Measurement of Intracellular Water Preexchange Lifetime in Perfused "Brains on Beads" System

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Introduction

Knowledge of the intracellular water preexchange lifetimes in central nervous system cells is important for many experimental and theoretical studies, especially for modeling tissue water diffusion and interpreting dynamic contrast enhancement data. Previously, we determined the intracellular water preexchange lifetime of HeLa cells (110 ms) by selectively and directly observing the intracellular water ^{1}H MR signal in a perfused, microbead-adherent cell system (1). This approach has now been extended to rat neurons and astrocytes, which have intracellular water preexchange lifetimes of ~30-40 ms.

Materials and Methods

Cell Culture: Pure neurons, pure astroctyes or mixed neurons/astroctyes were obtained from rat pups and grown on microbeads of 125-215 μm diameter. Microbeads were transferred to a 6.0-mm-ID glass tube and perfused with pre-warmed and oxygenated Tyrode solution.

MR Experiments at 11.7 T: A 100-μm thick slice orthogonal to the flow direction was selected with a slice-selective spin echo pulse sequence. Water ^{1}H MR signal from flowing extracellular media within the slice was suppressed by both time-of-flight effects and phase dispersion. For ^{1}H MR measurement of the intracellular water exchange-modified longitudinal relaxation time ($T_{1,obs}$), a slice-selective inversion pulse was inserted before the slice-selective spin echo sequence. For ^{1}H MR measurement of the inherent intracellular water spin-lattice relaxation time ($T_{1,in}$), packed cell pellets were prepared by centrifuging fresh cells (not on beads). $T_{1,in}$ experiments were performed at 37 degree C within 30 min of centrifugation.

MR Experiments at 4.7 T: ^{1}H MR $T_{1,obs}$ measurements of mixed cells were performed before and after the addition of a high concentration of Gd-BOTPA (Bracco) to the media. The study was performed on a 4.7-T system to avoid the marked bulk magnetic susceptibility (line broadening) effects present with this system when media containing a high concentration of Gd-BOTPA is employed at 11.7 T.

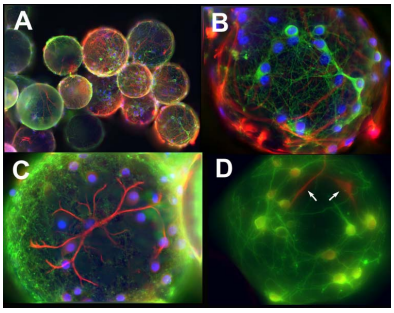

Fig.1 Fluorescence micrographs of rat nervous system cells on microbeads. Panels A through C show mixed neurons (green) and astrocytes (red) culture. The blue dots are cell nuclei. Panel D shows a pure neuron culture. This bead has an astrocyte (arrows), though most microbeads did not.

Results and Discussions

Fluorescence micrographs of rat neurons and astrocytes are shown in Fig. 1. Based on cell counts, the astrocyte preparations were estimated > 99% pure, and the neuronal preparations > 90% pure. A qualitative comparison showed no morphological differences between cells cultured on beads and in standard tissue culture. In experiments with cell-free beads, the water signal amplitude diminished to the noise level as the flow rate of the perfusion media increased (open circles, Fig. 2). However, in the equivalent experiments with mixed cells on beads, the water signal amplitude reached a plateau, with residual signal representing intracellular water (solid squares, Fig. 2). In addition, $T_{1,obs}$ of mixed cells did not change significantly as the T_1 of the media was markedly reduced from 3,040 ms to 14 ms with the addition of Gd-BOTPA, confirming little or no contamination of intracellular water signal by extracellular water signal (data not shown).

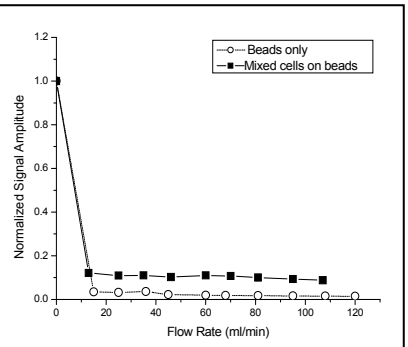

Fig. 2 Normalized signal amplitude *vs.* media flow rate for beads only (open circles) and a mixed neuron/astrocyte cell population on beads (solid squares). At flow rate ~80 ml/min, the signal from beads only is at the noise level. TE = 25 ms.

For each cell group, Table 1 lists its intracellular water exchange-modified ^{1}H longitudinal relaxation time ($T_{1,obs}$), inherent spin-lattice relaxation time ($T_{1,in}$), and preexchange lifetime (τ_{in}) derived using $(T_{1,obs})^{-1} = (T_{1,in})^{-1} + (\tau_{in})^{-1}$. Our study suggests only a small difference for τ_{in} between neurons and astrocytes. The similar τ_{in} value obtained from mixed cells is consistent with this observation. The τ_{in} values reported here are longer than those reported for rat brain (15 ms) by Pfeuffer *et al.* (2) and for human red blood cells (6-9 ms) by Herbst *et al.* (3), but much shorter than that for rat brain reported by Quirk *et al.* (550 ms) (4) and by Prantner *et al.* (200 ms) (5). The reason for this discrepancy is not clear. It may be related to the difference in measurement methodology and/or cells in culture *vs.* cells in tissue. The membrane permeability (P) can be estimated using the relationship: $P = V_{in} / (A\tau_{in})$, where A and V_{in} are the surface area and volume of the cells. While literature reports are not consistent regarding the surface-to-volume ratios of neurons, astrocytes, and their component substructures, a comprehensive electron microscopy study by Pilgrim *et al.* (6) reported surface-to-volume ratios of 5.9 and 12.5 μm^{-1} for neurons and astrocytes, respectively, from rat supraoptic nucleus. Combined with the preexchange lifetimes measured herein, these ratios yield membrane permeabilities of 3.77 μm/s and 2.45 μm/s for neurons and astrocytes, respectively, values within the (rather wide) range reported for other mammalian cell systems (4).

Conclusions

Intracellular water preexchange lifetimes for perfused microbead-adherent neurons and astrocytes were measured by a method that allowed selective detection of the intracellular water signal. The values are similar for the two types of cells. Knowledge of these values provides important information for modeling ^{1}H MR signal from brain. For example, the results suggest that intra/extracellular water is in "intermediate exchange" for typical diffusion and DCE studies.

Table 1 The intracellular water exchange-modified ^{1}H longitudinal relaxation time ($T_{1,obs}$), inherent ^{1}H spin-lattice relaxation time ($T_{1,in}$), preexchange lifetime (τ_{in}) and membrane permeability (P) for rat neurons, astrocytes and mixed neuron/astrocyte cells.

	$T_{1,obs}$ (ms) (Mean±SD, n=3)	$T_{1,in}$ (ms) (n=1)	τ_{in} (ms)	P (μm/s)
Neurons	43.9 ± 5.6	1782	45.0	3.77
Astrocytes	32.1 ± 1.1	1780	32.7	2.45
Mixed Cells	32.4 ± 1.0	1795	33.0	

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References

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