In Introduction, the mammalian brain slice is an established model for the measurement of water distribution and diffusion. We have previously demonstrated the utility of the model with NMR imaging [1] and have described changes in water diffusion following osmabrain exposure [2]. It is important to differentiate diffusion changes per se from compartmental size changes and this requires an estimate of cell volume. Here we describe a method of determining the cell volume fraction of perfused rat hippocampal slices using gadodiamide and T1 imaging.

Methods

We assume brain tissue contains extracellular (ECS) and intracellular (ICS) spaces. Given that water is in fast exchange between these compartments, signal recovery following an inversion pulse is monoexponential [3]. The T1 of the ECS may be shortened by administration of gadodiamide, resulting in a whole tissue T1 described by:

$$\frac{1}{T_1} = \frac{1}{T_{1W}} + (1 - f_{ICS})[Gd]$$

(1)

where T1(0) is tissue T1 in the absence of gadodiamide, fICS is the ICS spin population fraction (1-fECS), τr is relativity of gadodiamide and [Gd] is the concentration of gadodiamide in ECS.

Rat hippocampi were isolated using standard methods and cut into 0.5 mm thick coronal slices. The slices were then placed in artificial cerebrospinal fluid (aCSF). The brain slice was placed in a perfusion chamber and lowered into a standard 10 mm NMR tube [1]. Data were collected using a Doty microimaging probe interfaced to a Varian 600 MHz instrument. Images were acquired using an inversion recovery spin echo sequence. The images were collected at a resolution of 0.23 x 0.23 mm with a 0.3 mm imaging slice.

The relaxation of gadodiamide in aCSF was measured using a series of doped samples (0–4 mM). In 4 brain slices data were obtained using aCSF doped with 0, 1 and 4 mM gadodiamide. In a further 4 slices, data were obtained at 0 and 1 mM gadodiamide and then with 60 mM mannitol doped with 1 mM gadodiamide. In each slice estimates of T1, T1(0) and fICS were obtained from a series of 11 images acquired over a range of inversion time (TI = 0.015–4.93 s).

Results

The relaxation (mean ± SE) of gadodiamide in aCSF was estimated at 3.75 ± 0.02 mM s⁻¹. The brain slices showed monoexponential T1 recovery at all concentrations of gadodiamide (Fig. 1). The mean ± SD value of fICS was estimated as 0.59 ± 0.05 (n=8). The additional of 60 mM mannitol produced a 36% decrease in fICS (p<0.001, n=3). One slice was excluded due to motion during perfusion.

Discussion

The monoexponential nature of the T1 recovery and linear increase in relaxation rate with gadodiamide concentration support our assumption of fast exchange in the brain slices [3]. Addition of mannitol produced the anticipated decrease in fICS. However, fICS does not directly represent cell volume fraction. If we assume that the relative spin densities of the ECS and ICS are 0.95 and 0.71 respectively [4], our estimate of cell volume fraction becomes 0.66. This value is similar to that obtained using a radiotracer technique [5] though still less than that obtained by iontophoresis [6]. Differences in the method of slice preparation are known to alter ECS volume and our result represents an average both across and through the slice.

A significant advantage of the NMR imaging approach is in the ability to produce maps of cell volume fraction (Fig. 2). Calculating the change in relaxation rate in each voxel demonstrates considerable intraslice heterogeneity of compartmentation. Our data further demonstrate the potential of the brain slice model for understanding the origins of NMR signals.

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References