neonatal brain, due to the small dimensions and the complex geometry of the gyri and nuclei. Thus, conclusions about brain maturation based on MR spectroscopy have so far relied on observations of resonance peaks that contain contributions from both of these tissue types. With the technique used in our study, recordings are made primarily near the surface, resulting in a relatively high proportion of gray matter signal.

Use of a repetition time that is short compared with the T1 relaxation times results in partial saturation of the signals (2). For age-dependent saturation of the resonances to be assessed, additional measurements of the T1 values are necessary.

Overall, our study indicates that it should be possible in clinical practice to record standard curves for metabolites detected by MR spectroscopy, permitting assessment of phospholipid synthesis (PME, PDE) and energy status (PCr, NAD) in the developing human brain. These spectroscopic observations could possibly represent a maturation index that could be used as a supplement or an alternative to the myelination score determined with MR imaging (9). The most prominent changes in P-31 MR spectra occur before 60 weeks postconceptional age, whereas the most impressive changes observed with MR imaging occur after 60 weeks. Thus, the combination of MR imaging and MR spectroscopy increases the time period during which maturation of the human brain can be observed. However, the fact that the most prominent changes in MR imaging and MR spectroscopy occur at different times indicates that one does not observe identical chemical mechanisms. For the sum of the α-ATP peak and an additional resonance at its shoulder at −8.3 ppm, an increase is observed during development of the human brain. To our knowledge, this is the first report of in vivo observation of an increase of this resonance and of its correlation with the increase in PCr/β-ATP ratio. The temporal changes of the resonance at −8.3 ppm could be explained by variations in the concentration of NAD. This suggestion is supported by the fact that in some of the spectra, two components at the position of the α-ATP resonance are partially resolved (Fig 1). Finally, we found that pH values in the brains of infants tended to be higher than those in adults (6). This can be explained only partially by the different formula (16) used in the present study and concurs with the observation presented in earlier publications (2,6). ■

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