High Resolution Sodium Imaging of Isolated Neurons

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Introduction

In recent years, Magnetic Resonance Microscopy (MRM) has been applied to the high resolution imaging and localized spectroscopy of isolated cells (1,2). Providing resolutions below 40 μm, these efforts have demonstrated the diverse intracellular environments that can be probed by proton (^{1}H) MRM to provide insight into the compartmental diffusion and relaxation of subcellular water as well as the *in situ* identification of intracellular metabolites. These findings potentially impact the quantitative interpretation of MR signal changes (e.g. the redistribution of intra- and extracellular water and metabolites in response to pathology) in a clinical setting. MRM and localized MR spectroscopy in isolated single neurons suggest that there is an unexpected heterogeneity in the distribution of the organic osmolyte betaine in the L7 neuron found in the abdominal motor ganglion of the sea hare, *aplysia Californica* (3). The cytoplasm of the isolated L7 neuron displays a high concentration of betaine while the nucleus shows no evidence of the osmolyte. This finding is surprising given the porosity of the nuclear envelope and the relative inactivity of betaine in metabolism.

In this study, the intracellular distribution of the inorganic osmolyte sodium is assessed by MRM through the acquisition of three-dimensional (3D) microimages by direct observation of ^{23}Na. These efforts are made possible through (a) the use of a specially constructed, double-tuned Radio Frequency (RF) microcoil and (b) the application of a unique, ultra-widebore 21.1-T magnet. The link between the intracellular distributions of sodium and other osmolytes in the single neuron may shed light on the osmoregulatory processes in response to a wide range of insults or perturbations.

Methods

Isolated Single Neurons: The L7 neuron from the abdominal ganglion of late juvenile *Aplysia* (75-125 g) was isolated as prescribed previously [1,2]. Each neuron was examined individually in a buffer of artificial seawater (ASW). In the absence of perfusion, analysis was performed within 7 hours of dissection to ensure membrane viability.

RF Microcoil and Magnet system: To acquire sodium and proton images from the same neuron preparation, a susceptibility-matched RF microcoil was constructed to resonant at both ^{1}H (900 MHz) and ^{23}Na (2338 MHz) frequencies simultaneously (Figure 1). Double resonance in this microcoil was achieve through the use of one lumped element trap on each input. This coil was constructed on a fused silica capillary having an outer/inner diameter of 850/700 μm. Isolated neurons were placed within sealed glass capillaries having an outer/inner diameter of 700/530 μm.

MRI: All MR data were acquired using a 105-mm, 21.1-T vertical magnet constructed at the National High Magnetic Field Laboratory and equipped with a Bruker Avance Console and Micro 2.5 gradient system. To provide high resolution anatomical templates, high resolution ^{1}H images were obtained using 3D gradient-recall echo (GRE) sequence (TE/TR = 5/100 ms) at resolutions of 40x40x100, 40x40x40 and 15x15x15 μm over 4.25, 6 and 24 min acquisition times. Using the same RF microcoil and isolated neuron, high resolution ^{23}Na images were acquired using a 3D GRE sequence (TE/TR=2.5/100 ms) at resolutions of 40x40x100 & 40x40x40 μm over 2.5 and 7.5 hrs, respectively. Additionally, multiple ^{23}Na 3D GRE datasets were acquired in 2.5 hrs at 40x40x100-μm resolutions at TE values ranging form 2.5-15 ms to assess intracellular ^{23}Na T2 relaxation.

Results and Discussion

Figure 2 shows the proton and sodium images acquired from a single neuron. In the 1H image, the hyperintense region of the within the cell represents the nucleus. A similar hyperintensity corresponding to the nucleus is also evident in the sodium image, indicating a higher 23Na signal in this important organelle. Additionally, 23Na images (Figure 3) acquired with incremented TE display a T2 relaxation that is high in the nucleus but substantially lower in the cytoplasm, which corresponds well with previous reports of 1H T2 relaxation.

This data suggests that sodium in the nucleus experiences a significantly different environment than sodium in the cytoplasm. Efforts are underway to quantify the actual concentration of sodium in these intracellular compartments in reference to the 460-mM concentration of sodium in the external ASW. Interestingly, the apparent distribution of sodium seem to counterbalance the apparent concentration of the organic osmolyte betaine.

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Figure 1. Double-tuned 1H/23Na RF microcoil

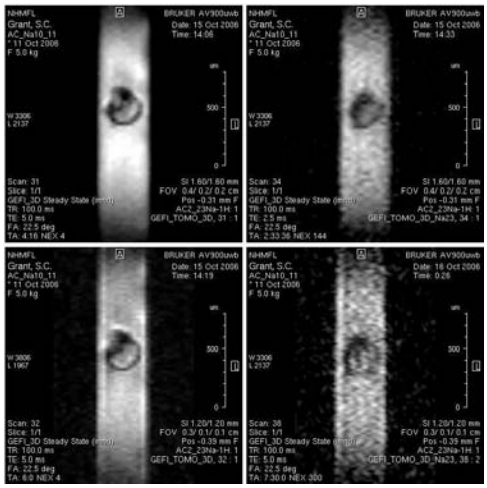

Figure 2. MRM of an isolated single neuron
Left column: 1H images; Right column: 23Na images
Top row: 40x40x100 μm; Bottom row: 40-μm isotropic

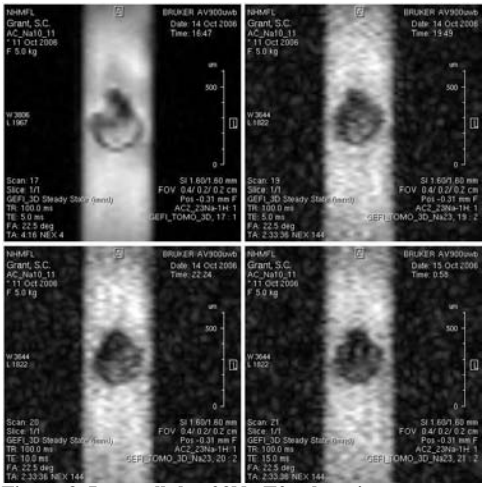

Figure 3. Intracellular 23Na T2 relaxation
Upper top-left: 1H image
Clockwise from top-right: 23Na with TE=5,10&15ms