Relayed Magnetization Transfer from Nuclear Overhauser Effects and Chemical Exchanges Observed by the *In Vivo* ^{31}P MRS in the Rat Brain

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Introduction The magnetization transfer (MT) effects among NMR resonances of PCr, γ-ATP and Pi have been commonly observed by *in vivo* ^{31}P MRS in the brains [1,2]. These MT effects caused by selective saturating any one of PCr, γ-ATP and Pi resonances have been attributed to the chemical exchanges catalyzed by the creatine kinase (CK) and ATPase enzymes (see Fig. 1). Consequently, ^{31}P MT techniques have been used to measure chemical exchange rate constants and fluxes of the CK and ATPase reactions by saturating γ-ATP resonance. Beside the expected reductions in the Pi and PCr signals upon saturating γ-ATP resonance, one particularly interesting phenomenon, *i.e.* decreases of signal intensity in α-ATP and β-ATP, was also observed [1, 2]. Nevertheless, the reason leading to these magnetization reductions is still not fully understood. The purpose of this study is to identify the possible sources which result in the magnetization reductions of α-ATP and β-ATP when γ-ATP is saturated and its possible impact on the measurement of chemical exchange rates using the three-site exchange system of PCr↔γ-ATP↔Pi.

Experiment Male Sprague-Dawley rats (260-380 g) anesthetized with 2% isoflurane were used for this study. *In vivo* ^{31}P spectra from the rat brain were interleavely acquired without and with steady-state saturation of Pi, PCr, γ-ATP, α-ATP, and β-ATP resonance, respectively (see Figs. 1 and 2). All NMR experiments were carried out at a 9.4T/31cm horizontal magnet (Magnex Scientific, UK) interfaced with a Varian INOVA console (Varian Inc., Palo Alto, CA).

Results The ^{31}P MRS in the absence of saturating any metabolite's resonance from a rat brain was illustrated in Fig. 2a. The magnetization transfer effects were illustrated in the differenced spectra from Fig. 2b to Fig. 2f when one of Pi, PCr, γ-ATP, α-ATP, and β-ATP resonance was selectively saturated. For example, saturating resonance of γ-ATP leaded to significant magnetization reductions of Pi and PCr, meanwhile, the decreases of signal intensity of α-ATP, and β-ATP were also visible in Fig. 2d. This differenced spectrum presents a complex MT network.

Discussion and Conclusions The direct off-resonance saturation effects were undetectable in our experiments because of ideal performances of the saturation pulse train applied [1]. This is clearly evident in Fig. 2b showing that when Pi was saturated, the down field resonances of PC and PE (around 6.5ppm) had no significant changes in signal intensity. Thus, any magnetization reductions as observed in our study will be attributed to chemical exchange effects or nuclear Overhauser effects (NOEs). PCr, γ-ATP and Pi form a coupled three-spin system through the chemical exchanges of PCr↔ATP↔Pi. Therefore, saturating any one of these spins can result in magnetization reductions of the other two coupled spins. This phenomenon is demonstrated by Fig. 2b (saturating Pi), Fig. 2c (saturating PCr) and Fig. 2d (saturating γ-ATP). When saturating γ-ATP (Fig. 2d), the magnetization transfer to the β-ATP resonance may result from β-ADP through adenylate kinase (AK) (i.e. ADP+ADP⇔ATP+AMP), CK or ATPase reactions because β-ADP is saturated together with γ-ATP due to the overlapped chemical shifts of these two resonances (see Fig. 2a). However, it is more difficult to explain the transfer effect to α-ATP based on chemical exchanges. One possibility is to attribute this transfer to negative ^{31}P NOE. To identify this possibility, the β-ATP resonance was saturated (see Fig. 2f), the magnetization reductions at resonance of α-, γ-ATP and PCr were clearly visible. Because β-ATP resonance was not overlapped with any other resonances, the only possible reason of α-ATP signal intensity reduction comes from negative ^{31}P NOE upon β-ATP resonance saturation.

There are two reasons which can be used to prove that there exists negative ^{31}P NOE between γ-ATP and β-ATP. First, the magnetization reduction of PCr upon saturating β-ATP only can be achieved through the relayed chemical exchange effect from γ-ATP. Secondly, although it is possible NMR peak reduction at resonance of γ-ATP can be attributed to the reduction of resonance of β-ADP. But [ADP] in the rat brain is about 0.3 mM [3], so usually β-ADP is undetectable. Therefore, the current experiments demonstrated a complex magnetization transfer network (*i.e.* spin diffusion) through the relayed chemical exchanges and NOEs (illustrated in Fig. 1). Nevertheless, because of the relatively low concentration of ADP (compared to [ATP]=3mM, [PCr]=5mM, [Pi]=1.3mM [3]), any possible NOEs and chemical exchange effects coming from the ADP spins could be ignored. On the other hand, for saturating γ-ATP to measure chemical exchange rates of Pi↔ATP↔PCr reactions, relative to magnetization reduction ratios of PCr and Pi, the reduction ratio of α- and β-ATP was very small, which should not significantly affect the measured chemical exchange rates when spin diffusion effect was ignored.

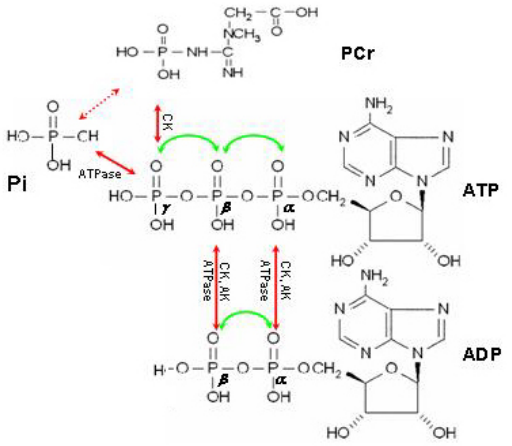

Figure 1. The molecular structures of PCr, ATP, ADP and Pi; and the possible MT pathways via NOEs (illustrated by the green lines) and chemical exchanges catalyzed by CK, ATPase and AK, respectively (illustrated by the red lines). The dashed line indicates an indirect chemical exchange.

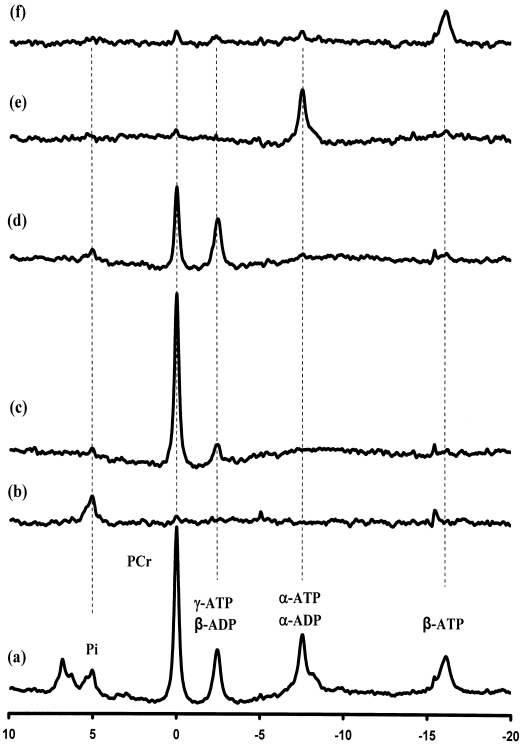

Figure 2. (a) ^{31}P control spectra acquired from a rat brain in the absence of saturation. The difference spectra between the control spectrum and the spectrum with the saturation on Pi (b), PCr (c), γ-ATP/β-ATP (d), α-ATP/α-ADP (e) and β-ATP (f), respectively. The saturation time was 7.6 s with 9 s repetition time and each spectrum was obtained by 128 scans. The glitches around -15 to -16 ppm are artifacts.

References [1]. Du F, et al. *MRM* 2007; [2]. Radda G, et al. *FEBS Lett*. 1982; [3]. Siesjo B, Brain Energy Metabolism, A Wiley-Interscience Publication, 1978.

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