^{23}Na MRI Detection of Cellular Responses to Osmotic Perturbation and Cell Viability

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Introduction

Cells actively maintain ion distributions that differ between the intracellular and extracellular environment and are critical for cell volume regulation and physiological responses. Therefore, disruptions in ion distributions, especially sodium (^{23}Na), result in cellular damage and eventual loss of cell viability as is seen in pathologies such as stroke, neurodegeneration and cancer. ^{23}Na magnetic resonance imaging (MRI) has shown that increases in ^{23}Na signal intensity correlate with neurodegeneration in Alzheimer's disease (1) as well as the onset time in stroke (2). However, the contributing mechanisms underlying these changes, including an increase in intracellular sodium, changes in ^{23}Na MR relaxation and/or loss of cell viability are not well understood. In this study, ^{23}Na MRI of an entire neural ganglion from *aplysia Californica* was used to characterize cellular responses to osmotic perturbation as well as cell viability over extended time periods.

Experimental

Abdominal ganglia from the sea slug aplysia Californica were dissected from the living animal without chemical digestion. A single ganglion was washed with isotonic artificial sea water (ASW; in mM: 460 mM NaCl, 10.4 KCl, 55 $MgCl_2$, 11 $CaCl_2$, and 15 HEPES) and loaded into a 2.5-mm o.d. glass capillary containing isotonic, hypertonic or hypotonic ASW. Hypotonic and hypertonic perturbations were introduced by changing the isotonic NaCl concentration (460 mM) to 345 mM and 545 mM, respectively. MR imaging was performed at 11.75 T using a homebuilt, double-tuned ^{1}H/^{23}Na solenoidal coil with a diameter of 3 mm immersed in a nonprotonated susceptibility matching fluid to reduce magnetic field distortions. After ^{1}H scout images to position the ganglia, quantitative ^{23}Na MR images were acquired at 20 C using a 3D gradient recalled echo (GRE) sequence at a resolution of 89x89x400 µm. A region of interest (ROI) was drawn along the contour of the ganglion as well as in the ASW medium, and the mean signal from the ROIs was quantified. A total of 33 images were acquired over a 48-h imaging period to assess changes in ^{23}Na signal intensity at extended times.

Results

The ganglionic sac appeared to remain intact under osmotic perturbation; however, loss of cell viability, evident through an increase in ganglia volume and increased ^{23}Na signal intensity, began to occur after 10 hours. No apparent changes were seen in the ASW medium indicating that the increasing signal in the ganglia is due to physiological changes. Further, ganglia in extracellular environments of decreased osmolarity responded with a more rapid change in signal intensity.

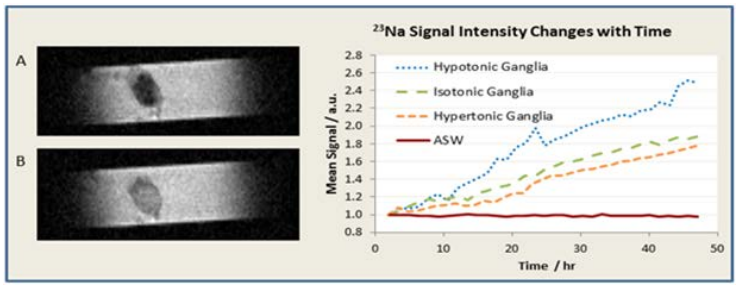

Figure 1. Left: ^{23}Na 3D GRE MR Images (TE/TR: 2.7/20 ms) of an abdominal ganglion in isotonic ASW 2 hours post dissection (A) and 48 hours post dissection (B). Right: Graph of 23Na signal intensity as a function of time after dissection illustrating the rise in ganglionic ^{23}Na signal intensity (ASW control was unchanged). ^{23}Na signal intensities from the ROIs were normalized to the initial time point.

Conclusions

^{23}Na MRI was able to visualize isolated neural ganglia and increases in signal intensity were evident with loss of viability. Osmotic perturbations resulted in different cellular responses. Further analysis is underway to quantify changes in ^{23}Na T_1 and T_2 relaxation to determine if the signal intensity increases are due to differing relaxation properties or increases in the ganglionic sodium concentration.

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References

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