

Intracellular pH by ³¹P MRS: It falls during brain maturation and is higher in the cerebellum than the cerebrum

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Introduction

An understanding of normal pHi in healthy human tissue at different developmental ages and in different organs is of fundamental scientific interest. Acute pHi changes are of clinical relevance for diagnosing disease states, because pHi often occur in a predictable manner. For example, some kinds of cancer and degenerative muscle diseases could be detected using non-invasive pHi measurements by ³¹P MRS. The biochemical analysis of biopsy samples is the classical method for the determination of pHi [1]. However, tissue biopsies cannot be obtained from e.g. human brains. This problem can be solved by non-invasive in-vivo ³¹P MRS [2].

Methods

MR Scanner: All measurements were performed on a 2.35 Tesla MEDSPEC 24/40 (Bruker/Spectrospin) and/or a 1.5 Tesla S15 Gyroscan (Philips) and/or a 4.7 T SISCO Imaging Spectrometer (Varian). Home-built Helmholtz-coils and 8-cm-surface-coils were used for the brain and calf muscle measurements, respectively.

Pulse sequence: Image-guided spectroscopy was performed for the brain measurements, using the volume-selective pulse sequence ISIS and adiabatic rf-pulses. For the muscle measurements, a simple pulse-acquire sequence was applied. Fully relaxed spectra were collected with a repetition time of 12 s (4 times the longest T₁) and 64 scans were averaged in order to enhance the signal-to-noise ratio.

Determination of the pHi: The chemical shift difference between inorganic phosphate (Pi) and phosphocreatine (PCr) was determined as follows: The positions of the peaks were not just equated with the peak maximum, but were determined by fitting the time signal of the FID with an iterative least-squares method [3]. This allows for a much more accurate pHi, because the crude peak-maximum method is very sensitive to systematic errors due to baseline effects and to statistical errors due to poor signal-to-noise ratios. However, the sophisticated time-signal-fitting method is robust with respect to both sources of errors. Exponentially decreasing model signals were fitted to the experimental signals. By Fourier transformation, these model signals were converted into Lorentzian lines. Iteration was started with a list of seven single model signals, one signal for each in-vivo resonance of ³¹P. The first two milliseconds of the experimental signal were omitted for fitting in order to avoid the strong but fast-dephasing signal of the almost immobile membrane phospholipids. In brain spectra, 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 [4]. This problem does not occur in muscle spectra, where the peak width of Pi was fitted.

Statistics: A binomial t-test was made to check the significance of the differences between the mean pHi of the different groups investigated.

Results

Table 1 lists the mean intracellular pH and its standard deviations found in healthy human tissue.

a) Age dependence in brain:	b) Organ dependence in adult brain:	c) Sex dependence in adult muscle:
Neonates: 7.09 ± 0.05 (n=12)	Cerebellum: 7.02 ± 0.06 (n=13)	Men: 7.05 ± 0.02 (n=9)
Adults: 7.00 ± 0.04 (n=13)	Cerebrum: 6.95 ± 0.05 (n=14)	Women: 6.97 ± 0.03 (n=8)
Difference (0.09) is highly sig. (p<0.001)	Difference (0.07) is highly sig. (p<0.001)	Difference (0.08) is highly sig. (p<0.001)

Because pHi measurements by ³¹P MRS are intrinsically non-invasive, we were able to make repeated measurements of single volunteers and obtained pHi differences of only 0.01 units. This is the intra-individual standard error of the measurement. The inter-individual standard deviation within a group examined, i.e. the mean pHi difference between the volunteers, varied between 0.02 and 0.06 units (see Table 1).

Discussion

We observed a fall in human cerebral pHi by 0.09 units during postnatal maturation. This result compares favorably with the pHi diminution of 0.12 units found in dog brains during postnatal development [1] and follows the same tendency found in preterm humans (pHi fall of 0.05 units between 28 and 42 weeks of postconceptional age) [5]. In mammalian muscles the pHi is generally assumed to be independent of sex. This presumption is mainly based on observations made by biochemical analysis of animal tissue. Our finding suggests a different equilibrium of the creatine kinase reaction (PCr + ADP + H⁺ ↔ Cr + ATP) in male and female muscle and, thus, a theoretically lower PCr-to-ATP ratio in case of a lower pHi level (= higher [H⁺]). However, we found the opposite: the PCr-to-ATP ratios found muscle tissue were higher for women (1.17 ± 0.08) than for men (1.07 ± 0.09). The same intriguing finding is observed in developing brain tissue, where the PCr-to-ATP ratio was higher for neonates (1.0 ± 0.1) than for adults (1.3 ± 0.1). Because the creatine kinase reaction is depending on so many parameters, the simple analysis made here may be too superficial.

References

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