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Comparison of potassium and sodium binding *in vivo* and in agarose samples using TQTPPI pulse sequence

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ABSTRACT

Potassium and sodium specific binding *in vivo* were explored at 21.1 T by triple quantum (TQ) magnetic resonance (MR) signals without filtration to achieve high sensitivities and precise quantifications. The pulse sequence used time proportional phase increments (TPPI). During simultaneous phase-time increments, it provided total single quantum (SQ) and TQ MR signals in the second dimension at single and triple quantum frequencies, respectively. The detection of both TQ and SQ signals was performed at identical experimental conditions and the resulting TQ signal equals 60 ± 3% of the SQ signal when all ions experience sufficient time for binding. In a rat head *in vivo* the TQ percentage relative to SQ for potassium is 41.5 ± 3% and for sodium is 16.1 ± 1%. These percentages were compared to the matching values in an agarose tissue model with MR relaxation times similar to those of mammalian brain tissue. The sodium TQ signal in agarose samples decreased in the presence of potassium, suggesting a competitive binding of potassium relative to sodium ions for the same binding sites. The TQTPPI signals correspond to almost two times more effective binding of potassium than sodium. *In vivo*, up to ~69% of total potassium and ~27% of total sodium can be regarded as bound or experiencing an association time in the range of several milliseconds. Experimental data analyses show that more than half of the *in vivo* total sodium TQ signal could be from extracellular space, which is an important factor for quantification of intracellular MR signals.

1. Introduction

Potassium and sodium are major ions *in vivo* and their MR signals have great potential to convey valuable information about cell functioning [1–5]. The difference in concentration of potassium and sodium between intracellular and extracellular sites is an important indicator of cellular energy metabolism [6–8] and the concentration gradients give rise to neuronal membrane potentials essential for nerve activity.

In addition to *in vivo* studies of the single quantum (SQ) signals from sodium in brain and heart, the triple quantum (TQ) MR signals from sodium and potassium have come under investigation [9–18] because of their potential to reveal changes in intracellular ion content without using contrast agents. For example, after calibration, the TQ filtered (TQF) sodium MR signal can measure changes in the intracellular sodium concentration in an isolated perfused rat heart without shift reagents [19].

Sodium and potassium nuclei have spin S = 3/2, thus these ions interact with the surrounding electric field gradients of the macromolecules *in vivo* with small (<200 Hz) quadrupole interactions. The effect of such interactions on the signal cannot be observed as separate satellite MR peaks. However, there are exceptions as were detected for potassium in muscle [20], sodium in cartilage [21] and the excised bovine optic nerve [22]. The fraction of such

Abbreviations: TQ, triple quantum; SQ, single quantum; MQ, multiple quantum; TPPI, time proportional phase increment; FT, Fourier transformation; RF, radio frequency; TQ^A, TQ MR peak area; SQ^A, SQ MR peak area; A_{SQF}, SQ MR signal amplitude, fast relaxing component; A_{SQL}, SQ MR signal amplitude, slowly relaxing component; A_{SQ}, total SQ MR signal amplitude, sum of the fast and slowly relaxing components; A_{TQ}, TQ MR signal amplitude; T_{2F}, T_2 relaxation time, fast component; T_{2L}, T_2 relaxation time, slow component; t-max, position of the TQ signal maximum; ns, number of steps.

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MR signals is very small in the rat head relative to the total MR signals of sodium or potassium and was not observed in our experiments. *In vivo* macromolecules provide many electro-negative interaction sites for sodium and potassium. These macromolecules are mainly proteins, nucleic acids and carbohydrates with exposed carboxyl groups (–COOH), hydroxyl groups (–COH) and phosphate groups (PO_4^{3-}). All these groups are involved in interactions with the positively charged ions of potassium and sodium with binding times in the range of several milliseconds. These interactions result in the TQ MR signal.

A widespread and accurate use of TQ signals is currently hindered by the low sensitivity of sodium and particularly potassium NMR signals. Historically, the application of TQF pulse sequences was the primary way to detect TQ signals. TQF detection is usually performed by the pulse sequence 90 - **τ** - 90-**δ**-90 (Fig. 1) using a specially selected phase cycling. The TQF pulse sequence interval "**τ**" is usually maximized for the most prevailing time of binding from a wide range of ion binding times existing *in vivo*; a typical value for interval "**τ**" is 2–6 ms. The TQF signal *in vivo*, detected by such pulse sequences is only a small fraction of the total single quantum (SQ) signal.

A dramatic improvement in the efficiency and accuracy of the TQ signal detection can be achieved by avoiding filtration using the TQTPPI method (Fig. 1). In comparison to a typical TPPI pulse sequence [23,24], where the phase increment is 90°, the largest phase increment to detect the TQ signal is 45°. Smaller phase steps are also acceptable, for example 30°. The TQTPPI has the same efficiency for a wide range of ion bindings. The TQ MR signals are acquired concurrently with the SQ peak. For example, during the 45° phase increment a full cycle (period of 360°) is covered by 8 phase steps. Thus, after Fourier transform (FT) in the second dimension, the SQ peak can be found at $1/(8 * \Delta\tau)$, which is well separated in the frequency domain from the TQ signal at $3/(8 * \Delta\tau)$.

The TQTPPI pulse sequence for sodium was previously demonstrated in test samples [25,26] and *in vivo* [27]. The current study is devoted to the application and analysis of the TQTPPI pulse sequence for potassium and sodium in a rat head at 21.1 T. The results were compared with the corresponding data from conventional TQF experiments. Additionally the same experiments were conducted in a model system of brain tissue consisting of 7.5% agarose. This concentration of agarose gives the MR relaxation parameters close to the *in vivo* results [28]. As will be shown, the model system was able to demonstrate a stronger and competitive binding of potassium relative to sodium for the same binding places.

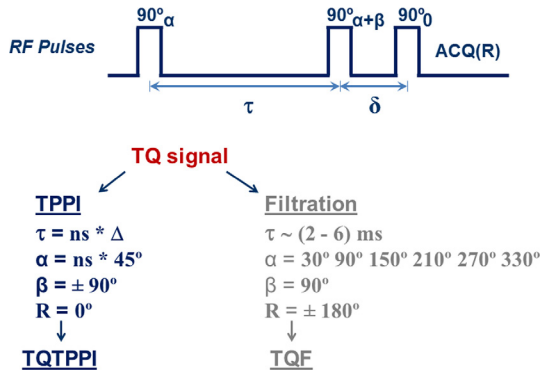

Fig. 1. Comparison of the TQTPPI and TQF pulse sequences. The TQTPPI pulse sequence has simultaneous increments of interval $\tau = ns * \Delta$ and RF phase $\alpha = ns * 45°$ (ns is a number of steps). The RF phase β has values of +90° and −90° for each value of τ and α. Both results are added together to suppress a double quantum signal. The receiver phase R remains unchanged. In the TQF pulse sequence, the interval "τ" has a fixed value in the range of 2–6 ms. The phase α changes throughout the cycle of 6 steps, while the receiver phase alternates ±180° with each RF phase step. A sum of all signals gives the TQF signal. A 180° pulse ($180_{\alpha+90°}$) at the middle of interval "τ" is not shown.

2. Methods

The experiments were performed on a 21.1 T magnet using Bruker MRI Avance III console (PV 5.1) and a 64 mm gradient coil (RR, Inc.). Volume MRI coils for potassium (41.8 MHz) and sodium (^{23}Na, 238 MHz) were approximately the same size with ID/L = 33/54 mm. A double tuned ^{23}Na/^{1}H RF coil was described previously [29]. The potassium coil was a single tuned RF coil of Alderman-Grant design [28].

A commonly used TQ filtering pulse sequence $90°_{\alpha} - \tau - 90°_{\alpha+\beta} - \delta - 90°_{0}$ was modified so that the phase "**α**" was incremented by 45° ($\alpha = ns * 45°$), at each step ns when the time delay ($\tau = ns * \Delta$) was incremented (Fig. 1). Before incrementing **τ** the phase "**β**" was alternated (±90°) and the results were added to suppress the double quantum (DQ) signal. The interval **δ** is usually selected to be the minimum allowed by the MR scanner hardware (~100 μs). The time increment Δ in the TQTPPI pulse sequence was 100–300 μs, the typical durations of the 90° pulse for sodium was 120 μs, and 140 μs for potassium. In both pulse sequences, TQTPPI and TQF, a $180°_{\alpha+90}$ pulse was used in the middle of the "**τ**" interval to compensate for the inhomogeneity of the magnetic field.

Free induction decays were acquired with "np" complex points, for potassium np = 2048, and for sodium np = 4092. The number of increments "ns" was selected in the range 128–1024. The large number of steps (>128) were selected to increase the accuracy of the spectra in the second dimension in some experiments. The spectral width was 25 kHz for potassium and sodium. The number of accumulations NA and repetition time TR were for potassium NA = 16, TR = 200 ms, for sodium NA = 1 and TR = 300 ms. The FID signals were phased and Fourier transformed (FT) in the first dimension. Then "ns" data points at the maximum of the central peaks of these spectra were selected (at the position of (np/2 + 1)). These points were used for a nonlinear fit in the time domain to derive amplitudes of the SQ and TQ signals. The same data were also FT transformed in the second dimension using the TPPI mode in which the phased real part was accompanied by zero filling for the omitted imaginary points. The area of the SQ signal and both positive and negative parts of the TQ peak areas were calculated. The SQ peak was normalized to 100%. Pre-processing of the above and other data was performed using MatNMR software v.3.9.94 [30]. All results of the time domain fit and spectral peak areas are presented as mean ± standard deviation.

The pulse sequence was first tested using model samples containing agarose gel 5% and 7.5% with added NaCl or KCl at a concentration of 154 mM. The agarose (Carl Roth, Karlsruhe, Germany) was dissolved in the above solutions and heated up to 90 °C with stirring. All model samples were placed in a plastic cylindrical container (diameter = 27 mm and length = 60 mm; volume = 25 mL). A competition between K^+ and Na^+ ions was observed when both NaCl (154 mM) and KCl (154 mM) were added to the agarose samples at the same time.

The TQTPPI potassium MRI signals were also acquired from polycrystalline potassium chloride (KCl). The results served as a reference emulating a 100% binding of potassium. In this specific case of a solid sample, a 180 degree pulse at the middle of "**τ**" interval was not used.

The TQTPPI/TQF pulse sequences were applied to detect potassium and sodium TQ signals in five male Fisher 344 rats (weight ~ 200 g). The animals were anesthetized by breathing a