Absolute Metabolite Quantification of ^{31}P MRS Spectra in the Rat Brain *In Vivo* at 17.2 Tesla using LCModel

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Purpose

^{31}P MRS allows the study of *in vivo* brain energy metabolism by directly measuring key high-energy phosphate compounds such as adenosine triphosphate (ATP) and phosphocreatine (PCr). Moreover, there is a growing interest in the study of changes in phosphomonoesters (PMEs) and phosphodiesters (PDEs) levels in neurodegenerative or psychiatric afflictions. In this study, ^{31}P MR spectra acquired in the rat brain *in vivo* at 17.2 T were analyzed in the frequency domain using LCModel[1] and a basis-set of simulated spectra using prior knowledge of chemical-shifts and J-coupling values[2,3]. ^{31}P MR spectra were acquired by employing a BISTRO[4] outer volume suppression (OVS) scheme for localization. Metabolite quantification was realized using brain tissue water as an internal concentration reference[5].

Methods

MRS Acquisitions. A total of 7 Dark Agouti male rats (300 ± 50g) were studied under isoflurane anesthesia (1-2% in pure O_2). Body temperature was monitored and maintained at 37° ± 0.5°. All experiments were performed on a 17.2 T/26 cm Bruker BioSpec MRI scanner (Ettlingen, Germany) using a ^{1}H/^{31}P dual-resonance coil consisting of a 20-mm single-loop ^{31}P surface coil and a butterfly ^{1}H geometrically decoupled surface coil (RAPID Biomedical GmbH). The tune and matching of both coils were fixed. Anatomical images were acquired with a RARE sequence. Local B_0 field homogenization was done with the ^{1}H channel using FASTMAP and Bruker shimming routines. ^{31}P MR Spectra were acquired with a pulse-acquisition (FID) sequence (100 µs square pulse, 12.8 kHz spectral width (SW), 1024 points, TR= 2 s, 906 averages) from a volume of interest (VOI) encompassing most of the brain (5x7x7 mm^3). The VOI was defined by a set of 8 OVS bands using the BISTRO scheme (HS8 adiabatic pulses: 3 ms duration, 6.6 kHz SW, HS envelope of 8 pulses). To correct for T_1-weighting, T_1 relaxation times were measured on 4 animals using the same localization sequence (TR= 2 s, 320 averages) and the inversion-recovery method (HS8 adiabatic pulse: 3 ms duration, 6.6 kHz SW; 10 inversion delays: 4, 176, 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 ms). The carrier frequency was centered on the PCr resonance frequency for all ^{31}P acquisitions. As proposed by Bottomley *et al*[5], a non-suppressed water spectrum was acquired from the same VOI with the equivalent FID-OVS sequence (100 µs square pulse, TR = 10 s, 8 averages) to be used as internal reference of concentration for the ^{31}P data. The scaling factor C_{PH} accounting for ^{1}H and ^{31}P sensibility differences was estimated from *in vitro* measurements (50 mM phosphoric acid sample) directly after each experiment using identical coil positioning and VOI. *In vitro* measurements were corrected independently for T_1 relaxation effects.

Data Analysis. ^{31}P MR spectra were zero-filled before their analysis with LCModel[1]. T_1 relaxation times were calculated by fitting the resonance apparent concentrations to a 2-parameters mono-exponential recovery function. Absolute concentrations were obtained by setting the LCModel parameter *FCALIB* to the value $[W]*C_{PH} / S_W$, where $[W]$ is the water concentration from the brain and S_W the reference ^{1}H water signal. Overall, the C_{PH} values were rather stable between experiments, with a variation of 16% (C_{PH} = 0.0021 ± 0.0003, *mean* ± *std*, n=6).

Results and Discussion

Figure 1 shows a typical ^{31}P MR spectrum acquired at 17.2T in the rat brain and its individual components. Notably, resonances for GPC, GPE and PCh were shifted by 0.31 ppm with respect to the reference values[2]. For fitting our spectra, no differential linewidth broadening of individual resonances was applied but will be considered for an improved quantification, namely of the ATP resonances, as suggested by Deelchand et al[6]. Quantification of individual PMEs and PDEs was achieved individually for Glc-6-P, GPC, GPE, PCh, PE as well as iP. Metabolite concentrations were in agreement with previous ^{31}P and ^{1}H studies done in the rat brain[6,8] and PCr concentration was determined as 4.01 ± 0.54 mM. The T_1 relaxation times for PCr (1080±120 ms) and ATP-γ (410±80 ms) were used to correct PCr and ATP concentrations. Notably, these T_1 times were shorter than the values predicted by Lu *et al*[7] (PCr: 1430 ms, ATP-γ: 530 ms). ATP-γ, GPC, GPE, PE, PCh, PCr and iP presented CRLB values below 10% in the absence of inversion pulses and ranged from 4% to 50% for TI between 176 to 1000 ms due to elevated noise levels with respect to the inverting resonances.

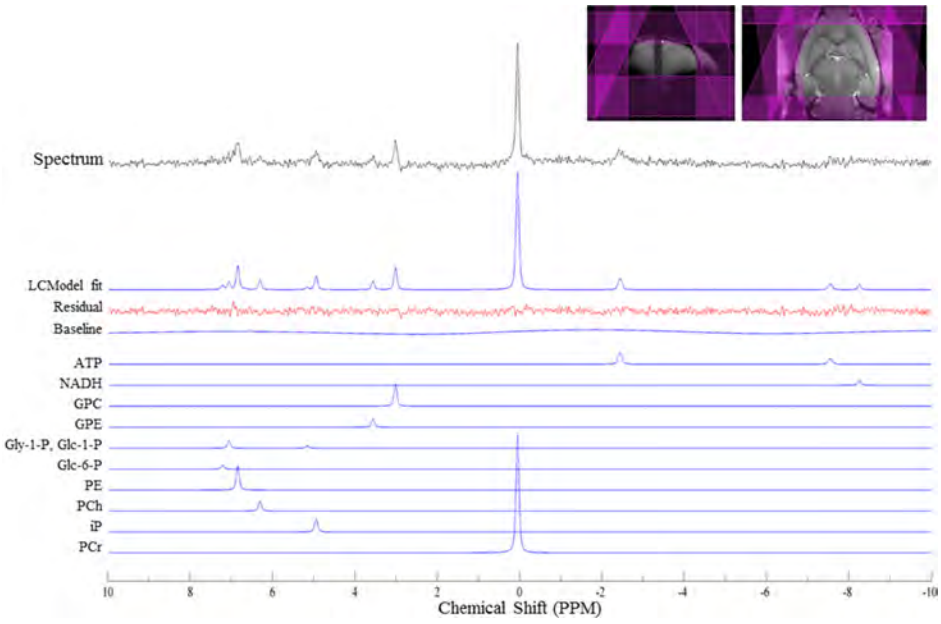

Figure 1. ^{31}P MR spectrum acquired in the rat brain *in vivo* at 17.2T. 11 phosphorylated metabolites were detected with CRLBs below 25%. The LCModel fit, baseline and residual are shown. No filtering was applied. The VOI was determined by placing 8 suppression bands implemented with a BISTRO module as shown in the anatomical images(top). Shown metabolites are: ATP, nicotidamide adenine dinucleotide (NADH), glycerolphosphorylcholine (GPC), glycerolphosphorylethanolamine (GPE), glycerol-1-phosphate (Gly-1-P), glucose-6 and -1-phosphate (Glc-6-P, Glc-1-P), phosphorylethanolamine (PE), phosphorylcholine (PCh), inorganic phosphate (iP) and PCr.

In conclusion, this study shows the feasibility of quantification of 11 different metabolites using LCModel on 31P spectra.

References

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