Published in final edited form as:
Adv Exp Med Biol. 2018 ; 1074: 375–380. doi:10.1007/978-3-319-75402-4_46.

Impact of MCT1 Haploinsufficiency on the Mouse Retina

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Abstract

The monocarboxylate transporter 1 (MCT1) is highly expressed in the outer retina, suggesting that it plays a critical role in photoreceptors. We examined $MCT1^{+/-}$ heterozygotes, which express half of the normal complement of MCT1. The $MCT1^{+/-}$ retina developed normally and retained normal function, indicating that MCT1 is expressed at sufficient levels to support outer retinal metabolism.

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Keywords

MCT1; Retina; Lactate; Transgenic mice; Electroretinogram

46.1 Introduction

The retina is among the most metabolically active tissues in the body and relies on glucose in both glycolytic and oxidative pathways to support the high-energy demands of visual transduction and outer segment renewal. Like cancer cells, the retina utilizes aerobic glycolysis to generate lactate (Weinhouse 1976). And the majority of glucose transported into the outer retina is metabolized to lactate (Wang et al. 1997). This raises the question: what becomes of the lactate? In the brain there is considerable evidence to suggest glucose is metabolized by astrocytes through aerobic glycolysis to generate lactate that is shuttled to adjacent neurons for oxidation (Pellerin and Magistretti 2012). A lactate shuttle could also support metabolism in the outer retina (Poitry-Yamate and Tsacopoulos 1991; Poitry-Yamate et al. 1995). In vitro studies showed isolated Müller glial cells (MGCs) metabolized glucose and released lactate into the media. When photoreceptor cells were added, lactate levels in the media decreased, suggesting photoreceptor cells utilized the lactate (Poitry-Yamate and Tsacopoulos 1991; Poitry-Yamate et al. 1995).

The cloning of MCT1 (SCL16A1) paved the way for identification of other members of the MCT family and the development of antibodies, siRNA, and inhibitors for studying their tissue-specific expression and activity (Adijanto and Philp 2012). Several MCT isoforms are expressed in the retina, and their specific distribution provides insight into the production and utilization of lactate in the outer retina. MCT1 is found in many cells but is expressed at the highest levels in cells that oxidize lactate such as type l muscle fibers and cardiac muscle. In the retina, MCT1 is expressed in the inner segment of photoreceptor cells and endothelial cells and in the apical membrane of the retinal pigment epithelium (RPE).

MCT1 is a heteromeric transporter that requires the accessory protein CD147 (*Bsg*) for trafficking to the plasma membrane (Kirk et al. 2000). Like other heteromeric transporters, the absence of one subunit results in the targeting of the other subunit for degradation. Mice lacking CD147 ($Bsg^{-/-}$) have severely reduced electroretinograms (ERGs), progressive photoreceptor degeneration (Hori et al. 2000), and reduced levels of MCT1, MCT3, and MCT4 in the retina (Philp et al. 2003). These findings indicate that aerobic glycolysis and the lactate shuttle are essential for supporting photoreceptor cell activity and viability. To understand the specific contribution of MCT1 in supporting visual function, we examined the ocular phenotype of $MCT1^{+/-}$ mice, which have axonopathy due to disruption of the lactate shuttle between oligodendrocytes and motor neuron axons (Lee et al. 2012).

46.2 Materials and Methods

46.2.1 Mice

The generation of *MCT1* mutant mice has been described (Lee et al. 2012). While MCT1 homozygous mutant mice do not survive, mice used in these studies were generated by crossing $MCT1^{+/-}$ and C57BL/6J (WT) mice.

46.2.2 Visual Electrophysiology

The methods used to record ERGs and visual evoked potentials (VEPs) have been described (Yu et al. 2012). In brief, after overnight dark adaptation, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) after which ERGs or VEPs were recorded to strobe flash stimuli presented in a ganzfeld bowl.

46.2.3 Western Blotting

RPE and retinas were microdissected from mouse eyes and homogenized in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS) containing protease inhibitors (Sigma) as described (Philp et al. 2003). Detergent soluble lysates (5 μg) were loaded onto a NuPAGE® 4–12% Tris-acetate gel (Invitrogen) for electrophoresis. Proteins were subsequently transferred onto PVDF membranes using XCell II™ Blot Module (Invitrogen). Nonspecific binding sites were blocked with TBS (+0.1% Tween 20) containing 5% w/v powdered milk. Antibodies used in this study were cyclophilin A antibody (Upstate Cell Signaling Solutions), Glut1 (Abcam), CD147 (G19; Santa Cruz), and MCT1 (Philp Lab) (Philp et al. 2003).

46.2.4 Immunofluorescence and Imaging

Mouse eyes were fixed using methanol/acetic at −80 °C as described (Sun et al. 2015). Cryosections (8 μm) were collected on glass slides and labeled with anti-MCT1 and CD147 antibodies as described (Philp et al. 2003).

46.3 Results

Figure 46.1a presents frozen sections of an $MCT1^{+/-}$ retina analyzed at 14 months of age. MCT1 antibody labels the apical membrane of the RPE, photoreceptor cell inner segments, and capillaries in the outer and inner plexiform layers. The overall appearance of the $MCT1^{+/-}$ retina showed no structural abnormalities, and there was no evidence of photoreceptor degeneration. Similar results were obtained by OCT imaging (not shown). Immunoblot analysis demonstrated that the $MCT1^{+/-}$ RPE and retina are both haploinsufficient for MCT1, as compared to WT littermates (Fig. 46.1b).

To examine if there was a functional impact of MCT1 haploinsufficiency, we examined visual electrophysiology. ERGs recorded from $MCT1^{+/-}$ mice under dark-adapted conditions had a waveform that was not different from WT littermates (Fig. 46.2a). The amplitudes of the major ERG components did not differ in amplitude from those of WT littermates (Fig. 46.2b), indicating normal function of photoreceptors (a-wave) and rod bipolar cells (b-wave). Similar results were obtained for the light-adapted ERG (not shown).

We also examined transmission through the visual pathway, by recording VEPs over the visual cortex. The VEP of *MCT1*$^{+/-}$ mice had a normal waveform (Fig. 46.2c), and implicit time measurements were not different from WT littermates (Fig. 46.2d).

46.4 Discussion

In the central nervous system, MCT1 is expressed in oligodendrocytes and facilitates lactate transport to the axon to support their energy demands. The *MCT1*$^{+/-}$ haploinsufficient adult mice exhibit widespread axonopathy in the central nervous system that does not result from demyelination or oligodendrocyte death (Lee et al. 2012). In the retina and RPE, we and others have shown MCT1 is primarily expressed in the RPE and photoreceptor cells suggesting that lactate provides a key energy substrate for these cells (Philp et al. 2003). Mice expressing a single *MCT1* allele had reduced levels of MCT1 in the retina, indicating the absence of a mechanism to upregulate expression of the single allele present in *MCT1*$^{+/-}$ mice or to stabilize the protein for a longer period of time. Despite this reduction, we detected no changes in retinal structure. Moreover, our visual electrophysiology data indicate that a single allele of *MCT1* generates sufficient protein to support normal function of photoreceptors and bipolar cells, as the ERG a- and b-waves of *MCT1*$^{+/-}$ mice were comparable to WT. Transmission through the *MCT1*$^{+/-}$ visual pathway was also comparable to WT. These results, coupled with the absence of visual abnormalities in patients with *GLUT1* haploinsufficiency (Levy et al. 2010), indicate that the retina enjoys high expression of transporters related to supplying this tissue with metabolic substrates.

Acknowledgments

This work was supported by grants from NIH (R01EY12042) and Department of Veterans Affairs (I01BX2340) and by the Foundation Fighting Blindness and Research to Prevent Blindness.

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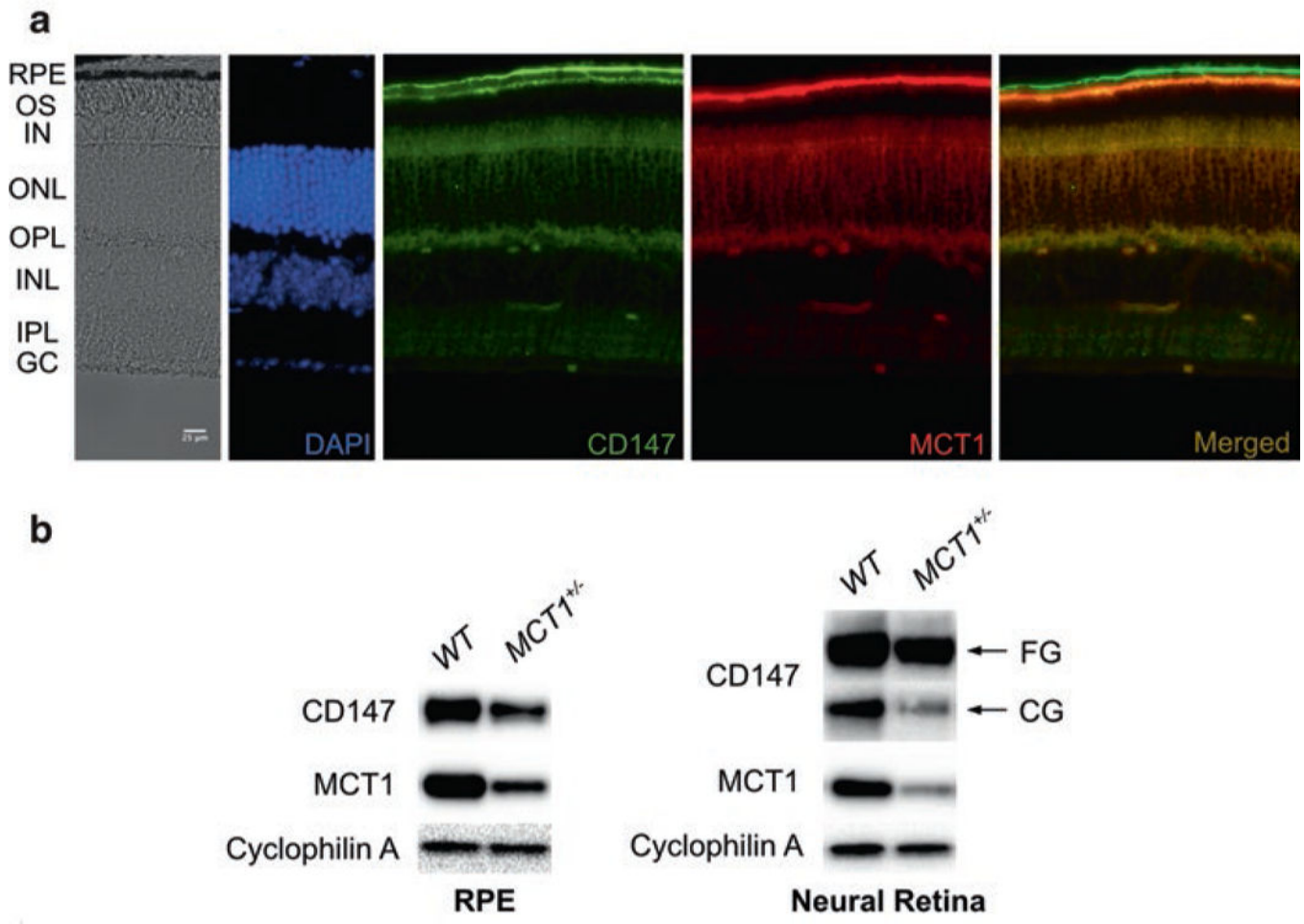

Fig. 46.1.

MCT1$^{+/-}$ retina has normal structure but reduced levels of MCT1. (**a**) Frozen section of 14-month-old *MCT1*$^{+/-}$ retina. Retinal structure is normal, and while the abundance of MCT1 is decreased relative to age-matched WT littermates, the distribution of MCT1 is not changed. (**b**) Immunoblot analysis of RPE and retinal lysates demonstrates greater than 50% reduction in MCT1 levels in *MCT1*$^{+/-}$ as compared to WT mice

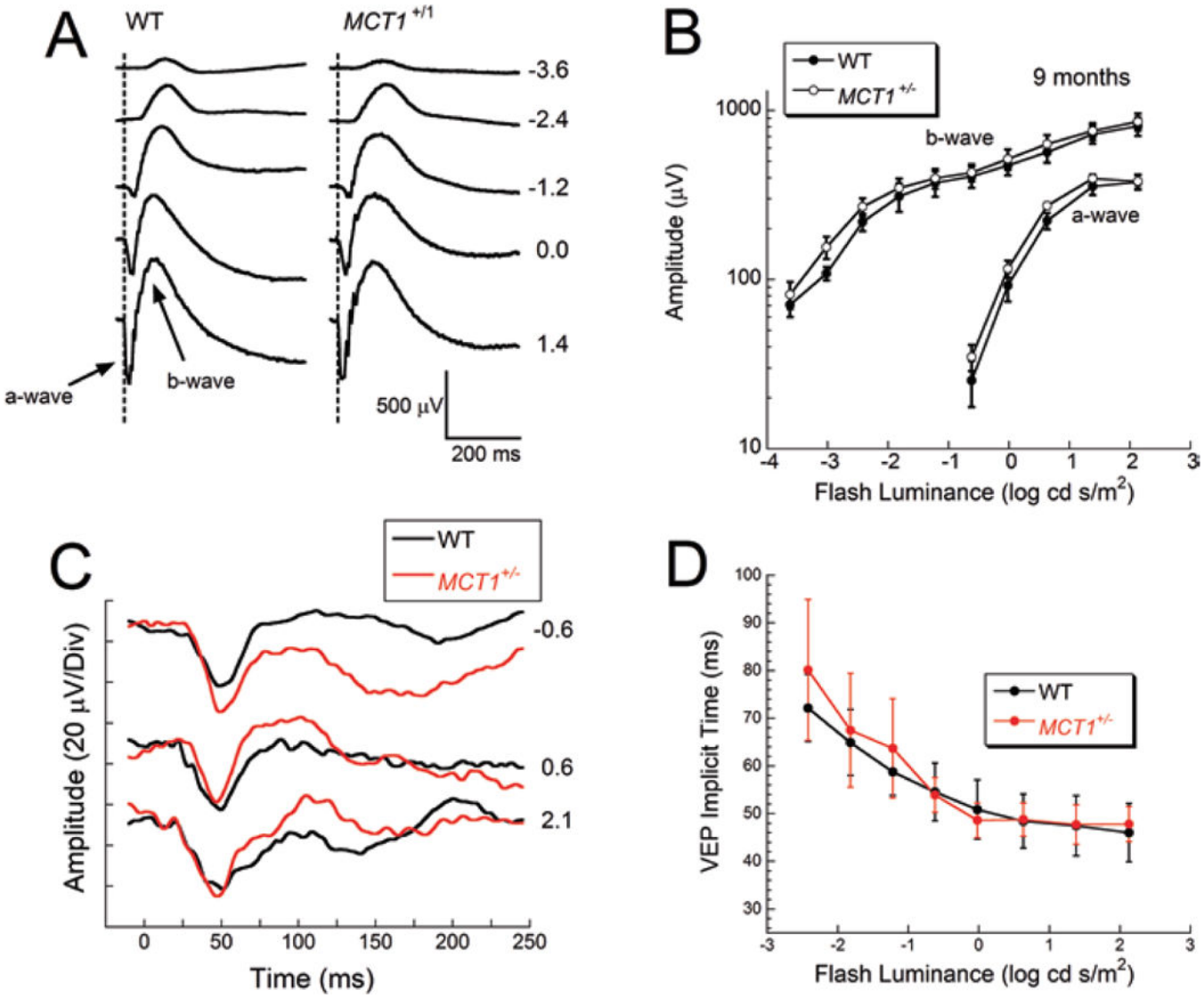

Fig. 46.2.

Normal visual electrophysiology in *MCT1*$^{+/-}$ mice. (**a**) Dark-adapted ERGs obtained from 9-month-old *MCT1*$^{+/-}$ and WT littermates. (**b**) Amplitude of the major components of the dark-adapted ERG. Data points indicate average ± sem from four mice per genotype. (**c**) VEPs obtained from 8-month-old *MCT1*$^{+/-}$ and WT littermates. (**d**) Implicit time of the major VEP component plotted as a function of flash luminance. Data points indicate average ± sem from 15 WT and 11 *MCT1*$^{+/-}$ mice