Influence of Mn2+ Concentration on Transport Rates

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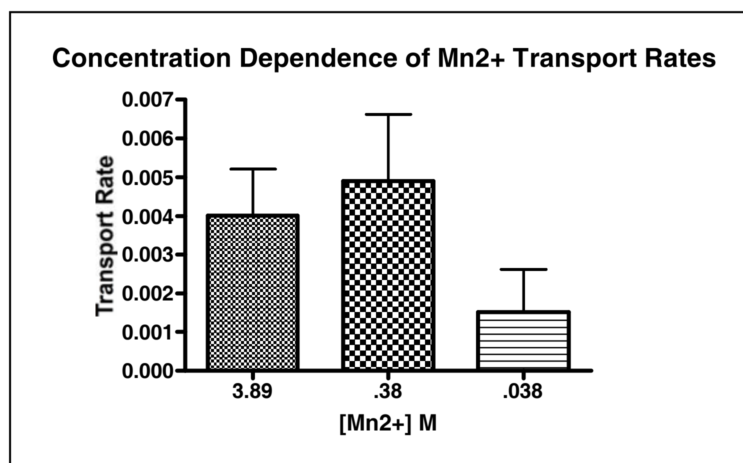
Introduction: The use of Manganese Enhanced MRI (MEMRI) tract tracing to evaluate axonal transport rates has become an area of increased interest [1,2]. However, it is not clear if discrepancies in the amount of Mn^{2+} to a brain region either by lavage or brain injection would result in different transport rates. We addressed this question by assessing the transport rates of Mn^{2+} along the olfactory receptor neurons after nasal lavage at several different concentrations.

Methods: Mice were anesthetized with a combination of 7.5 mg/ml of ketamine and 0.5 mg/ml xylazine at a dose of 0.17 ml/10g body weight. Following anesthesia either 3.89 M, 0.39 M or 0.039 M $MnCl_2$ was pipetted into the nasal cavity of the mouse at a total volume of 4 µl (2µl/naris). Mice were allowed to recover on a warming pad for about 45 minutes, allowing the loading of Mn^{2+} into the olfactory receptor neurons located in the olfactory epithelium. They were then sedated with 2% isoflurane in 100% Oxygen and then imaged for 80 minutes. The zero time point for imaging was at 60 minutes post Mn^{2+} exposure. T_1-weighted, spin-echo 2D data sets were acquired of the mouse brain using a horizontal bore 9.4T Bruker Advance imaging spectrometer with a micro-imaging gradient insert and a 30mm birdcage RF coil. Mice were anesthetized and maintained on 1 – 2 % isoflurane in a seterotaxic holder for the duration of the imaging experiment. The imaging parameters were as follows: Multi-Slice/Multi Echo 2D imaging protocol, matrix dimensions=128x128; FOV=3.0 cm x 3.0; slice thickness=1 mm; repetition time (TR)=504.1ms; echo time (TE)=8.2 ms; NA=2, number of images=15, time per image=2 min. The short TR ensures a heavily T_1-weighted image that will provide positive signal enhancement in regions with an accumulation of the paramagnetic Mn^{2+}. Because axonal transport is a temperature dependent process, the body temperature of the mouse was monitored and maintained at 37°C using an air heater. 4 axial slices were selected with the first slice aligned with the leading edge of the olfactory bulb. Each slice spans 1 mm. In all studies, slice 2 of the 4 slices was assayed for axonal transport and the dorsal lateral portion of the olfactory bulb was selected as a region of interest (ROI). Changes in the signal intensity of this ROI were measured using Bruker's Paravision software and plotted using Microsoft Excel. All signal intensities were normalized to non-enhanced muscle outside of the brain. A least squares method was used to determine the change in signal intensity over time, reflective of the rate of transport of Mn^{2+}. A t-test was utilized to assess significance between the two groups.

Results: Our results indicate that at there was not a significant difference between the transport rates at 3.89 M (N = 6) and 0.38 M (N = 4) (p = 0.67) [Figure 1]. However, at 0.038M (N = 4), we observed a decrease in the transport rates that did not quite reach significance (p = .10) [Figure 1].

Figure 1:



Discussion: We have shown that at concentrations within a 10-fold dilution, there is not a significant difference between the rates of Mn^{2+} transport. At concentrations that ranged within a 100-fold dilution, there was a definite decrease in the rate that did not quite reach significance most likely due to the sample size. These data indicate that within a specific population of mice, most likely, transport rates will be reliable as it is unlikely that the applied concentrations (e.g. either by lavage or injection) will vary by up to 10-fold.

However, caution must be regarded when assessing mouse mutants as these data also indicate that if there is a substantial decrease (> 10-fold) in neuronal Ca^{2+}/Mn^{2+} uptake, these decreased rates of uptake may be misinterpreted as decreased axonal transport rates. When assessing axonal transport rates utilizing MEMRI in mouse mutants, additional studies are mandatory to rule out deficits in loading.

References:

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