Intracellular Diffusion in Normal and Ischemic Rat Tissues *via* ^{133}Cs MR at 12 T

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Introduction: The apparent diffusion coefficient (ADC) of CNS tissue water *in vivo* is remarkably sensitive to injury. Water ADC decreases by 30-50 % within minutes of an ischemic event. The biophysical mechanisms responsible for this phenomenon remain poorly understood, and compartment-specific diffusion information is an important step in unraveling those mechanisms. Since evaluation of compartment-specific water diffusion is complicated by compartmental (intracellular/extracellular) exchange, we have selected the $^{133}Cs^+$ ion to infer the motion of intracellular water indirectly. ^{133}Cs is ~100% naturally abundant and is an MR-active physiologic analog of potassium. It is taken up by cells *via* potassium channels and K^+/Na^+ATPase . It is concentrated in the intracellular space and resides at high enough concentrations that it is observable *via* MR. Further, the vast majority of the $^{133}Cs^+$ ions are "unbound," so that its ADC reflects that of intracellular water ((1) and references therein). In this study ^{133}Cs MR has been used to compare the intracellular kinetic environment of brain to that of muscle under healthy and ischemic conditions.

Materials and Methods: Thirteen 250 – 350 g, male Sprague-Dawley rats underwent dietary loading of $^{133}Cs^+$ (2) for a minimum of one week prior to being scanned. Experiments were performed on an 11.74-T, horizontal bore scanner (Magnex Scientific/Varian Instruments). The experimental setup for animals undergoing brain study (n=10) has been described previously (3). Animals for skeletal muscle study (n=3) were positioned on their sides, with one leg secured to a level platform. A 1.0-cm by 1.4-cm ellipsoidal 2-turn ^{133}Cs surface coil (65.5 MHz) was positioned on the thigh, above the gastrocnemius, soleus and biceps femoris muscles. The leg and platform was then inserted into a 4-cm ^{1}H birdcage coil (Stark Contrast, Erlangen, Germany) for imaging and voxel planning. Coupling between the two coils was minimal because of the large frequency difference. ^{133}Cs diffusion measurements were made with a LASER sequence. To minimize echo time, localizing slice-selective pulses were applied along a single direction, selecting a slab parallel to the plane of the RF coil (Figure 1). The MR signal obtained under this circumstance includes contributions from both brain a temporalis muscle. The volume of muscle included in the voxel is small, but the fractional contribution to the total signal is quite large due to a 5-fold higher ^{133}Cs concentration in muscle tissue as compared with brain (4). ^{133}Cs ADC in brain/temporalis was estimated using six *b*-values ranging from 0.001 to 2.951 ms/μm^2 (tr=3.8 s, te=44 ms, δ=10 ms, Δ=22 ms). The high concentration of ^{133}Cs in thigh compared to brain enabled acquisition of ten *b*-values over the same range. Global ischemia was induced within the magnet *via* cardiac arrest induced by a 2M KCl bolus delivered through a tail vein catheter. Ten minutes following arrest, the diffusion measurement was performed again, using an array of six (or ten) *b*-values sufficient to induce more than a one *e*-fold reduction in diffusion attenuated signal (tr=3.8 s, te=48 ms, δ=12 ms, Δ=24 ms). Following death, the tissues were kept warm by blowing warm air over the body and head and by circulating warm water underneath the animal. ^{133}Cs diffusion measurements in thigh were performed again at 30 and 60 minutes after induction of ischemia (Figure 2).

Results: The chemical shift difference between muscle and brain allow separate modeling and analysis of both populations from a single spectrum (Figure 1a). ^{133}Cs in the muscles of the thigh gives a single resonance (Figure 1b). The measured ^{133}Cs ADCs in healthy and ischemic brain, temporalis muscle and thigh muscle are presented in Table 1. The diffusion signal attenuation data for all peaks were modeled with Bayesian probability theory, using a single exponential function. Temporalis muscle and muscles of the thigh display a similar diffusion response to ischemia, decreasing negligibly. ^{133}Cs ADC in brain, however, plummets drastically, decreasing by 74 %. Diffusion measurements in thigh were continued for one hour after induction of ischemia, and no precipitous ADC decrease was noted. This is in agreement with recently published water diffusion data from hindlimb skeletal muscle (5).

Discussion: Intracellular ^{133}Cs concentration in brain and muscle, when established through long-term dietary administration, has been estimated to be nine and forty times higher than extracellular concentration in brain and muscle, respectively (4). It has also been shown to leak slowly from injured tissue (4), so, to the extent that it is solvated, ^{133}Cs should reflect the motion of water in the intracellular compartment both before and after injury. Thus, there are two interesting items to note. First, because water in brain is roughly 80 % intracellular, these results imply that the widely-observed 30-50% decrease in brain water ADC following ischemia may be accounted for by an even larger ADC decrease in the intracellular fraction of brain tissue water. Second, the biophysical determinants within the intracellular space of brain that result from ischemic challenge seem to be distinct in magnitude and/or temporal response from those in the intracellular space of muscle. This underscores the great complexity of CNS tissue.

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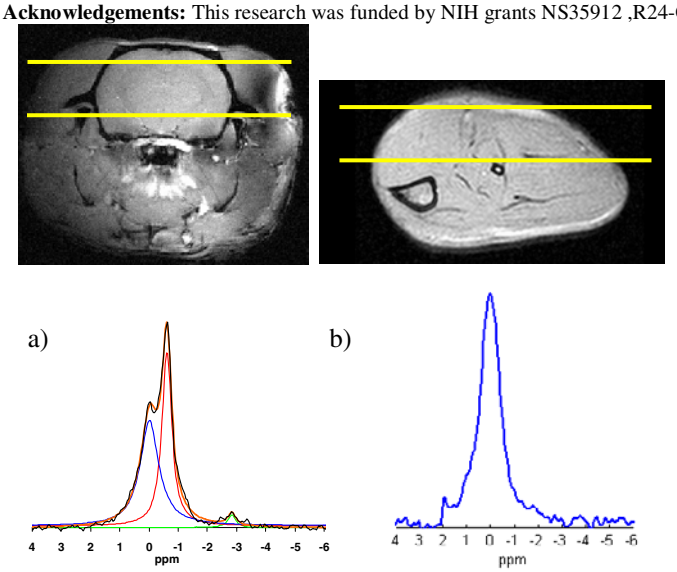

Figure 1. $^{133}Cs^+$ spectra of a) brain with strong contributions from temporalis muscle, and b) gastrocnemius, soleus and biceps femoris muscles. Spectra were obtained *in vivo*, and were acquired from regions approximated by the yellow lines above each spectrum. For a) 512 transients, total acquisition time=21 minutes; for b) nt=16, total acquisition time =~40 s. Signal from brain is modeled in red in a). Muscle signal is shown in blue in both spectra. Note that only a single resonance is present in b) (The small peak at 2 ppm is a dc offset artifact).

		brain	Temporalis m.	thigh m.
Normal:	ADC	0.93	1.00	0.72
	Av unc	0.18	0.13	0.03
	Std dev	0.37	0.15	0.08
Ischemic:	ADC	0.24	0.99	0.68
	Av unc	0.04	0.28	0.03
	Std dev	0.12	0.15	0.07
%	ADC decrease	74	1	6

Table 1. Single gradient-direction ADCs (μm^2/ms) for the $^{133}Cs^+$ resonances in brain, temporalis muscle, and thigh muscle. The average uncertainty (Av unc) in each estimate is given, as is the inter-animal standard deviation for each measurement.

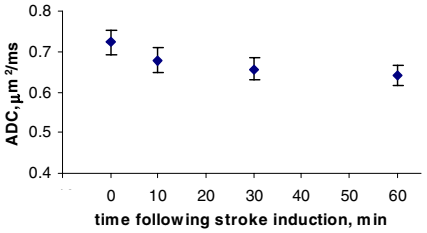

Figure 2. $^{133}Cs^+$ ADC in rat thigh muscle *vs.* time following ischemia (n=3). The point at time=0 represents the measurement in normal tissue.

References:

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