In vivo assessment of axonal transport in diabetic mice using Manganese-Enhanced MRI (MEMRI)

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Introduction:

Clinical studies have suggested that people afflicted with diabetes often develop olfactory dysfunction [1-3]. However, the mechanism by which this dysfunction occurs remains unknown. In rats, insulin receptors are highly expressed in the olfactory region [4] suggesting that this region could be susceptible to changes in insulin levels. One experimental model for diabetes is produced by the administration of streptozotocin (STZ) which causes a selective destruction of the beta cells of the pancreas. This results in a deceased production of insulin and high levels of serum glucose (hyperglycemia). It has been demonstrated *in vitro* that STZ-treatment causes impairment in axonal transport in both central and peripheral neurons [5]. However, the examination of axonal transport deficits in diabetic mice has been done mostly *in vitro*. Thus, the aim of this study is to assess if hyperglycemia correlates with axonal dysfunction in the olfactory system *in vivo* utilizing Manganese-Enhanced MRI (MEMRI).

Methods:

Induction of diabetes: C57BL/6 (23-25g) mice were injected intraperitoneally (IP) with 170mg/kg streptozotocin (STZ) made in sodium citrate buffer pH4.5. Body weight and glucose levels were checked before and after STZ-injections. Mice are considered diabetic if glucose levels were higher than 200mg/dl. In this study we examined: mice with no STZ injection (n= 3); mice injected with STZ with glucose levels below 300mg/dl (n=2); and glucose levels ranging 338-598mg/dl (n= 10).

Manganese Enhanced MRI (MEMRI): Axonal transport analysis was performed using MEMRI in the olfactory bulb. The use of Mn2+ as a paramagnetic agent allows for the positive signal enhancement in regions that uptake Mn^{2+} in T1-weighted images. C57BL/6 mice and mice that received an IP injection of STZ one week prior were anesthetized using 5% isoflurane in 100% oxygen. Following anesthesia, 4µl (2µl/naris) of a 0.77g/ml solution of $MnCl_2$ was pipetted into the nasal cavity of each mouse. Mice were allowed to recover for 45 min in a warming pad which also facilitated the uptake of Mn^{2+} into the olfactory receptor neurons in the olfactory epithelium. Mice were placed in a horizontal bore 9.4T Bruker Advanced imaging system and maintained in 1-2% isoflurane for the remainder of the imaging session. The body temperature of the mice was monitored and maintained at 37°C using an air heater. This is critical as the rate of axonal transport is temperature-dependent. The imaging parameters were as follows: multi-slice/multi echo 2D imaging protocol; matrix dimensions = 128 x 128; FOV: 3.0cm x 3.0cm; slice thickness = 1mm; repetition time (TR) = 500ms; echo time (TE) = 10.2 ms; NA = 2; and number of repetitions = 15 for a total image time of 32 min. First scan was started 60 min after Mn^{2+} lavage (zero time point). Four axial slices (1 mm thickness; 1mm interslice thickness) were selected with the 1st slice aligned within the edge of the olfactory bulb (slice #4, sagital slice). For the axonal transport analysis, the region of interest (ROI) was always localized within the slice #2 out of 4 in the dorsal olfactory bulb. The actual pixel which corresponded to the ROI was localized about 50% of the length along the lateral portion of olfactory bulb. Changes in the pixel intensity were measured using Paravision software (v 3.02) and the changes in slope were compared among groups and plotted using Microsoft Excel. All signal intensities were normalized to non-enhanced muscle outside the brain.

Results:

We observed that all the mice in both control groups (no STZ and non-diabetic STZ-injected mice) have slopes around or above 0.02 (Fig. 1). In contrast, 50% of the STZ-injected mice with glucose levels above 338 mg/dl showed a decrease in the slope (below 0.02, Fig. 1). These preliminary data suggest that there is a trend toward a deficit in axonal transport in mice with high glucose levels.

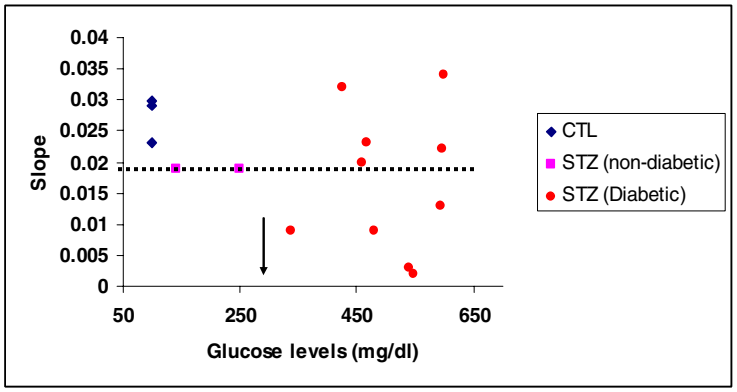

Figure 1. Distribution of the slopes representing the changes in axonal transport of Mn^{2+} in the olfactory bulb. ♦ control mice (no STZ); ■ STZ-treated (non-diabetic) mice; and ● STZ-treated (diabetic) mice. The Y-axis represents the normalized slope values of Mn2+ signal intensity over time and the X-axis represents blood glucose levels. Arrow represents ~300mg/dl level at which mice are considered diabetic. Cut off line at 0.02 represent the level above which all control mice are distributed.

Conclusions and Future Directions:

Our data suggests a deficit in axonal transport in a subset of diabetic mice as early as 1 week after STZ injections. We expect that chronic (1-2 months) STZ treatment will cause greater axonal dysfunction. This methodology holds the potential to be extremely useful in the longitudinal analyses of olfactory neuropathy in a rodent model of diabetes.

References:

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