

Evaluation of Intracellular Diffusion in Normal and Globally-Ischemic Rat Brain via ^{133}Cs NMR

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The question of whether the apparent diffusion coefficient (ADC) of intracellular water changes after brain injury was addressed by using ^{133}Cs as an indicator to report on the state of the intracellular environment. Cesium is an NMR-detectable potassium analog that accumulates in the intracellular space and is detectable in rat brain after being added to the animal's diet. The ADC of cesium was measured before and after the death of the rat. The cesium ADC fell from $0.91 \pm 0.05 \times 10^{-3} \text{ mm}^2/\text{s}$ (mean \pm SEM, $n = 5$) in the alive rat to $0.71 \pm 0.05 \times 10^{-3} \text{ mm}^2/\text{s}$ within 20 min (the best time resolution of the experiment) of the death of the animal and stayed at this value for at least 3 h ($P < 0.001$). Assuming that the ADC of cesium reflects motion in the intracellular environment, these results support the idea that there are changes associated with cell injury that would cause a reduction in the ADC of intracellular water. Hence, one factor contributing to the decrease in water ADC after brain injury is a change in the ADC of intracellular water.

Key words: diffusion; cesium; stroke.

INTRODUCTION

It is widely recognized that the apparent diffusion coefficient (ADC) of water in central nervous system tissue changes rapidly after injury, particularly with stroke (1, 2), seizure (3–5), excitotoxic injury (6, 7), spinal cord trauma (8), and spreading depression (9); yet the precise mechanism(s) for these changes is not well understood. The ADC is referred to as an "apparent" diffusion coefficient because the water motion it describes is a complex combination of Brownian motion, possibly cell streaming, and/or movement of molecules across cell membranes. It is also generally accepted that the ADC of water in the intracellular space is smaller ("slower") than that of water in the extracellular space (1–3, 6). Under most conditions, the ADC then reflects a weighted average of the ADCs of water in the intracellular and extracellular spaces (vide infra). Based on these assumptions, several possible explanations for the rapid change in ADC after

brain injury have been put forth. They include: (i) movement of water from the extracellular space to the intracellular space in association with cell injury and cytotoxic edema (1–3, 6), (ii) reduction in the movement of water molecules across cell membranes after cell injury (10), (iii) reduction in the ADC of extracellular water because of increased restriction of diffusion in the extracellular space associated with cytotoxic edema (11, 12), and (iv) reduction of the intracellular ADC (3) due to either a loss of intracytoplasmic macromolecular motion or an increase in the viscosity of the intracellular milieu. At present, most authors feel that the first explanation given above is the correct one (1–3, 6). The second explanation has been largely discounted because of evidence that the movement of water molecules across membranes is of insufficient magnitude to explain the observed changes in ADC (3, 11, 13). Some data supporting the third explanation are derived from diffusion studies done at extremely short diffusion times (12), and this explanation has yet to gain widespread acceptance. To our knowledge, the study described herein is one of the first designed to directly address the fourth possibility. Herein, we describe experiments in which we used ^{133}Cs as an NMR-detectable indicator to report on the intracellular environment and address the question of whether the ADC of intracellular water changes with cell injury in rat brain.

Cesium was chosen for this study because it is an NMR-detectable potassium analog that accumulates in the intracellular space (14, 15). Data from our laboratory suggest that after long-term administration of cesium to rats, there is roughly 40 times more cesium present in the intracellular space than in the extracellular space of brain (16). In addition, and in contrast to potassium, cesium leaks rather slowly from cells after their death (16 and unpublished data, vide infra). These properties allow us to detect a signal that arises mainly from intracellular cesium, both while the animal is alive, and also for a short time after death.

It is likely that for a given compartment (e.g., the intracellular space), the motion of cesium is related to that of water. This is because in aqueous solution, cesium exists as aquated Cs^+ ions (each with a first hydration shell of six water molecules) that rarely encounter other cesium ions in solution. As a result, cesium ions interact not with other cesium ions, but mainly with water molecules and, to a certain extent, anions such as Cl^- . Consequently, the movement of cesium is linked to the movement of the water in which it is contained. (Formally, the diffusion coefficient of aquated cesium is inversely related to the viscosity of the media in which it is contained.) The diffusion coefficient of cesium in aqueous solution is similar to the self-diffusion coefficient of water [$1.9 \times 10^{-3} \text{ mm}^2/\text{s}$ for ^{133}Cs in 2 M CsCl (17) versus

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2.1×10^{-3} mm²/s for water (18), both at 25°C] and, as will be seen below, the ADCs for cesium in rat brain are quite close to those for water. While it is clear that the diffusion of cesium in aqueous solution is not exactly the same as the self-diffusion of water, it is likely that changes in the ADC of intracellular cesium reflect similar changes in the ADC of intracellular water as both are related to the motion of the intracellular milieu. In the study described below, we evaluate the ADC for cesium in rat brain before and after brain injury (death of the animal) in an effort to obtain information regarding changes in the intracellular environment.

MATERIALS AND METHODS

Animal Preparation

Five female Sprague-Dawley rats weighing between 80 and 100 g were fed a low-potassium diet (Purina Mills, Inc., Richmond, IN) and drinking water containing 20 mM KCl plus 20 mM CsCl for 10 to 20 days before the NMR experiment (15). Rats weighed 150 to 200 g at the time of the NMR experiment. The animals were anesthetized with 2.0 to 2.5% (v/v) halothane in 100% O₂. A catheter was placed in the femoral vein. The trachea was cannulated and the animal was mechanically ventilated using a Harvard Apparatus model 680 rodent respirator. The animal's body temperature was monitored via rectal probe (Yellow Springs International, Yellow Springs, OH), and body temperature was maintained near 37°C with a feedback circuit and pad containing circulating warm water. A small hole was drilled in the skull and a fiberoptic temperature probe (Luxtron Corp., Santa Clara, CA) was inserted into the anterior portion of the frontal lobe of the brain to monitor "brain temperature." This was maintained near 37°C after the death of the animal by either blowing warm, moist air over the animal's head or circulating warm water through plastic tubing wrapped around the animal's head.

NMR Spectroscopy

The animal's head was placed in an NMR transmitter/receiver set consisting of a Helmholtz transmitter and surface coil receiver. Both coils could be tuned to the ¹H resonance frequency (200 MHz) for shimming as well as the ¹³³Cs resonance frequency (26.2 MHz) for detection of the cesium signal. The receiver coil consisted of a 14-mm diameter, two-turn surface coil positioned over the animal's head to maximize the signal from the brain. A second, antisense coil was positioned equidistant from the transmitter coil and was electrically connected to the receiver (sense) coil so as to provide optimum radio frequency isolation between transmitter and receiver coils through induction of offsetting voltages in the sense and antisense coils during operation of the transmitter coil (19). The magnetic field gradient set used consisted of a laboratory-built Maxwell pair oriented along the z axis of the magnet (perpendicular to the coronal plane of the animal).

The experiments were done using an SIS-200 (SISCO) console and a 4.7-Tesla Oxford magnet with a 40-cm bore. After the animal was placed in the magnet, mag-

netic field homogeneity was optimized by maximizing the ¹H free induction decay lifetime. Typical ¹³³Cs linewidths were 20–40 Hz. Measurements of cesium diffusion were made with a standard Stejskal-Tanner sequence (20) that was gated to the R wave of the animal's EKG using an EKG unit manufactured by SISCO. The echo time was 72 ms. The gradient pulses were each 28 ms in duration (δ), and the time interval between gradient pulses (Δ) was 36 ms. The gradient strength (G) was varied from 0 to 23 Gauss/cm, corresponding to b values of 0, 87, 458, 743, 1086, and 1480 s/mm² (see Eq. [2] below). Sixty-four transients were collected for each spectrum. Transients were collected four at a time at each gradient strength in an interleaved fashion to avoid systematic errors that could be introduced by drift in the value for ADC, which would occur if the ADC were changing after the animal's death. The delay between acquisitions was an optimal 2.35 s, which corresponds to 1.27 times the T_1 for ¹³³Cs in rat brain (16, 21). The signal from the first 10 scans was discarded to allow for equilibration of spin-lattice relaxation before the acquisition of data. Each individual measurement of diffusion coefficient (i.e., acquisition of 64 transients at each of the six b values) took approximately 20 min. After measurements of the cesium ADC in the alive, anesthetized animal, the animal was killed with an overdose of Nembutal administered intravenously. The mechanical ventilator was left on and ADC measurements were made continuously for 3 h after the death of the animal.

In addition, one further ADC measurement was made with each of the alive, anesthetized rats with 32 transients and 10 s between acquisitions (fully relaxed) to allow comparison between ADC values measured with and without full spin-lattice relaxation.

Data Analysis

Cesium signal amplitudes were estimated using Bayesian probability theory (22–25). These amplitudes were then fitted to the following expression using a least-squares method (RS/1 software system, BBN Software Products) to determine the ADC (20).

$$S_i/S_0 = \exp(-b_i \cdot D) \quad [1]$$

where S_i is the signal intensity at some value b_i , S_0 is the signal intensity with $b = 0$, and D is the diffusion coefficient. The parameter b_i is given by the equation:

$$b_i = \gamma^2 G_i^2 \delta^2 \left(\Delta - \frac{1}{3} \delta \right) \quad [2]$$

where γ is the magnetogyric ratio, G_i is the gradient strength, δ is the duration of each gradient pulse, and Δ is the time between application of the two gradient pulses. Evaluation of ADC values at different time points was done using either analysis of variance (ANOVA) or Student's t -test.

RESULTS

Figure 1 shows representative data from a single determination of the ADC for cesium. When the ADC was

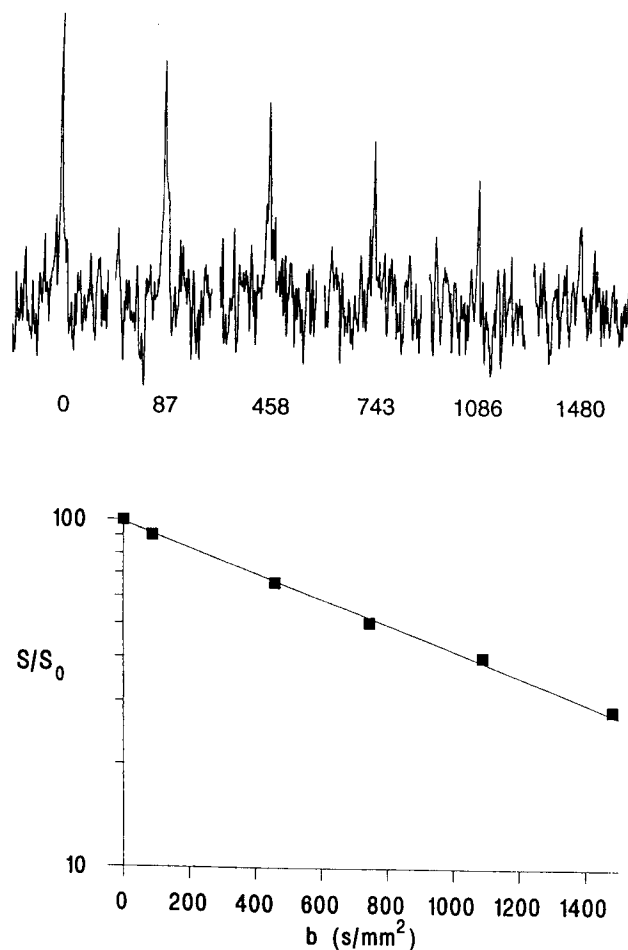


FIG. 1. (TOP) A series of ^{133}Cs spectra obtained from a live animal. The spectra shown represent Fourier transforms of the time domain data for which an exponential apodization filter was used, resulting in 20 Hz line broadening (see the text for further details on data acquisition). The numbers beneath each spectrum represent the b value (in units of s/mm^2) at which each spectrum was obtained. Note that Fourier transforms are used here for display purposes, but the signal amplitude estimations used for data analysis and for the plot at the bottom of the figure were done using Bayesian analysis as described in the text. (BOTTOM) A semilog plot of normalized signal intensity (S/S_0) versus b value corresponding to the data shown in the top part of the figure. The line represents the best nonlinear least squares fit to the data and corresponds to an ADC of $0.86 \times 10^{-3} \text{ mm}^2/\text{s}$.

measured in an alive rat under conditions of full spin-lattice relaxation, the value obtained was $1.02 \pm 0.04 \times 10^{-3} \text{ mm}^2/\text{s}$ (mean \pm SEM, $n = 5$). This was significantly larger than $0.91 \pm 0.05 \times 10^{-3} \text{ mm}^2/\text{s}$, which was obtained with a 2.35-s delay between acquisitions (paired t -test, $P < 0.01$).

As can be seen in Fig. 2, the ADC value fell within 20 min (the best temporal resolution of the experiment) after the death of the animal and remained at a value roughly 30% less than that obtained from the live animal. The ADC values from the live animals were significantly different from those obtained after the animals' deaths (ANOVA, $P < 0.001$), and the values obtained after death were not significantly different from one another (ANOVA, $P > 0.1$).

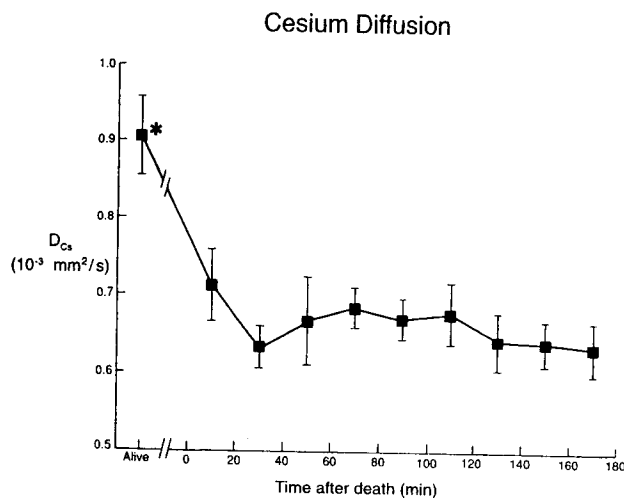


FIG. 2. A plot of cesium ADC versus time. The first data point represents measurements from live animals. Times were measured from the time of the animal's death. Each data point was collected over 20 min time and consists of 64 free induction decays with a time interval between acquisitions of 2.35 s (see text for details). The error bars represent standard errors of the means. The asterisk denotes that data point that is significantly different from the others by ANOVA ($P < 0.001$).

DISCUSSION

Before attempting to interpret the data from this study, it would be useful to address the issues of: (i) temperature, (ii) the diffusion time used for these experiments, (iii) leakage of cesium from the intracellular to extracellular space after the death of the animal, and (iv) the effect of the acquisition parameters on the fraction of the detected signal arising from intracellular (as opposed to extracellular) cesium. With regard to temperature, we found that the brain temperature of rats this size falls as much as 7°C within the first 20 min after death despite maintaining the animal's rectal temperature at 37°C with circulating warm water (unpublished data). While diffusion coefficients tend to decrease with temperature, decreased temperature is generally not thought to be a significant factor in the decrease of water ADC with brain injury (26–28). Despite this, we chose to control brain temperature in these studies to avoid any potential confounding effects introduced by changes in brain temperature.

The "diffusion time" (t_{dif}) represents the time during which the ADC measurement is sensitive to movement of the molecule under consideration, in this case cesium. As one might expect, it is related to the time between the first diffusion gradient, which dephases the spins based on their position in space, and the second diffusion gradient, which rephases the spins (to a degree dependent on how far they have moved) before formation of the spin echo. The diffusion time is given by the equation:

$$t_{dif} = (\Delta - \delta/3) \quad [3]$$

where Δ is the time interval between diffusion gradient pulses and δ is the duration of each pulse. This time interval has an effect on the value obtained for ADC in systems in which there are boundaries that restrict the movement of the molecule under consideration (29). In

general, the longer the diffusion time, the more likely molecules are to encounter a restricting boundary. This is because longer diffusion times allow molecules to move over greater distances before the rephasing gradient is applied. The distances over which molecules move during a single-gradient-direction NMR diffusion experiment can be expressed as the mean square displacement, $\langle x^2 \rangle$, in the direction of the applied gradient. This is given by the equation (30):

$$\langle x^2 \rangle = \sqrt{2} (\text{ADC}) t_{\text{diff}} \quad [4]$$

where ADC is the ADC. (Note that the factor of $\sqrt{2}$ becomes $\sqrt{6}$ if one considers three-dimensional movement.) For infinitely short diffusion times, molecules are unlikely to encounter restrictive barriers—the ADC measured is maximum and presumably represents unrestricted motion within the circumscribed (e.g., intracellular) space. For extremely long diffusion times, all molecules will eventually encounter barriers and, unless this phenomenon is taken into account, the ADC measured from the signal attenuation curve will be much lower than that measured at short diffusion times.

For cesium, cell membranes likely represent a boundary across which the molecules cross only very slowly (vide infra). In the study described here, the diffusion time was 27 ms. Using the ADC for cesium in live rat, this gives a mean square displacement of about 7 μm , which is on the order of the size of a single cell body (remember that the sensitive volume of the receiver coil included both white and gray matter in these studies). To correct for restrictions of diffusion requires that ADC measurements be made at several diffusion times, which was impractical for this study. But based on studies of motion of *N*-acetyl aspartate, choline, and creatine (29), which are intracellular constituents of neurons and glia, it is likely that there was a component of restriction in the ADC values reported in this study. This means that the ADC values reported may be underestimates of the motion of cesium inside cells by a small amount.

With regard to leakage of cesium from the intracellular to extracellular space after the death of the animal, this leakage takes place rather slowly (16) in rat brain. In addition, we estimate that for isolated, cesium-loaded cardiac muscle less than 10% of the intracellular cesium leaks out over the first 3 h after death of the tissue (unpublished data). But however slowly cesium leaks from dead or dying cells, some leakage must have taken place over the time course of these experiments. If significant leakage were taking place, it would tend to increase rather than decrease the ADC of cesium in this experiment. While it is possible that the ADC for extracellular cesium decreases after the death of the animal as a result of increased restriction of the extracellular space, it is likely that the ADC of molecules in the extracellular space remains greater ("faster") than that in the intracellular space even after the death of the animal. Because we observed a decrease in the cesium ADC after the animal's death, it is unlikely that the result is due to leakage of cesium. In fact, the decrease observed might have been even greater in the absence of leakage.

It is likely that the acquisition parameters used had an effect on the relative proportions of signal arising from the intracellular and extracellular spaces. Both spin-spin (T_2) and spin-lattice (T_1) relaxation should be considered. In this study, relatively long echo times were used. This was done because the magnetogyric ratio for ^{133}Cs is roughly one eighth that of ^1H . As can be seen from Eq. [2], this decreases the value for b by roughly a factor of 64 for a given gradient strength (G) and duration (Δ and δ) when comparing ^1H measurements to ^{133}Cs measurements. To compensate for this, long gradient pulses were used (increasing the values for Δ and δ), requiring long echo times. However, long echo times also favor signal from spins with relatively long T_2 relaxation times. For rat brain at 4.7 Tesla, we estimate that the T_2 relaxation time for intracellular cesium is on the order of 53 ms (16), while that for extracellular cesium is on the order of 1000 ms (unpublished data). Using these relaxation times, one can calculate that the ratio of $\sim 40:1$ for intracellular to extracellular signal would decrease to $\sim 10:1$ at an echo time of 72 ms. The second consideration is T_1 relaxation. When acquiring data under conditions that do not allow for full spin-lattice relaxation, signal from spins with relatively short T_1 relaxation constants tends to dominate. We chose a time interval between data acquisitions of 2.35 s to optimize the signal-to-noise ratio from intracellular cesium (21), which has a T_1 relaxation time of 1.85 s under our experimental conditions (16). The T_1 of extracellular cesium, on the other hand, is on the order of 3 s (unpublished data). Thus, the signal amplitude ratio of $\sim 10:1$ would increase to $\sim 12:1$ when data are acquired with a delay of 2.35 s. Overall, it is likely that over 90% of the signal observed in these experiments arose from intracellular cesium.

The finding that the cesium ADC measurements from live animals differed depending on the acquisition parameters is an intriguing one. The ADC measured under conditions of full spin-lattice relaxation was greater than that measured under conditions of partial saturation by about 10%. By acquiring data under conditions of full relaxation, the weighting described above for conditions of partial magnetization saturation no longer applies. Thus, signal acquired under conditions of full spin-lattice relaxation would tend to have a greater contribution from spins with a longer T_1 relaxation constants (i.e., extracellular cesium) than signal acquired under conditions of partial saturation. If extracellular cesium has a larger ADC than intracellular, one would predict that the ADC measured under fully relaxed conditions would be larger than that measured under conditions of partial saturation, as was observed in this study. Hence, the observed difference in ADC is consistent with estimates of T_1 relaxation times and relative ADCs for intracellular and extracellular cesium. On the other hand, it is also possible that the intracellular cesium population is inhomogeneous with regard to T_1 and ADC, accounting for the differences in ADCs measured. Based on the data obtained in this study, there is no way to tell which explanation is correct.

The values for the ADC of cesium in the live rat were roughly half those for cesium in aqueous solution, which is consistent with data from electron spin resonance

studies that suggest that the diffusion coefficients of small molecules in the intracellular space are about half those for the same molecules in an aqueous solution (31). Furthermore, the ADC value (corrected for restricted diffusion) for inorganic phosphate in rat muscle (32) at 37°C is $\sim 1.0 \times 10^{-3} \text{ mm}^2/\text{s}$, which is in good agreement with the values reported in this study. However, measurements of ADCs of other small molecules in the intracellular space often give values that are somewhat lower than what we obtained for cesium [for a review of these studies, see reference (29)]. From studies of non-neural tissues, for example, the ADC (corrected for restricted diffusion) of lithium inside red blood cells at room temperature is on the order of $0.5 \times 10^{-3} \text{ mm}^2/\text{s}$ (33), while that for sodium is about $0.05 \times 10^{-3} \text{ mm}^2/\text{s}$ (34). Calcium in fish muscle cytoplasm at 25°C gives an ADC (measured with non-NMR methods) of $0.25 \times 10^{-3} \text{ mm}^2/\text{s}$ (35). On the other hand, the ADC value we report for cesium in rat brain after injury, $0.6 \times 10^{-3} \text{ mm}^2/\text{s}$, is identical to that obtained in our laboratory for excised rat heart at 37°C, from which cesium leaks very slowly after cell death (unpublished data). From studies of neural tissue, data from rat brain at 37°C have been obtained for *N*-acetyl aspartate, choline, and creatine. Each of these molecules gives a value (measured at very short diffusion times of 17 ms) of $\sim 0.3 \times 10^{-3} \text{ mm}^2/\text{s}$ (36). Overall, there is a moderate degree of variability in ADCs for different molecules inside various cells, with the ADCs we report for cesium in rat brain being at the high end of the reported values. It is clear that ADC values depend, at least in part, on the particular cell type and molecule being studied.

There is no reason to expect, a priori, that the ADC values for cesium obtained in this study should be the same as those for intracellular water, although we are interpreting the changes in cesium ADC as specific to the intracellular space and as reflecting perturbations that affect both water and cesium motion in parallel. On the other hand, it is interesting to compare the ADC values because, as noted previously, the true diffusion constants are essentially equivalent in dilute aqueous solution (perhaps fortuitously). The values obtained for cesium in live rat ($\sim 0.9 \times 10^{-3} \text{ mm}^2/\text{s}$) are higher than estimates of diffusion coefficients for intracellular water in tumor cells in culture, which are on the order of $0.2 \times 10^{-3} \text{ mm}^2/\text{s}$ (29). The water ADC estimates are derived from biexponential fits of signal attenuation data. The biexponential fits give two diffusion coefficients for the system under study; the larger is interpreted as corresponding to "extracellular" water and the smaller to "intracellular" water. However, it is possible that values obtained in this fashion are an underestimate of the intracellular water ADC. In the only study of neurons of which we are aware—isolated neurons from *Aplysia californica*—diffusion constants of $0.28 \times 10^{-3} \text{ mm}^2/\text{s}$ and $1.5 \times 10^{-3} \text{ mm}^2/\text{s}$ were obtained at room temperature for water in the cytoplasm and the nucleus, respectively (37). This study raises the possibility that, because of the multicompartmental nature of the system under study, biexponential fits of diffusion data from cell suspensions do not properly separate out intracellular and extracellular diffusion. For example, in this case water in the cell nu-

cleus, having a relatively high diffusion coefficient, may be misidentified as "extracellular." In any event, these values from the intracellular space of *Aplysia* neurons are on the same order as those we obtained for cesium.

While we are using the results of this study of cesium to draw inferences about the changes in ADC of water, we are assuming some important differences in the behavior of the two molecules in brain tissue. Specifically, we assume that cesium is in "slow exchange" between intracellular and extracellular compartments during these experiments. We make this assumption because cesium ions are likely to pass through cell membranes much less readily than water molecules. As a result, it is likely that a given cesium molecule remains in a given compartment (intracellular or extracellular) during the 27-ms diffusion time of the ADC measurements of this study. For such a system, it is theoretically possible to obtain a biexponential signal decay curve in which the two slopes represent the ADCs of the two compartments (with the caveat discussed in the preceding paragraph). No attempt was made to fit the data from this study to a biexponential expression because it was unlikely that we would be able to effectively detect a signal arising from such a small extracellular population and because the signal-to-noise ratio did not permit acquisition of enough data points for a realistic biexponential fit. Hence we used a monoexponential fit and assumed that the ADC represents movement of cesium in the intracellular space. Water molecules, on the other hand, pass through membranes more readily than cesium. As a result, it is possible that a given water molecule has sampled both compartments, perhaps even more than once, during the course of a water ADC experiment. For such a system in "fast exchange," only a single ADC is detected and it represents a weighted average of the ADCs of the two compartments. (Note that the concept of "restricted diffusion" for water is compatible with the idea that water can cross membranes if one assumes that the membrane barriers still act to slow the movement of water from one compartment to the next.) To what extent this phenomenon takes place for water depends on factors such as diffusion times, relative membrane permeabilities of the cells under study, and the relative volume fractions of the different compartments. This effect would be more likely for those experiments in which the diffusion time is longer, such as in imaging studies done on human subjects. In any event, by virtue of its low membrane permeability, cesium offers insight into the intracellular milieu without the presence of confounding effects caused by sampling the extracellular space.

The main finding of this study was a decrease in cesium ADC after the death of the animal. While we feel that this change in cesium ADC reflects changes in the intracellular environment that affect cesium motion, an alternative explanation relates to the active transport of cesium across cell membranes. Cesium, being a potassium analog, is transported across cell membranes from the extracellular to intracellular space by the sodium-potassium ATPase pumps (albeit with approximately 70% of the efficiency of potassium; unpublished data from cardiac muscle). These pumps function to maintain the ionic gradients necessary for resting membrane po-

tential, and they are especially active in neurons because the firing of an action potential leads to movement of potassium out of cells. This potassium must then be actively transported back inside the cell. For the sake of discussion, cesium (or potassium) can be considered as being in one of two pools during the diffusion measurement. The first pool consists of cesium in the intracellular space that does not encounter the membrane pumps during the diffusion measurement. The second pool is made up of cesium that is actively transported by the pumps during the diffusion measurement. It is conceivable that the reason that the cesium ADC decreases after the death of the animal is that when the cells die, the sodium-potassium pumps stop functioning and the displacement of cesium is reduced, causing a decrease in ADC. The likelihood that this explanation is correct depends on how great a proportion of the total cesium is actually transported by the pumps during the diffusion measurement. As we will see, the vast majority of cesium molecules probably do not encounter a pump during the diffusion measurement.

To illustrate this point, we will examine transport of potassium across the axonal membrane of neurons as a consequence of an action potential. The brain substance within the sensitive volume of the receiver coil during these experiments consists of both neurons and glia. Neurons, because they are electrically active, lose potassium at a greater rate than glia and must pump this potassium back across the cell membrane to maintain the necessary concentration gradient. In addition, the axon of the neuron is very electrically active, as this is the portion of the cell in which action potentials are propagated. Furthermore, the axon has a relatively high surface area to volume ratio as compared with a cell body; and because the pumps are located in the cell membrane, axons would be expected to transport a relatively large proportion of their intracellular potassium. Taken together, this suggests that transport of potassium across the axonal membrane is the "worst case" for ion transport in a central nervous system cell. But even in this case, it has been calculated that the change in intracellular potassium that takes place with each action potential is roughly 1/10,000,000 of the internal potassium content (38). As a result, even for axons firing action potentials at 1000 Hz [the maximal firing rate for mammalian axons (39)], the amount of potassium transported during the 27-ms diffusion time of this study would be quite small. Extending the argument to cesium, this cation is overwhelmingly present in the pool that does not encounter membrane pumps during the diffusion measurement. Therefore, it is likely that the sodium-potassium pumps play a very minor role in the overall motion of cesium, and the ADC of cesium does not reflect pump activity to any detectable degree. This makes it extremely unlikely that the fall in cesium ADC associated with the death of the animal is due to changes in sodium-potassium pump activity.

As a result, the finding that cesium ADC falls quickly after the death of the animal strongly suggests that there are changes in the intracellular environment that affect cesium motion. Active intracellular processes that could possibly account for such changes include: cell stream-

ing, axoplasmic flow, and organelle movement (40, 41). As it has been shown that the fall in water ADC with cerebral ischemia coincides with energy failure (42), it is possible that energy failure brings about a loss of intracellular motion that causes a decrease in intracellular cesium ADC. Such a process would also affect the intracellular water ADC, and hence the decrease in water ADC observed with brain injury may in part be explained by a decrease in the intracellular water ADC. Another possible explanation for the fall in cesium ADC is a change in the viscosity of the intracellular contents, such as might occur in conjunction with dissociation of microtubules (43). It is important to note that the mechanism suggested herein for the decrease in water ADC does not exclude contributions from other mechanisms, such as shifts of water from the extracellular to intracellular compartment or trapping of extracellular water. In addition, the findings in this study do not necessarily apply to other conditions under which the ADC for water in the brain is known to decrease, such as status epilepticus.

In studies done essentially coincident with the study described here, two other research groups have obtained data, which are consistent with our results. Wick *et al.* (44) have evaluated the ADCs of the metabolites *N*-acetylaspartate, *myo*-inositol, creatine, and choline using ^1H spectroscopy of rat brain during stroke and reperfusion. They find that the ADC values of these metabolites fall after stroke, suggesting a significant reduction in intracellular mobility. In a similar but separate study, van der Toorn *et al.* (45) also used ^1H spectroscopy to evaluate motion of *N*-acetylaspartate, creatine, and choline in rat brain before and after cerebral ischemia. Their results are qualitatively very similar to those of Wick *et al.*, as they report a reduction in the metabolite ADCs after stroke. While these studies and ours strongly suggest that a decrease in intracellular ADC occurs with brain injury, confirmatory studies with other NMR-detectable markers (using endogenous or exogenous molecules present in the intracellular or extracellular space) are necessary.

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