Cellular Responses to Tonicity: A High Field ^{1}H and ^{23}Na MR Microscopy Study

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Introduction MRI relaxation and diffusion properties are sensitive to the physiological state of cells and tissues. In particular, cerebral ischemia is associated with a reduced apparent diffusion coefficient (ADC) and increased T_2 relaxation parameter in ^{1}H MRI (1) making it useful for clinical diagnosis. Further, increases in sodium signal intensity in ^{23}Na MRI correlate with several neurodegenerative diseases (2-3) as well as ischemia (4), which can be used to identify stroke and predict onset time (5). However, the contributing mechanisms underlying these changes, including possible increases in intracellular sodium and/or lengthened ^{23}Na T_2 relaxation, are not well understood. It has been hypothesized that alterations in cell regulatory mechanisms result in cell swelling, which disrupts tissue microstructure and ionic distributions. Osmotic perturbations have been used on single neurons and neural tissue models to mimic these cell volume changes. Evaluated with ^{1}H MRI, previous tissue models include the isolated turtle cerebellum (6) as well as human (7) and rat (8) hippocampal slices. Generally, hypotonic perturbations mimic tissue ischemia and result in an increase in ^{1}H signal intensity, a decrease in ^{1}H ADC, and an increase in ^{1}H T_2, while all trends are reversed for hypertonic perturbation. Single cell work has focused on the large L7 neuron from the abdominal ganglia in the sea hare *Aplysia californica*, which has been shown to have distinct nuclear and cytoplasmic compartments with differing relaxation and diffusion properties (9,10). Therefore, it would be useful to develop a tissue model comprised of large cells in which the intracellular contribution to the volume averaged signal could be determined. In this study, abdominal *Aplysia* ganglia were used due to its simple anatomy with a small collection of relatively large neurons up to 300 μm in diameter. By examining the cellular response in the context of a tissue environment, much can be observed concerning cell volume regulation and the influence of changing ionic concentration distributions, notably sodium, on cell swelling in disease states.

Methods The abdominal ganglia from *Aplysia* were dissected from the living animal. The ganglia were washed with isotonic artificial sea water (ASW; in mM: 460 NaCl, 10.4 KCl, 55 $MgCl_2$, 11 $CaCl_2$, 15 HEPES) and loaded into a 2.5-mm o.d. capillary containing isotonic, hypertonic or hypotonic ASW. Hypertonic and hypotonic perturbations were introduced by changing the isotonic sodium chloride concentration (460 mM) to 545 mM and 345 mM, respectively. All MR imaging was performed at 11.75 T utilizing a homebuilt, double-tuned ^{1}H/^{23}Na solenoidal coil having a diameter of 3 mm immersed in a non-protonated susceptibility matching fluid to reduce magnetic field distortions (11). Three ganglia dissected from different *Aplysia* were imaged simultaneously, and each run was repeated three times to provide a total sample size of 21 ganglia. Imaging was performed in separate studies to quantify the proton T_1, T_2, T_2* and ADC immediately following dissection on viable ganglia as well as sodium T_1 and T_2* after the loss of cell viability. Quantitative ^{1}H images were acquired using multi-slice spin echo (SE) and gradient recalled echo (GRE) sequences with an in-plane resolution of 40x40 μm and slice thickness of 100 μm. Quantitative ^{23}Na images were acquired using only 3D GRE sequences at a resolution of 89x89x400 μm. Proton and sodium datasets were acquired from the same ganglia so that coordinated regions of interest (ROIs) could be drawn to quantify relaxation and ADC from approximately identical volumes across individual ganglia. Relaxation and ADC values were generated from ROI signal means fitted using a non-linear regression algorithm applied to a single exponential function.

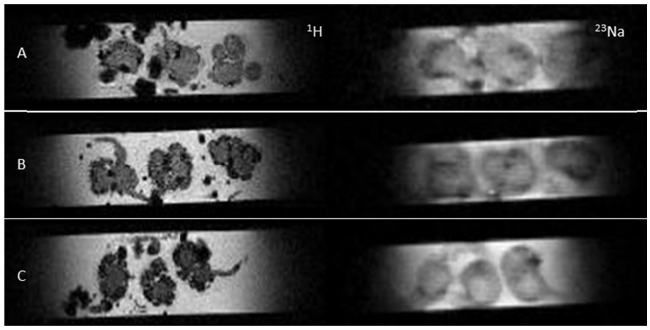

Figure 1. MR images of three neural ganglia in A) hypotonic, B) isotonic or C) hypertonic ASW by row. Left column: ^{1}H GRE (TE/TR: 18/5000 ms); Right column: ^{23}Na 3D GRE (TE/TR: 3.5/250 ms).

Results With tonicity changes (Fig 1), the ganglionic sac remains intact although alterations in the signal intensity (particularly with respect to ^{23}Na) are evident. Proton T_1 relaxation (Table 1) did not change significantly with osmolarity; however, ^{1}H T_2 and T_2* relaxation both decreased with increasing tonicity. All relaxation parameters increased after cell death and loss of viability. No significant changes in the ADC values were observed. Sodium measurements revealed a decrease in T_1 with hypertonicity but no apparent change in T_2* with tonicity changes.

Table 1. Proton and sodium relaxation parameters with altered ASW tonicities.

ASW Tonicity (NaCl conc. in mM)	^{1}H T_1 / ms	^{1}H T_2 / ms	^{1}H T_2* / ms	^{1}H ADC / μm^2/ms	^{23}Na T_1 / ms	^{23}Na T_2* / ms
Hypotonic (345)	1917.4 ± 77.4	22.98 ± 1.22	17.00 ± 1.08	1.08 ± 0.17	49.27 ± 1.57	10.64 ± 3.21
Isotonic (460)	1889.8 ± 671.3	19.50 ± 0.95	12.33 ± 1.04	1.20 ± 0.38	44.12 ± 8.24	9.91 ± 2.73
Hypertonic (545)	1901.7 ± 295.7	17.37 ± 2.53	9.50 ± 1.11	1.15 ± 0.55	42.78 ± 5.28	11.44 ± 1.27

Discussion The trends observed for ^{1}H relaxation follow those seen previously in osmotically perturbed *Aplyisa* neurons (9) and neural tissues (6-8) with an increase in T_2 with reduced tonicity, possibly due to an increased intracellular volume fraction. The lack of a clear trend in ADC values may be due significant volume averaging between the nuclear, cytoplasmic and interstitial compartments of the ganglia, which may react differently to tonicity changes and should be better modeled with a multi-compartmental approach. Changes in sodium relaxation are evident in compromised ganglia but significant changes are seen only between the hypo- and hypertonic conditions. Ongoing work with perfused ganglia will allow for perturbation studies to be performed as a means of quantifying the sensitivity of ^{23}Na techniques to transient ionic redistribution during ischemia.

Acknowledgements & References MR data was collected at the FAMU-FSU College of Engineering, The Florida State University. Funding provided by the American Heart Association (SE Division) and FSU Department of Chemical & Biomedical Engineering. **(1)** van Dorsten, F., et al. (2002). *Magn Reson Med*. **(2)** Inglese, M., et al. (2010). *Brain*. **(3)** Mellon, E., et al. (2009). *Amer J Neurorad*. **(4)** Lin, S., et al. (2001). *Stroke*. **(5)** Petkova, M., et al. (2010). *Radiology*. **(6)** O'Shea, J., et al. (2000). *Magn Reson Med*. **(7)** Shepard, T., et al. (2003). *Magn Reson Med*. **(8)** Blackband, S., et al. (1997). *Magn Reson Med*. **(9)** Hsu,E., et al. (1996). *Amer J Physiol-Cell Physiol*. **(10)** Grant, S.C. et al. (2001). *Magn Reson Med*. **(11)** Webb, A.G. & Grant S.C. (1996) *J Magn Reson Ser B*.