The Intracellular Water Preexchange Lifetime of Neurons and Astrocytes Are Different and Decrease Rapidly under Oxygen-Glucose-Deprivation Conditions

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INTRODUCTION Knowledge of intracellular water preexchange lifetime (τIN) in central nervous system (CNS) cells is of fundamental importance for interpreting diffusion MR results over different time scales. Herein, by employing a previously described “Brains on Beads” methodology1, we measure the τIN in cultured rat neurons and astrocytes under normal conditions (oxygen-glucose-perfusion) and upon oxygen-glucose-deprivation (OGD).

METHODS Neurons or astrocytes from newborn rat cerebral cortex were cultured on polystyrene microbeads (125 – 212 μm in diameter). Fig. 1. Microbead-adherent cultures were perfused at 36.5°C with oxygenated, glucose-containing, Tyrode’s solution inside an 8-mm ID glass tube. In some experiments, OGD was achieved by replacing oxygen with nitrogen and glucose with 2-deoxy-D-glucose. MR data were acquired on an 11.74-tesla Agilent/Varian DirectDriveTM scanner using a slice-selective inversion-recovery (IR) spin-echo sequence. The slice-selective spin-echo sequence greatly suppresses signal from flowing medium. Fifteen inversion times were used, logarithmically spaced from 5.5 ms to 5 s. Bayesian analysis was employed to estimate the water 1H signal amplitude and to model the IR data (http://bayesiananalysis.wustl.edu).

RESULTS AND DISCUSSION IR data from medium flowing through cell-free packed microbeads were well modeled as a mono-exponential. The flow-dominated apparent τT was estimated to be very short (10-30 ms) and was sensitive to flow rate (data not shown). In the presence of microbead-adherent cells, however, IR data were well modeled as a bi-exponential, Fig. 2, thus defining two relaxing components (A and B) by their different τT’s. Component A, with an observed τT varying between ~0.3 – 0.7 s depending on the experimental condition, was not detected in the cell-free experiment and contributed to an average of 25% of the total signal. The average τT of component B (τT,B) was 48 ± 7 ms, similar to that of the flowing medium in cell-free experiment. Therefore, the long-Τ component (A) was assigned to intracellular water population. Using the Bloch-McConnell equations describing longitudinal relaxation in the two-compartment slow-exchange regime, Eq. [1], and an intrinsic intracellular water τT, τIN, of 1.8 s as previously reported2, τIN was determined as 0.88 ± 0.24 s for neurons (n = 9) and 0.66 ± 0.17 s for astrocytes (n = 13). OGD experiments were employed to mimic cerebral hypoxia-ischemia. The τIN in both types of cells decreased within 30 min after OGD onset, Fig. 3. At 75 min after OGD onset, neuron and astrocyte τIN decreased to 0.48 ± 0.07 s (n = 3) and 0.18 ± 0.04 s (n = 5), respectively. The decrease in τIN, interpreted as an increase in apparent cell-membrane water permeability, may reflect disruption of energy-dependent ion pumps but could also indicate disruption of aquaporin function and/or change in membrane structure upon OGD. Importantly, τT,B remained unchanged in each OGD trial, wholly consistent with the interpretation that component B represents the flowing medium. Non-mono-exponential decay of the diffusion-weighted MR signal in CNS is often ascribed to contributions from water populations with distinct apparent diffusion coefficients. In a simplified two-compartment-exchange model, τIN determines in what exchange regime the diffusion-weighted data should be assessed3. The diffusion times typically used in clinical studies place them in the slow-exchange regime. However, as τIN decreases significantly shortly following injury, the transmembrane exchange moves towards a more intermediate-exchange regime. This should be taken into account when interpreting diffusion data. Moreover, the intrinsic, non-negligible difference between neuron and astrocyte τIN, especially in injured cells, should also be taken into account in the analysis of time-scale-sensitive data.

CONCLUSION To our knowledge these are the first MR measurements of intracellular water preexchange lifetime in cultured neurons (0.88 ± 0.24 s) and astrocytes (0.66 ± 0.17 s). These lifetimes decreased quickly upon oxygen-glucose-deprivation to 0.48 ± 0.07 s and 0.18 ± 0.04 s, respectively.

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