Quantitative MEMRI Mapping of Ion Channel Regulation by Visual Cycle Activity in Rodent Photoreceptors In Vivo

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Introduction: As gene therapies for correcting outer retina defects, such as visual cycle abnormalities in Leber's Congenital Amaurosis, enter Phase II trials there are the following unmet needs: grading progression of regional retinal dysfunction before the appearance of photoreceptor degeneration, identifying retinal regions most likely to benefit from treatment intervention, and prognostically measuring focal rescue efficacy following different dosing schedules and concentrations. To this end, we tested the hypothesis that the extent of outer retina uptake of manganese is a quantitative biomarker of photoreceptor ion channel regulation by visual cycle activity. At intermediate light intensities, photoreceptors respond to background light levels with a proportionate closure of the ion cation channels (1). It is not yet known if such a graded response is reflected in the extent of outer retinal manganese uptake in rodents. We also examined the sensitivity of MEMRI to specific inhibition of the visual cycle at the level of RPE65. Here MEMRI was used to study dark-adapted control mice and mice systemically pre-treated with retinylamine (2). A single application of retinylamine produces a long-lasting but impermanent inhibition of 11-*cis*-retinal production without associated retinal degeneration (2). We chose to examine overnight dark adapted animals to optimize detection sensitivity to conditions associated with either light or disease-induced channel closure. In addition, we also investigated, before degeneration was apparent, dark-adapted mice with a nonsense mutation in exon 3 of the RPE65 gene ($RPE65^{rd12}$) which produces retinal dystrophy by 7 mo of age, and $RPE65^{rd12}$ mice treated with systemic 11-*cis*-retinal (3;4).

Methods: The following groups were studied: control Sprague Dawley rats (204 - 276 g) adapted to three different background light intensities (1.8 ± 0.7 Lux (n = 10, mean ± SEM), 51.3 ± 11.7 Lux (n = 5), 250.2 ± 19.3 Lux (n = 6)), dark-adapted control mice (C57BL/6, WT) without any treatment, WT mice systemically pre-treated with retinylamine, and dark-adapted $RPE65^{rd12}$ mice (C57BL/6 background) with and without systemic 11-*cis*-retinal pre-treatment. In all cases, rodents were anesthetized and examined with MEMRI after i.p. manganese administration (5). MEMRI data were analyzed for central retinal thickness and intraretinal ion channel regulation as previously described.(5).

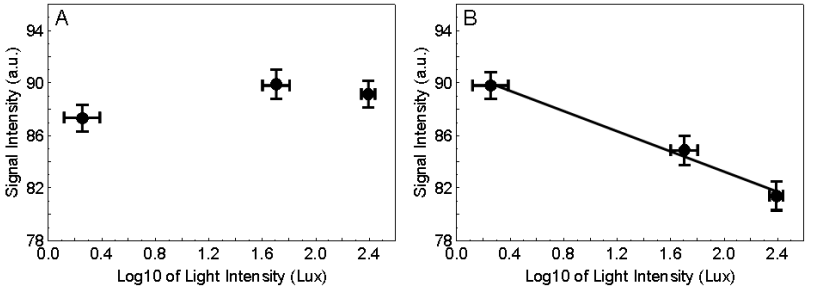

Figure 1: Summary of changes in MEMRI signal intensity in rats as a function of light intensity for A) inner retina, and B) outer retina. Error bars are SEM.

Results: No differences ($P > 0.05$) in retinal thickness were noted within any arm of this study. In rats, manganese uptake was proportional ($r = -0.99$, $P = 0.034$) to the log10 of the background light intensity for outer retina but not for inner retina (Figure 1). Specific inhibition at the level of RPE65 activity, either acutely with retinylamine or chronically in $RPE65^{rd12}$ mice, reduced ($P < 0.05$) outer retinal manganese uptake compared with that in WT mice (Figure 2). In $RPE65^{rd12}$ mice, outer retinal manganese uptake returned to normal ($P > 0.05$) following 11-cis retinal treatment (Figure 2). Inner retinal uptake was normal in untreated or 11-cis treated $RPE65^{rd12}$ mice but supernormal ($P < 0.05$) in retinylamine treated mice, perhaps as an acute response to the retinylamine (data not shown).

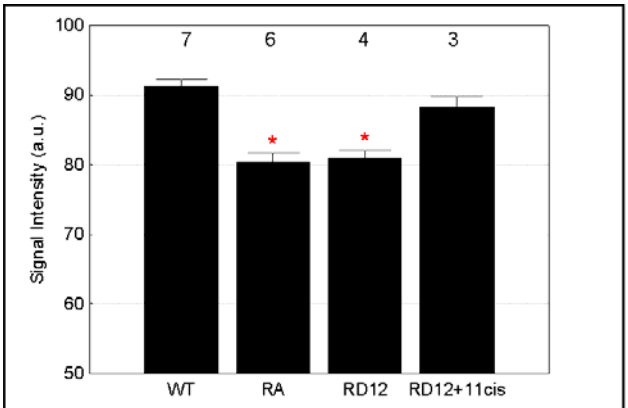

Figure 2: Summary of visual cycle manipulations on outer retinal manganese uptake. WT = control mice, RA = control mice treated with retinylamine, RD12 = $RPE65^{rd12}$ mice, RD12+11cis = $RPE65^{rd12}$ mice treated with 11-cis retinal. *, $P < 0.05$ compared to WT values. Error bars are SEM. The y-axis scale starts at a signal intensity of 50 because this is the pre-manganese baseline level determined from uninjected mice (data not shown)

Conclusions: The present data support measuring the extent of manganese uptake in the outer retina as an analytic non-invasive metric of visual cycle regulation of photoreceptor ion channel activity in vivo.

Acknowledgements: NIH EY018109, NIH Mouse Metabolic and Phenotyping Centers Pilot and Feasibility Program, the Juvenile Diabetes Research Foundation, and an unrestricted grant from Research to Prevent Blindness.

References: (1) J Physiol 2003 552(3):763-76; (2) PNAS 2005 102(23):8162-7; (3) Mol Vis 200511:152-62; (4) J. Biol. Chem 2002 277(43):40491-8; (5) IOVS 2006 47 (6):2668-74.