Phosphorus $J$-Coupling Constants of ATP in Human Brain

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Proton decoupled $^{31}$P NMR spectroscopy of the occipital brain of healthy volunteers was performed with a 1.5 T whole-body imager. By use of two-dimensional chemical-shift imaging in combination with slice-selective excitation well resolved localized spectra (38 ml) were obtained within 34 min from which the homonuclear $^{31}$P-$^{31}$P $J$-coupling constants of ATP could be determined: $J_{\alpha\beta} = 16.1$ Hz $\pm$ 0.2 Hz and $J_{\beta\gamma} = 16.3$ Hz $\pm$ 0.1 Hz (mean $\pm$ SEM, $n = 14$). Both, the $J$-coupling constants and the chemical-shift difference between $\alpha$- and $\beta$-ATP ($\delta_{\alpha\beta} = 8.61$ ppm $\pm$ 0.01 ppm) were used to calculate the concentration of intracellular free magnesium. The concentrations are $0.39$ mM $\pm$ 0.09 mM by using the average of both coupling constants of each spectrum, which is in fair agreement with $0.32$ mM $\pm$ 0.01 mM obtained from the chemical shift of $\alpha$ and $\beta$ phosphate resonances, which is the more accurate result.

Key words: $^{31}$P ATP $J$-coupling constants; human brain; intracellular free magnesium.

INTRODUCTION

In vivo $^{31}$-phosphorus NMR spectroscopy is often performed to determine the intracellular pH (e.g., 1–3) or the concentration of intracellular free magnesium ($[Mg^{2+}]$), e.g., 2–9). The latter information can be obtained by using the chemical-shift differences between some of the phosphorus signals (2–9) or the $^{31}$P-$^{31}$P homonuclear $J$-coupling constants $J_{\alpha\beta}$ and $J_{\beta\gamma}$ of ATP (2, 5, 7, 9). Using the chemical-shift differences for the determination of $[Mg^{2+}]$ careful assessment is mandatory since the chemical-shift differences depend not only on $[Mg^{2+}]$ but also on intracellular pH. Since the coupling constants of ATP are independent of pH in the range between pH = 7 and 8 (7), they seem to represent a useful measure for the determination of $[Mg^{2+}]$. However, the coupling constants obtained under broadband proton decoupling in model solutions vary only in the range between approximately 19.5 Hz for totally uncomplexed and approximately 15.5 Hz for totally magnesium complexed ATP (2, 5, 7). Therefore, it was recognized almost 2 decades ago that the coupling constants represent a less sensitive measure for the fraction of ATP complexed to magnesium $([\phi])$ and the corresponding $[Mg^{2+}]$ than the chemical-shift difference $\delta_{\alpha\beta}$ between $\alpha$- and $\beta$-ATP (5). In contrast, a recent study revealed that between human calf muscle and human myocardium, a difference in the ATP coupling constant $J_{\alpha\beta}$ exists that is 11 times greater than expected from the difference in $[Mg^{2+}]$ between these two tissues and, moreover, that a difference between $J_{\alpha\beta}$ and $J_{\beta\gamma}$ of 1.1 Hz exists in human calf muscle, which cannot be explained by magnesium complexation alone (9). These findings again raise the question whether or not the $J$-coupling constants are a useful way to the determination of $[Mg^{2+}]$.

The goal of this paper is an accurate determination of the $J$-coupling constants of ATP in human brain and their evaluation towards magnesium complexation.

METHODS

NMR Spectroscopic Investigations

Examinations were conducted on a Siemens Magnetom SP 63 Helicon whole-body imager operating at 1.5 T with Larmor frequencies of 25.74 and 63.60 MHz for $^{31}$P and $^1$H spectroscopy, respectively. The imager is equipped with a second RF-channel for proton decoupling. Transmission and reception was performed with a 100-mm double-resonant single-turn surface coil.

The heads of the volunteers were positioned such that for each head, the occipital brain was located above the center of the surface coil. Flow-rephased gradient echo proton images were then acquired to control the position and to determine the volume of interest. Subsequent nonlocalized shimming was also performed on protons leading to water linewidths between 8 and 11 Hz within less than 5 min.

A two-dimensional phosphorus chemical-shift imaging sequence (2D-CSI, 10) in combination with slice-selective excitation was used to perform a complete three dimensional localization of 38 ml volume elements. A detailed description of this pulse sequence is provided in Ref. 9 where the identical sequence was used for calf muscle investigations. The measurements were conducted with a pulse angle of 140° in the coil center and a TR of 1 s. To improve the signal-to-noise ratio and the resolution of the spectra and to eliminate the influence of
the J-couplings to protons. WALTZ4 proton decoupling (11) was applied during the first 50 ms of data acquisition in the CSI measurements. The decoupler frequency was adjusted to the water resonance. The acquired signals were recorded with 1k data points and a dwell time of 0.5 ms.

Data Analysis

We used only one volume element of the acquired array of 8 × 16 volume elements from each CSI measurement. This volume element was located within the occipital brain as close as possible to the surface coil but with enough separation from the large blood vessel sinus sagittalis and the skull to prevent blood signals and broad bone resonances in the spectra.

The time-domain signal of this volume element was multiplied with a Gaussian function (t1/2 = 150 ms), zero-filled to 4k, and Fourier transformed. The phase-corrected spectra were fitted with Gaussian lines for the metabolite resonances and a third order polynomial for baseline-correction. The fit for γ-ATP was performed with two Gaussian lines of equal linewidth while for α-ATP an additional line with free linewidth was fitted to take the NAD signal into account.

Calculation of the intracellular concentration of free magnesium ([MgJ+]) was performed following Gupta and Moore (6) as introduced in Ref. 9 by using the chemical shift difference δMg and a dissociation constant of MgATP $K_D = 53 \mu M$. To calculate [MgJ+] from the ATP J-coupling constants, a linear decrease of both coupling constants from 19.5 to 15.5 Hz in the range from totally uncomplexed to fully complexed ATP (2, 5, 7) was used leading to

$$[MgJ^+] = K_D \left( \frac{1}{0.25/\text{Hz} - 3.875} - 1 \right), \quad 15.5\text{Hz} < J$$

[1]

in close analogy to the formulas established by Gupta et al. for δMg (6).

The intracellular pH was determined by using the chemical shift of P1 relative to creatinephosphate (PCr) at 0.00 ppm and the Henderson-Hasselbalch equation in the form published by Petroff et al. (3).

RESULTS AND DISCUSSION

Fourteen examinations of 12 healthy volunteers with ages ranging from 23 to 74 years (mean age 37 years) were performed with the protocol introduced (two volunteers were studied twice).

Figure 1 shows a typical spectrum of the occipital human brain. The ATP J-β coupling constants can be determined from such well resolved proton decoupled spectra by fitting the resonance lines of γ- and α-ATP. The coupling constants are (mean ± standard error of the mean (SEM)) Jγβ = 16.1 Hz ± 0.2 Hz and Jαβ = 16.3 Hz ± 0.1 Hz with linewidths (FWHM) for γ- and α-ATP of 10.8 Hz ± 0.2 Hz and 10.0 Hz ± 0.2 Hz, respectively. A relatively small error was obtained for the coupling constants because the ATP multiplets are well resolved and because the signal-to-noise ratio is far beyond the threshold of the fit. In addition, the use of a fit routine instead of peak picking prevents from an underestimation of the coupling constants due to peak overlap. With a low signal-to-noise ratio and greater linewidths, however, the determination of the J-coupling constants would be more difficult or even impossible, if the multiplets are not resolved as it is often the case in investigations conducted at higher field strengths. The $^{31}$P J-coupling constants Jγβ and Jαβ in human brain are not very different, although the α-ATP doublet is better resolved in the spectra than γ-ATP. This finding results not only from the 0.2 Hz smaller coupling constant Jγβ but also from the 0.8 Hz broader linewidths that were obtained for γ-ATP.

An interpretation of the coupling constants Jγβ and Jαβ toward magnesium complexation in vivo was conducted by using the average of both coupling constants of each spectrum and Eq. [1], leading to $[MgJ^+] = 0.39 \pm 0.09 mM$.

The determination of [MgJ+] was also conducted by using δαβ. The corresponding chemical shift of α-ATP was determined by fitting three independent resonances. Their fitted chemical shifts (relative to PCr at 0.00 ppm) and linewidths (FWHM) are: δ = 15.57 ppm ± 0.01 ppm and FWHM = 10.7 Hz ± 0.6 Hz for the left line, δ = 16.17 ppm ± 0.01 ppm and FWHM = 13.6 Hz ± 0.6 Hz for the central line, and δ = 16.76 ppm ± 0.02 ppm and FWHM = 12.8 Hz ± 0.1 Hz for the right line. The chemical shift of the right line showed twice the SEM as the left and central line. This fact is due to the multiplet pattern of β-ATP, which seemed to show a small “fourth line” between the right and the central line in some spectra. A co-addition of all spectra was therefore conducted to show whether such a signal is also present at a higher signal-to-noise ratio. However, additional signals could not be evaluated with the present signal-to-noise ratio and the slightly decreased resolution of the added...
spectrum. On the other hand, it was not possible to exclude the existence of one or more additional small signals especially underneath the region of the right line because this region of β-ATP was not resolved in the added spectrum.

Despite the greater error of the right line, the central line of the β-ATP multiplet is well resolved and its chemical shift could be determined accurately with a small SEM of 0.01 ppm. The resulting chemical shift difference $\delta_{\text{at}} = 8.61 \pm 0.01$ ppm leads to $[\text{Mg}^{2+}] = 0.32 \text{ mM} \pm 0.01 \text{ mM}$, which is in excellent agreement with Halvorson et al. (8) who also found 0.32 mM for healthy human brain.

The $[\text{Mg}^{2+}]$ values derived from coupling constants and $\delta_{\text{at}}$ are in acceptable agreement, at least with respect to the order of magnitude. However, it is obvious that the coupling constants lead to a much greater SEM. The greater accuracy using $\delta_{\text{at}}$ is a result of the fact that the full range of magnesium complexation from fully uncomplicated to fully complexed ATP corresponds to a change of only 4 Hz in the coupling constants (19.5-15.5 Hz, 2, 5, 7) but to a change in $\delta_{\text{at}}$ of 2.5 ppm (10.85-8.35 ppm, 6) or 66 Hz at 1.5 T.

A further problem when using the coupling constants for determination of $[\text{Mg}^{2+}]$ was recognized in a recent study (9). Between human calf muscle and human myocardium, a difference in the ATP coupling constant $J_{xy}$ was found, which is 11 times greater than expected from the difference in $[\text{Mg}^{2+}]$ between these two tissues and, moreover, a difference between $J_{xy}$ and $J_{yz}$ of 1.1 Hz was found in human calf muscle, which could not be explained by magnesium complexation alone. This led to the question whether a possible influence of the interaction of ATP with other molecules as for example the enzymes in living cells has to be taken into account (9). In human brain neither such a great difference between both coupling constants nor such a great difference between the magnesium concentrations derived from the coupling constants and $\delta_{\text{at}}$ was observed. Nevertheless, the existence of effects other than magnesium complexation that influence the $J$-coupling constants of ATP in human brain can not be excluded.

Besides the interrelationship between the magnesium complexation and the $J$-coupling constants of ATP the knowledge of the coupling constants is important for spin-echo based phosphorus spectroscopic investigations. This is because $T_2$ determinations and quantitative determinations of ATP concentrations using spin-echos have to be corrected for $J$-modulation (13-15).

Finally, the chemical shift of inorganic phosphate $\delta_{\text{Pi}} = 4.85$ ppm $\pm 0.01$ ppm leads to pH $= 7.05 \pm 0.01$, which is identical to the value found by Luyten et al. (12).

**CONCLUSION**

The phosphorus coupling constants of ATP in human occipital brain are $J_{xy} = 16.1$ Hz and $J_{yz} = 16.3$ Hz. These values can be used to correct $J$-modulation effects in quantitative analysis of phosphorus spectra that are acquired with spin-echo based techniques. An evaluation of these coupling constants toward magnesium complexation of ATP leads to similar concentrations of intracellular free magnesium as derived from the chemical-shift difference between $\alpha$- and $\beta$-ATP, but the latter method leads to more accurate results.

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