Measurement of intravascular Na\(^+\) during increased CBF using \(^{23}\)Na NMR with a shift reagent

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ABSTRACT: Sodium ions are intimately involved with neural activity. Thus, it is highly desirable to devise a way of mapping brain activity via sodium imaging. Sodium ions exist in the extravascular and intravascular spaces. To separate the two components, the shift reagent Tm(DOTP)\(^5\) was intravenously introduced into rats. Intravascular sodium changes in the rat brain were measured during increased blood flow induced by hypercapnia using volume-localized \(^{23}\)Na-NMR. The intravascular sodium changes, equivalent to cerebral blood volume changes, are significant during hypercapnia conditions and correlate well with the increase in arterial pCO\(_2\). This suggests that the intravascular sodium change is dominant in total \(^{23}\)Na spectroscopy or imaging of the brain during blood flow increase induced by external perturbation. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: \(^{23}\)Na-NMR; shift reagent; brain activation; localized spectroscopy; hypercapnia; functional MRI

INTRODUCTION

Existing functional MRI methods based on the BOLD effect\(^2,3\) provide indirect information on neuronal activity using the hemodynamic response to the neuronal activation. The spatial specificity of current functional MRI methods is closely dependent on vascular structures and intrinsic coupling between neural activity and hemodynamic response. To improve spatial specificity, \(^{23}\)Na-MRI and MRS methods have been proposed, since Na\(^+\) ions are intimately involved with neuronal activity through cross-membrane currents. For example, Good-\(\text{year et al}.\) obtained \(^{23}\)Na functional imaging at 4T using a standard gradient-echo imaging technique during visual stimulation.\(^1\)

Na\(^+\) ions in the brain exist in the extravascular compartment, in which Na\(^+\) resides in the intracellular and interstitial space, and in the intravascular compartment, in which most Na\(^+\) ions are present in the blood plasma. During neuronal stimulation, sodium flux within the extravascular compartment increases due to cross-membrane currents, and cerebral blood volume will concomitantly increase, thus enhancing intravascular sodium signals. If the sodium signal change during increased neural activity occurs only at the extravascular space, sodium imaging will potentially provide high spatial specificity to neuronal activity, since the Na\(^+\) ion dynamics occur at the actual polarization/depolarization loci. However, if the intravascular signal change is significant, sodium functional imaging based on simple excitation techniques provides information mostly about cerebral blood volume (CBV) changes. In that case, the separation of neuronal-based and hemodynamic-based phenomena using \(^{23}\)Na-MRI is essential.

The most simple and dependable approach for obtaining compartment-specific information from \(^{23}\)Na-NMR involves the use of \(^{23}\)Na shift reagents, such as Dy(PPP)\(_2\)\(^{7-}\) and Dy(TTHA)\(_3\)\(^\text{-4}\), and more recently Tm(DOTP)\(^5\)\(^-5\). The mechanism through which the \(^{23}\)Na resonance is shifted has been broadly investigated and the reagents have been introduced in several in vivo \(^{23}\)Na-NMR studies.\(^6,12\) Although the use of shift reagents in vivo is made complicated primarily because of their moderate toxicity through their high affinity to Ca\(^{2+}\) and Mg\(^{2+}\) ions,\(^10\) it allows the simultaneous detection of Na\(^+\) in more than one compartment with simple detection schemes and the highest possible SNR.

In this study we intravenously administered the shift reagent HNa\(_4\)TmDOTP, allowing discrimination between the intravascular and extravascular sodium NMR...
signals in the rat brain. Then we directly measured the fractional intravascular sodium content upon increase in cerebral blood flow (CBF), caused by increase in inhaled CO₂ (hypercapnia). This provides a direct measurement of the relative cerebral blood volume (CBV). We used a modified version of the STEAM sequence, tailored for using extremely short TE and TM periods in order to obtain relatively high SNR 23Na-NMR spectra that were not contaminated by signal from surrounding muscles. Ultimately, we show the feasibility of directly measuring cerebral blood volume (CBV) changes in the rat brain in vivo, following regulation of the rat cerebral blood flow (CBF) by using the well-established hypercapnia model.

**MATERIALS AND METHODS**

**Animal preparation**

Six rats (250–300 g) were initially anesthetized with 2% isoflurane in a 3:7 O₂:N₂ mixture, and orally intubated. Then, both femoral veins and one femoral artery were catheterized with PE-50 tubing for drug administration and blood-gas sampling, respectively. End-tidal CO₂ level was controlled via a capnometer (Capnocheck, Pryon Co., WI) and body temperature was monitored through a rectal probe. The animal body temperature was kept constant using a feedback controlled water bath. Following the surgery, the anesthesia level was brought down to 1.5% and the animal was secured in a home-built head-holder with ear bars and a bite bar. A 350 mM solution of the shift reagent Na₄HTmDOTP (Macro cyclics, TX) was infused into the femoral vein using an infusion pump (Harvard Apparatus, South Natick, MA). During the first half-hour of infusion, the infusion rate was kept about 0.5 ml/h, and then increased to about 0.8 ml/h until the desired shift (~150 Hz) was achieved, typically after a total infusion time of about 2 h. This infusion rate results in a delivered dose of Na₄HTmDOTP of ~1.6 mmol/kg for a 300 g animal. Although addition of Ca²⁺ helps reduce the toxicity of the shift reagent, the Na₄HTmDOTP solution was not added with Ca²⁺ since adding Ca²⁺ reduces the observed shift, as observed by Bansal et al. The infusion rate was kept slow enough so that the physiology of the animals was not compromised.

**MR experiments**

Experiments were performed on a 9.4 T/31 cm horizontal MRI scanner (Magnex Scientific, UK) equipped with 30 G/cm gradients (11 cm I.D., 300 μs risetime, Magnex Scientific) and driven by a Unity INOVA console (Varian, CA). A home-built ¹H/²³Na double-surface coil (¹H figure-8 coil and ²³Na 1.5 cm diameter double loop coil, π/2 degree pulse at the ²³Na frequency 35 μs at 10 W and 140 μs at the ¹H frequency at the same power) was placed above the animal head. Shimming and positioning were performed using the ¹H coil. Shimming was performed using the automatic shimming procedure FASTMAP on an 11 × 8 × 8 mm³ voxel, until a typical linewidth of ~20 Hz for the water signal was achieved. Then a smaller voxel of 8 × 4 × 8 mm³ located at the top of the rat brain was selected for the acquisition of the ²³Na-NMR spectra. Localization of the ²³Na-NMR signal was achieved using a modified STEAM sequence that allows very short TE and TM periods. TE was set to 2 ms and TM was 0.9 ms.

The animal was then infused i.v. with an initial dose of 0.3 ml pancuronium bromide, and the isoflurane level was lowered to 1%. For the rest of the experiment the animal was infused i.v. with pancuronium bromide at 0.1–0.2 ml/h and the shift reagent infusion rate was regulated in real time so that the shift was kept roughly constant and the physiology of the animal was not compromised. Graded hypercapnia conditions were induced by setting the CO₂ concentration in the gas mixture at six different levels between 0 and 10%, so pCO₂ levels ranged between 25 and about 65 mmHg. At each CO₂ level, Three ²³Na NMR spectra were acquired (2048 scans, TR = 0.1 s).

**RESULTS**

Figure 1 shows a ¹H-MRI axial and coronal views of the rat brain, on which the voxel used for the localized ²³Na spectra is superimposed. Figure 2 shows three typical ²³Na NMR spectra taken before and after the shift reagent was infused. The bottom spectrum was taken before the infusion of the shift agent. As expected, only a single ²³Na NMR peak was observed, which contains extravascular and intravascular Na⁺ ions. The spectrum in the middle was taken without any localization after infusion of the shift agent. The non-shifted peak (right) is generated by the Na⁺ pools that are not in contact with the shift reagent, i.e. the extravascular (extra- and intracellular) Na⁺ in the brain and the intracellular Na⁺ in muscle tissue. The shift reagent does not cross the blood–brain barrier, hence the brain tissue ²³Na peak remains unshifted. The large shifted peak is assigned to Na⁺ present in the head muscles, where the shift reagent permeates through the vessel walls into the interstitial space. To eliminate muscle contribution to sodium NMR, localized spectrum (top) was acquired. The non-shifted peak is attributed to extravascular Na⁺ ion pool, whether the small shifted peak (approximately 10–12% of the large peak) is attributed to the intravascular-plasma Na⁺.

Since the hematocrit Na⁺ concentration, less than 10 mM, is fairly low in comparison to the Na⁺ concentration in the blood plasma, about 150 mM, the contribution of the hematocrit Na⁺ to the non-shifted Na peak is negligible.

Based on the changes in the relative area of the shifted peak and on the formula:
The changes in the intravascular volume are then calculated. In the formula above, $A_{iv}$ is the relative area under the shifted peak, $C_{iv}$, $C_{is}$ and $C_{ic}$ are the Na$^+$ concentrations at the intravascular, interstitial and intracellular spaces, and $V_{iv}$, $V_{is}$ and $V_{ic}$ are the respective volumes. It is assumed that the intracellular volume is 0.75 of the tissue volume, and that the initial Na$^+$ concentration ratio between the intracellular and extracellular space is 1:10. The increase in the extracellular and intravascular Na$^+$ concentration due to the infusion of the shift reagent solution alone, in which the Na$^+$ concentration is 1.65 M, is calculated in a similar way from the increase in the area of the non-shifted peak.

Figure 3 shows the relative intravascular volume calculated from the data acquired during the graded hypercapnia conditions, as a function of the arterial blood.
pCO2. The pCO2 was binned in 5 mmHg intervals, and the average and standard error were taken in each data bin for both the pCO2 and the relative i.v. volume values. Because of the variability in the baseline values for the relative area of the intravascular 23Na peak used in the calculation of the i.v. volume, those values were arbitrarily normalized to be 1 at normal pCO2 level (pCO2 = 30–35 mmHg).

The permeability of the vessel wall to Na+ ions does not significantly increase, or at least the exchange rate does not become comparable with the time scale of the 23Na-NMR, so no significant changes in the extravascular 23Na-NMR lineshape and shift were detected. The intravascular CBV monotonically increases with arterial pCO2. This result is consistent with previous observation of the relation between CBV and arterial pCO2.\(^{16}\)

**DISCUSSION AND CONCLUSIONS**

We have shown that the intravascular sodium signal, which is indicative of regional CBV, monotonously increases with the blood pCO2. Hence it can be inferred that, during increased CBF induced by external perturbations, including neural stimulation, the intravascular sodium signal is significantly modulated. Thus, sodium spectroscopy and/or imaging of total sodium ions in the brain contain information about hemodynamic changes. This finding makes a strong case for a 23Na-NMR method that yields compartment-specific information about Na\(^+\) ions, and in particular, the intra- and extracellular Na\(^+\) pools.

We used a shift agent to separate different compartments. Similar approaches were used by other investigators. Lee et al.\(^{9}\) and Bansal et al.\(^{10}\) showed that the shift reagent Na\(_4\)HTmDOTP, which does not cross the blood–brain barrier, can be intravenously infused into a live rat, thus allowing the use of 23Na-NMR to discriminate between the intravascular and extravascular Na\(^+\) ions pools in the rat brain. Alternatively, Eleff et al. have shown that the shift reagent Dyt(TTHA)\(^3\)– can be delivered to the parenchima of a dog brain through temporarily breaking the blood–brain barrier.\(^{11}\) In that case, the shifted peak is that of the extracellular Na\(^+\), giving rise to the possibility of measuring the dynamics between intra- and extracellular Na\(^+\) ions during various activation conditions. In that case, the change in cross-membrane ion currents following neuronal activation will be expressed in changes in the lineshape or shift of either of the 23Na-NMR resonances. Similarly, direct infusion of the shift reagent into the extracellular space allows separation between the intracellular and extracellular Na\(^+\) pools, and measure compartment-specific Na\(^+\) dynamics during brain activation.

One approach to distinguishing between the intracellular and extracellular sodium signals is to utilize 23Na triple quantum filtered (TQF) NMR. Triple quantum coherence (TQC) in 23Na-NMR can be generated when the motional correlation time of the Na\(^+\) ions falls much below the extreme narrowing limit (\(\omega_Q T_2 \ll 1\)), thus only slow-tumbling Na\(^+\) ions, i.e. those bound to macromolecules, can generate TQC that would pass the filter.\(^{17,18}\) In earlier works it has been shown that because the concentration of macromolecules in the intracellular space is significantly larger than that in the extracellular space, the triple-quantum-filtered 23Na signal is primarily originated by intracellular Na\(^+\) ions, and that extracellular 23Na-NMR signal is practically eliminated.\(^{19}\) TQF 23Na MR imaging has already been applied on animal\(^{20}\) and human\(^{21}\) models. TQF-23Na-NMR, possibly with diffusion enhancement, would be the method of choice for human studies, although the low SNR might dictate prohibitively long experiments. In addition, contamination of the TQC with single quantum coherence and small TQC signal generated by i.v. and extracellular Na may considerably complicate the analysis of TQF data.

Another approach to analyzing 23Na-NMR data in living tissue is that of relaxation analysis, and in particular T\(_2\) analysis. It is known that fast tumbling Na\(^+\) ions give rise to a monoexponential decay of the transverse magnetization, whether slow tumbling Na\(^+\) ions give rise to a biexponential relaxation with two distinct relaxation times, commonly denoted as T\(_2\)s and T\(_2\)f, with a population ratio of 2:3 accordingly.\(^{20}\) Analysis of the relaxation behavior yields information about the nature of the bonding of Na\(^+\) ions to macromolecules and the exchange process between ‘free’ and ‘bound’ Na\(^+\) ions.\(^{23–25}\) This particular approach is not very suitable for investigating neural activity for several reasons: it is substantially time consuming—the collection of data from a reasonably small voxel for relaxation analysis is prohibitively long. Then, it is unlikely that the possible changes in exchange rate between intra- and extracellular Na\(^+\) following neuronal activation would have a significant impact on the exchange process between free and bound Na\(^+\) in the intracellular space. In addition to these, analysis of multieponential behavior in a heterogeneous system is not accurate enough to yield numbers that would be statistically significant in the case where such differences are expected to be very small.

There are several problems associated with 23Na-NMR of living tissue. One is the limited SNR inherent to 23Na-NMR, which is a combined result of low concentration (particularly in the intracellular space), broad lines and a gyromagnetic ratio that is considerably lower than that of protons. This implies that temporally resolved 23Na-NMR studies of neural activation are practically not feasible. The other significant problem is the short relaxation times of 23Na, which not only result in low SNR, but also limit the range of the dynamic effects that can be investigated using 23Na-NMR. In particular, very slow chemical exchange processes that occur between two Na\(^+\) ion pools may become very hard to detect. An additional and very serious problem is the moderate...
toxicity of Tm(DOTP)5−, which in spite of being very efficient in terms of shift/mol, tends to compromise the physiology of the animal. In particular, the administration of the Tm(DOTP)5− into the interstitial space may result in seizure-like effects, due to sequestration of Ca2+ and Mg2+ ions. This might dictate in future studies an administration of smaller doses, resulting in smaller shifts, some compensation of Ca2+ ions, or using a less toxic, albeit less efficient shift reagent, such as Dy(TTHA)3 which appears to be less toxic.

In conclusion, it has been shown that increase in CBV is a major source of signal increase in 23Na-NMR of the brain and muscle by nuclear magnetic resonance spectroscopy with magnetic fields. 23Na-NMR will be useful to measure these changes. 23Na-NMR will be useful to provide information on cross-membrane ion currents, when the shift reagent is in contact with the Na+ ions in the interstitial space, rather than with the intravascular Na+ ions.

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REFERENCES


