Developmental Changes of Phosphorus Metabolite Concentrations in the Human Brain: A ^{31}P Magnetic Resonance Spectroscopy Study *In Vivo*

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ABSTRACT. Phosphorus magnetic resonance spectroscopy is a noninvasive method to investigate brain metabolism *in vivo*. ATP generally serves as an internal concentration standard for the quantification of the various phosphorus metabolites, because the ATP concentration in mammalian brains is assumed to be age independent. This presumption is based on observations made in the biochemical analysis of the developing rat brain. In the present study, metabolite concentrations were assessed with an external concentration standard. Each brain spectrum was quantified using a calibration spectrum that was acquired from a phantom after the *in vivo* brain measurement. Fully relaxed localized brain spectra were obtained from 16 neonates (2–28 d), 17 infants (6–20 mo), and 28 adults (22–58 y). The metabolite concentrations (in mmol/L) changed from neonates to adults: phosphomonoester from 4.5 to 3.5, inorganic phosphate from 0.6 to 1.0, phosphodiester from 3.2 to 11.7, phosphocreatine from 1.4 to 3.4, and ATP from 1.6 to 2.9. We conclude that 1) the ATP concentration in the human brain almost doubles between neonates and adults, and 2) ATP may not be used as an age-independent internal concentration standard. (*Pediatr Res* 35: 431–435, 1994)

Abbreviations

MR, magnetic resonance
MRS, magnetic resonance spectroscopy
VOI, volume of interest
CSF, cerebrospinal fluid
PME, phosphomonoester
PDE, phosphodiester
Pi, inorganic phosphate
PCr, phosphocreatine
pH_i, intracellular pH
[ATP], ATP concentration
PEt, phosphorylethanolamine
PCh, phosphorylcholine
T₁, longitudinal relaxation time
T₂, transversal relaxation time

An understanding of normal metabolite concentrations in the healthy human brain at different developmental ages is of fundamental scientific interest. Acute concentration changes are of clinical relevance for diagnosing disease states, because they often occur in a predictable manner.

Biochemical analysis of biopsy samples is the classic method for the determination of metabolite concentrations (1–5). However, it has two crucial disadvantages: 1) labile metabolites may be altered or destroyed during the tissue extraction procedure and their decomposition products may become more abundant, and 2) tissue biopsies cannot be obtained from healthy human brains. Both these problems can be solved noninvasively by determination of molar concentrations with *in vivo* MRS (6–10).

Although results obtained by MRS are usually expressed as ratios of peak areas such as PCr to ATP, molar concentrations can be determined accurately by MRS provided an appropriate quantification procedure is applied. Because each peak area is proportional to the concentration of the corresponding metabolite, the individual concentrations can be estimated if the concentration of at least one metabolite, which can then be used as an internal concentration standard, is known. In ^{31}P MRS studies on brain development, concentration estimates are generally assessed by assuming an age-independent [ATP] of about 2.8 mmol/L. This assumption is based on observations in the developing rat brain in which the cerebral [ATP] in neonatal and adult rats were found to be independent of age (1, 2). With reference to these findings in animals, an age-independent [ATP] was also assumed for the human brain (6). Several human ^{31}P MRS studies coincidentally found an [ATP] of about 2.8 mmol/L in the adult human brain (7–9).

The purpose of this study was to determine the molar concentrations of phosphorus brain metabolites with *in vivo* ^{31}P MRS in the human brain at different developmental ages and to test the hypothesis of an age-independent [ATP].

MATERIALS AND METHODS

Subjects. Sixteen term neonates (mean postconceptional age 42 wk, range 39–44 wk), 17 infants (mean age 11 mo, range 6–20 mo), and 28 healthy adult volunteers (mean age 32 y, range 22–58 y) were studied. Of the 33 pediatric subjects, five were clinically healthy and six had a small hemorrhagic lesion outside the VOI. Twenty-two of the 33 children were hospitalized for nonneurologic reasons, e.g. infectious diseases or gastrointestinal problems. The parents of all children gave their oral or written consent after thorough oral and written explanation of the MR examination procedure. They were usually present with their child during the whole examination. The neonates and infants were sedated with chloral hydrate (50–100 mg/kg) given orally

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or via a nasogastric tube. Oxygen saturation, heart rate, and ECG were continuously measured in all children. The study including the sedation has been approved by the local ethical committee and by the Swiss Federal Research Council.

Instrumentation. All neonates and infants and seven adults were examined on a 2.35-Tesla MEDSPEC 24/40 (Bruker/Spectrospin). In addition, measurements were made on 21 adults with 1.5-Tesla S15 Gyroscan (Philips). Because both spectrometers yielded almost identical mean cerebral metabolite concentrations with comparable standard deviations, the data of all 28 adults were treated together. Helmholtz type radiofrequency coils of different sizes were used as transmitter and receiver coils. For each volunteer, optimal pulse gains were applied using the previously determined relation between the matching capacitance and pulse gain required for the 90° pulse.

Localization. Image-guided spectroscopy was performed using the volume-selective pulse sequence ISIS (11). Figure 1 shows position and size of the volumes selected. VOI of 5³, 6³, and 7³ cm³ were selected in the brain of neonates, infants, and adults, respectively. The volumes composed approximately similar parts of the parietal lobes (≈45%), the frontal lobes (≈15%), the occipital lobes (≈10%), and the basal ganglia (≈20%) in all three groups. The contribution of gray and white matter was also approximately similar for the three age groups. The relative composition of the VOI changed with respect to the CSF, which contributed 18 ± 3% in neonates, 11 ± 2% in infants, and 10 ± 2% in adults. The amount of CSF and the composition of the VOI were calculated in four subjects of each age group by performing an operator-guided planimetric analysis of eight to 10 frontal and transversal T₂-weighted multislice MR images. This method of CSF assessment was validated by comparing our results measured in adults with those determined from matched brain sections in an anatomical atlas (the correspondence was better than ±12%). Because CSF contains almost no phosphorus (12), the metabolite concentrations obtained for neonates, infants, and adults were multiplied by a correction factor of 1/0.82, 1/0.89, and 1/0.90, respectively.

Quantification. Quantification was achieved with a calibration phantom used as concentration standard. The calibration measurement was performed immediately after the *in vivo* investigation. The brain and the calibration measurements were identical with respect to all measurement parameters, the size and location of the volume selected, and the load of the radiofrequency coil (Fig. 1). The coil load for the calibration measurement was adjusted to match that of the brain examination in the following way: the matching capacitance of the radiofrequency coil was left unchanged after the *in vivo* measurement, and the coil load of the calibration measurement was successively increased with a saline bottle that was brought closer to the phantom-loaded coil in a stepwise fashion until optimal matching was achieved

(Fig. 1). Head-shaped bottles containing 1, 2, or 4 L of 75 mmol/L K₂HPO₄ solution were used as phantoms for neonates, infants, and adults, respectively. The electrical conductivity of the solution [$\sigma \approx 0.58$ siemen/m (13)] was slightly lower than that of human tissue [$\sigma \approx 0.64$ siemen/m (14)] to allow fine adjustment of the coil load with the saline bottle. The initial T₁ of 9 s was shortened to 1 s through the addition of 0.04 g/L NiCl₂ (no T₂ shortening).

Data acquisition. The position of the VOI was selected from MR images, which were available from the preceding routine examination. The time required to prepare the spectroscopy session was about 10 min and the acquisition time of a spectrum was 13 min. Fully relaxed spectra were collected with a repetition time of 12 s (4 times the longest T₁) and 64 scans were averaged to enhance the signal-to-noise ratio. The spin-lattice relaxation times T₁ of the ³¹P metabolites were determined in four neonates and five adults with saturation-recovery and inversion-recovery sequences.

Data processing. The MRS signals (free induction decays) were fitted in the time domain with an iterative least-squares method (15). Exponentially decreasing model signals were fitted to the experimental signals. By Fourier transformation, these model signals convert into Lorentzian lines. The multiplets of ATP were fitted with special model signals taking the ³¹P-³¹P coupling into consideration. Iteration was started with a list of seven single model signals, one signal for each *in vivo* resonance. The first 2 ms of the experimental signal were omitted for fitting to avoid the strong but fast-dephasing signal of the almost immobile membrane phospholipids. After iterative fitting, the first 2 ms of the experimental signal were replaced by the corresponding model signal. The experimental signal collected after the first 20 ms was also omitted because of the decreasing signal-to-noise ratio. The peak width of Pi was not fitted but kept constant at 14 Hz full-width-at-half-maximum because it often degenerates due to strong peak overlapping with PME and PDE. In addition, the peak width of PDE was kept constant at 60 Hz full-width-at-half-maximum to prevent its broadening due to the almost immobile membrane phospholipids.

Statistical analysis. Mean values of the various metabolite concentrations were compared between the three age groups with the parametric two-sample *t* test. Values are expressed as means ± SD throughout this paper.

RESULTS

Figure 2 depicts three representative ³¹P brain spectra from one neonate (1 wk), one infant (1 y), and one adult (36 y). The chemical shifts are plotted along the abscissa and the peak intensities along the ordinate. The most obvious difference between the spectra of neonates and adults is a high PME but a

In Vivo Tissue Meas.

In Vitro Calib. Meas.

Fig. 1. Calibration strategy for the determination of ³¹P metabolite concentrations. The brain spectrum was quantified by means of a calibration spectrum acquired from a phantom of known concentration. Both spectra were localized with the volume-selective pulse sequence ISIS.

Fig. 2. Fully relaxed ^{31}P spectra from healthy human brain *in vivo*. The experimental spectrum, the calculated model spectrum, and the calculated model peaks are shown. The model spectrum is the sum of the model peaks, which were obtained by fitting the free induction decay in the time domain.

Table 1. Concentrations of ^{31}P metabolites in healthy brains of human neonates, infants, and adults

	<i>n</i>	Mean concentration $\pm$ SD (mmol/L = mmol/dm ³)					^{31}P total
		PME	Pi	PDE	PCr	ATP	
Neonates	16	4.5 $\pm$ 0.7*	0.6 $\pm$ 0.1†	3.2 $\pm$ 0.8†	1.4 $\pm$ 0.2†	1.6 $\pm$ 0.2†	14.9 $\pm$ 2.3†
Infants	17	3.6 $\pm$ 0.9	0.6 $\pm$ 0.1	4.2 $\pm$ 0.7	1.7 $\pm$ 0.3	1.8 $\pm$ 0.3	16.1 $\pm$ 2.5
Adults	28	3.5 $\pm$ 0.6*	1.0 $\pm$ 0.2†	11.7 $\pm$ 2.2†	3.4 $\pm$ 0.5†	2.9 $\pm$ 0.4†	29.3 $\pm$ 3.1†

* Significant difference: $p < 0.01$.

† Significant difference: $p < 0.001$.

low PDE in the former, and low PME but high PDE in the latter. This change of pattern is typically observed during brain maturation.

Table 1 lists the molar concentrations of the various human brain ^{31}P metabolites in neonates, infants, and adults. Note that the concentrations of ATP, Pi, PCr, and PDE all increased significantly by factors of 1.8, 1.9, 2.4, and 3.6, respectively,

from birth to adulthood. During the same period, PME decreased by only 22%, despite its apparent relative fall in the spectra. The mean concentration changes between neonates and adults were all significant.

Table 2 depicts relevant ratios of peak areas for the three age groups. From birth to adulthood, the PCr-to-ATP and the PCr-to-Pi ratios both increased by about 30%; the PDE-to-PME ratio increased by a factor of 4.7.

Table 3 lists age-dependent PME parameters. From neonates to adults, the chemical shift decreased by 0.21 ppm, the peak width increased by 60%, and the T_1 value decreased by 35%. This indicates a change in the chemical composition of PME. The T_1 values of the other resonances were 1.2 s for Pi, 1.4 s for

PDE, 2.8 s for PCr, 0.9 s for γ -ATP, 1.1 s for α -ATP, and 1.0 s for β -ATP; they did not change with age.

Table 4 lists the mean pH_i , which decreased significantly from 7.11 to 7.02 during brain development. The pH_i was calculated from the chemical shift difference of Pi relative to PCr (16).

Table 2. Ratios of peak areas in healthy brains of human neonates, infants, and adults

	n	Ratios of peak areas		
		PCr/ATP	PCr/Pi	PDE/PME
Neonates	16	0.9 $\pm$ 0.1*	2.4 $\pm$ 0.2†	0.7 $\pm$ 0.2†
Infants	17	1.0 $\pm$ 0.1	2.8 $\pm$ 0.3	1.2 $\pm$ 0.3
Adults	28	1.2 $\pm$ 0.1*	3.4 $\pm$ 0.4†	3.3 $\pm$ 0.9†

* Significant difference: $p < 0.01$.

† Significant difference: $p < 0.001$.

Table 3. Chemical shift, peak width, and T_1 time of PME in healthy human brain*

	n	PME parameters		
		Chemical shift (ppm rel. PCr)	Peak width (fwhm in Hz)	T_1 time (s)
Neonates	16	6.74 $\pm$ 0.03†	29 $\pm$ 3†	1.8 $\pm$ 0.3‡
Adults	28	6.53 $\pm$ 0.04†	46 $\pm$ 9†	1.2 $\pm$ 0.3‡

* PME is mainly composed of PEt (6.8 ppm) and PCh (6.3 ppm). Their relative contributions differ in the neonatal brain (PEt $\approx$ 70%, PCh $\approx$ 10%) and in the adult brain (PEt $\approx$ 30%, PCh $\approx$ 30%). ppm, parts per million; fwhm, full-width-at-half-maximum.

† Significant difference: $p < 0.001$.

‡ Significant difference: $p < 0.01$.

Table 4. pH_i in healthy brains of human neonates, infants, and adults

	n	Chemical shift difference $\Delta\delta$ (Pi - PCr) (ppm)	pH_i *
Neonates	16	4.93 $\pm$ 0.07	7.11 $\pm$ 0.06†
Infants	17	4.90 $\pm$ 0.08	7.09 $\pm$ 0.07
Adults	28	4.82 $\pm$ 0.08	7.02 $\pm$ 0.07†

* $pH_i = 6.73 + \log \frac{\Delta\delta - 3.28}{5.69 - \Delta\delta}$

† Significant difference: $p < 0.01$.

DISCUSSION

This study shows that the molar concentration of ATP in the human brain increases by a factor of 1.8 during maturation from 1.6 mmol/L in neonates to 2.9 mmol/L in adults. The adult value compares favorably to literature data from quantitative ^{31}P MRS (7–9), but no neonatal concentrations are available in the literature. The following two observations can be interpreted to be in agreement with our result of an [ATP] increase in the human brain. First, the nonwater proportion in human brain increases from 10% in neonates to 23% in adults (17) and the total phosphorus concentration rises from 54 to 109 mmol/L (17). Both changes are partly due to myelination but also to an increase of the concentration of cytosolic compounds (18, 19). Second, the cerebral metabolic rate rises 5-fold for oxygen, 10-fold for glucose, and even 20-fold for ATP during brain development (19) and therefore makes a developmental [ATP] increase plausible, although a higher energy turnover does not automatically imply a higher [ATP].

Although the adult [ATP] of about 2.9 mmol/L is similar in humans (7–9) and rats (4), the neonatal [ATP] seems to differ between the two species: it is about 2.5 mmol/L in rat pups (2, 4) and about 1.6 mmol/L in human neonates (present study) (Table 5). The higher [ATP] in rat pups than in human neonates could tentatively be explained by a higher specific energy demand resulting from rapid growth and high heat dissipation, assuming a higher [ATP] is related to a faster energy turnover. Rat pups are probably more mature with respect to brain energy metabolism than human neonates, although the brain morphology of 10-d-old rat pups is comparable to that of term human neonates (20).

Another aspect of paramount importance is the composition of the brain tissue investigated. In rats, the entire brain was investigated (cerebral hemispheres: $\approx$ 35%, brain stem and basal ganglia: $\approx$ 65%) (1, 2). On the other hand, in this study, we have mainly selected brain tissue from human cerebral hemispheres (cerebral hemispheres: $\approx$ 78%, basal ganglia: $\approx$ 22%). It is possible that, at birth, the [ATP] is higher in the brain stem and basal ganglia than in the cerebral hemispheres, because the neonatal metabolic rates of oxygen and glucose consumption are both

Table 5. Literature data of PCr and ATP concentrations at different stages of brain maturation

First author	Year	Method	Species	Age	Concentration (mmol/L = mmol/dm ³)	
					PCr	ATP
Mandel (1)*	1966	Biochemical analysis	Rat	7-d-olds		2.3
				Adults		2.4
Miller (2)*	1977	Biochemical analysis	Rat	10-d-olds	3.4	2.5
				Adults	3.6	2.4
Palmer (3)	1990	Biochemical analysis	Rat	7-d-olds	2.6	2.5
Onodera (4)	1986	Biochemical analysis	Rat	Adults	5.0	2.8
Nioka (5)	1991	Biochemical analysis	Dog	0- to 2-d-olds		2.8
				Adults		2.9
This study	1993	^{31}P MRS	Human	Neonates	1.4	1.6
				Adults	3.4	2.9

* The lower concentrations obtained in earlier biochemical studies are probably due to autolysis of PCr and ATP.

higher in the former than the latter regions (19). In adults, these regional differences have a tendency to level out (19). The hypothesis of a postnatal increase in the [ATP] in the cerebral hemispheres in contrast to a constant concentration in the brain stem and the basal ganglia would explain the discrepancies between our study and studies on rats. Thus, the diverging results in neonatal human and rat pups are possibly due to different brain regions being analyzed rather than to interspecies differences. It has to be pointed out that the chemical analysis assesses pure ATP, whereas ^{31}P MRS always detects all nucleoside triphosphates. Additionally, a certain amount of nucleoside triphosphate may be undetectable by ^{31}P MRS.

The PME and PDE signals are both composed of many different metabolites containing PME or PDE groups, respectively. PME is assigned to phospholipid precursors, whereas PDE is related to both phospholipid decomposition products ($\approx 20\%$) and phospholipids themselves ($\approx 80\%$) (21, 22). We found a relatively small diminution (only 22%) of PME concentration during maturation. This seems to be in contradiction to the reported 3-fold decrease of PEt (23, 24); PEt is a main component of PME. However, the two findings are consistent if the age-dependent chemical composition of the PME signal is considered. The PEt contribution to PME is about 70% in neonatal brain (23, 25) but only 30% in adult brain (25, 26). In contrast to PEt, the contribution of PCh rises from about 10% to more than 30% during brain maturation (24, 26, 27). In the present *in vivo* study, the change in the PME composition could be observed through the upfield shift of the PME resonance (Table 3). In addition, the broadening of the PME resonance line expresses the change from an almost monocomponent peak into a multicomponent peak (Table 3). Finally, the reduction of the T_1 relaxation time of PME can also be related to a reversion of the PEt and PCh contributions if a shorter relaxation time is assumed for PCh than for PEt (Table 3). All three findings indicate a change in the composition of PME. In contrast to PME concentration, PDE concentration rises steadily during the last quarter of gestation by a factor of 2.4 (10), from birth to adulthood by a factor of 3.7 (this study), and from adulthood to old age by a factor of 2 (28).

We observed a decrease in human cerebral pH_i of 0.09 U during postnatal maturation. This result compares favorably with the pH_i diminution of 0.12 U found in dog brains during postnatal development (5) and follows the same tendency found in preterm humans (pH_i fall of 0.05 U between 28 and 42 wk of postconceptional age) (29).

To verify our concentration estimates, control examinations were performed with a circular surface coil positioned under the back of the head of three neonates and three adults. A rectangular pulse performing a 180° flip angle in the coil center was applied to the occipital lobe. Although somewhat less precise, this surface-coil method is more robust than the ISIS method and thus less likely to suffer from any systematic error (30). The calibration phantoms used had a wall thickness approximately equal to the distance between skin and brain. Almost identical results were obtained using the ISIS sequence in our study and a surface coil in the control examinations: the respective [ATP] were 1.54 and 1.67 mmol/L in neonates and 2.85 and 2.98 mmol/L in adults. Therefore, we believe that the occurrence of severe systematic errors can be excluded.

In summary, we found that the [ATP] almost doubles during brain development of healthy human neonates to adults. Therefore, we conclude that ATP may not be used as an age-independent internal concentration standard in MRS studies measuring ^{31}P metabolites in the human brain.

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