An optimized protocol for measuring rate constant of creatine kinase reaction in human brain by ^{31}P NMR saturation transfer

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Introduction

The forward rate constant of the creatine kinase reaction was found to be a useful parameter for characterizing metabolic disorders in brain. It was shown on rat models that this parameter decreases in severe chronic ischemia (1) and in alcohol intoxication (2). The saturation transfer protocol used for animal experiments is not suitable for humans as the DANTE saturation pulse train, which is usually used for selective irradiation of the γ-ATP signal, causes inacceptably high specific absorption rate (SAR). Thus, for measurements on humans we replaced the DANTE sequence with a BISTRO pulse train (3,4). In our study we optimized the BISTRO sequence for a saturation transfer experiment, which served for determining forward rate constant of the creatine kinase reaction. Applicability of this technique was tested on a human subject.

Theory

The method of progressive saturation relies on measuring series of ^{31}P spectra with different saturation times of the γ-ATP resonance. As a result of chemical exchange, the phosphocreatine (PCr) signal decreases with the time constant T_{1S}. The pseudo-first-order rate constant of the reaction

$$PCr^{2-} + MgADP^{-} + H^{+} \Leftrightarrow MgATP^{2-} + Cr$$

catalyzed by creatine kinase is given as

$$k_{for} = (1- M^{\infty}/M^{0})/T_{1S}, \quad (1)$$

where $(T_{1S})^{-1} = (T_{1PCr})^{-1} + k_{for}$ and M^{∞} is the steady-state PCr magnetization after a long-term irradiation of γ-ATP. To account for possible direct saturation, the PCr magnetization measured with the saturation applied downfield, symmetrically about the PCr resonance, is used as M^{0}.

Method

Measurements were performed on a 3 Tesla Medspec DBX whole body scanner (Bruker Medical, Ettlingen, Germany). The signal was acquired from the occipital brain region of a healthy volunteer using a 10-cm ^{31}P/^{1}H double-tuned surface coil. Saturation of the γ-ATP signal was accomplished by repeating (1 to 5 times) the train of 7 adiabatic secant pulses (50 ms, bandwidth of 100 Hz) with variable amplitudes, and with the central frequency shifted 45 Hz upfield from the γ-ATP resonance. Saturation pulses were followed by 4 ms spoiling gradients, thus giving the total duration of the saturation module of 0.378 s. At the end of the saturation period, a hard excitation pulse of 200 μs was used. According to the number of repetitions of the module, the saturation time varied from 0.378 s to 1.89 s. The steady-state saturation was achieved using 5 repetitions of the saturation module with longer delays between pulses, which gave the total saturation time of 10 s. For each spectrum, 8×4 scans were accumulated in an interleaved mode, using the constant relaxation delay of 10 s.

Results

First the effect of direct saturation was examined. Fig. 1 compares signal intensities of the ^{31}P spectrum, in which saturation was applied symmetrically about the PCr resonance, with the spectrum obtained using the irradiation offset of 100 kHz. Since signal intensities of PCr and γ-ATP are almost the same in both spectra, the effect of direct saturation seems to be negligible. Fig. 2 shows a series of ^{31}P spectra acquired from a human brain. Using an exponential fit, T_{1S} = 1.57 s was calculated. In combination with the value M^{∞}/M^{0} = 0.45, Eq. (1) gave k_{for} of 0.28 s^{-1}. The maximum SAR was below 5 % of the maximum value allowed for this coil (4 W).

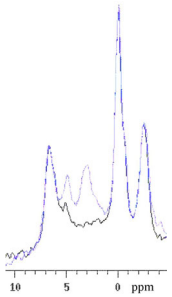

Fig. 1. Effect of direct saturation of the PCr signal by the BISTRO pulse train. The irradiation time was 1.89 s and the offset frequency was 175 Hz (black line) or 100 kHz (blue line) relative to the PCr resonance.

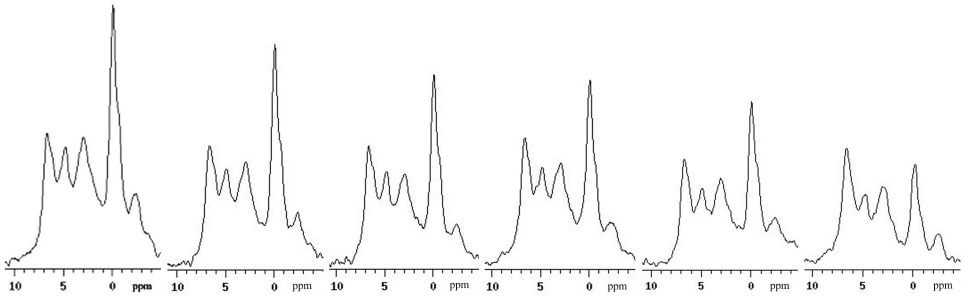

Fig. 2. A series of ^{31}P NMR spectra measured with different saturation times, left to right, 0.378 s, 0.756 s, 1.134 s, 1.512 s, 1.89 s and 10 s.

Conclusions

Our study demonstrates that the rate constant of creatine kinase in human brain can be measured by the progressive saturation method. The saturation profile of the BISTRO sequence is sharp enough to avoid direct saturation of the PCr signal when a proper frequency offset is used. Even an unintentional saturation of the inorganic phosphate resonance in the control experiment had no measurable effect on either γ-ATP or PCr signal intensities.

References

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