Mitochondrion 36 (2017) 77–84

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Melanopsin-expressing retinal ganglion cells are resistant to cell injury, but not always

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ARTICLE INFO

Keywords:
Melanopsin
Retinal ganglion cells
Mitochondria
Leber's hereditary optic neuropathy
Dominant optic atrophy
Optic atrophy
Light
Robustness
Alzheimer

ABSTRACT

Melanopsin retinal ganglion cells (mRGCs) are intrinsically photosensitive RGCs deputed to non-image forming functions of the eye such as synchronization of circadian rhythms to light-dark cycle. These cells are characterized by unique electrophysiological, anatomical and biochemical properties and are usually more resistant than conventional RGCs to different insults, such as axotomy and different paradigms of stress. We also demonstrated that these cells are relatively spared compared to conventional RGCs in mitochondrial optic neuropathies (Leber's hereditary optic neuropathy and Dominant Optic Atrophy). However, these cells are affected in other neurodegenerative conditions, such as glaucoma and Alzheimer's disease. We here review the current evidences that may underlie this dichotomy. We also present our unpublished data on cell experiments demonstrating that melanopsin itself does not explain the robustness of these cells and some preliminary data on immunohistochemical assessment of mitochondria in mRGCs.

1. Introduction

Melanopsin-expressing retinal ganglion cells (mRGCs) represent the third class of photoreceptors in the retina, the two other being rods and cones. The mRGCs are mainly involved in the non-image forming functions of the eye, with a crucial role in photoentrainment of circadian rhythms (Hannibal and Fahrenkrug, 2002; Hannibal, 2002; Berson et al., 2002; Hattar et al., 2002; Do and Yau, 2010). The discovery of the non-image forming system was prompted by the observation that in mice models of retinal degeneration, light was still able to photoentrain circadian rhythms (Foster et al., 1991; Freedman et al., 1999; Lucas et al., 1999), thus the existence of a novel photoreceptor was postulated. In 2002, after identification of the melanopsin photopigment, a G-protein coupled receptor with structural similarities to the opsin family of photoreceptors (Provencio et al., 1998, 2000), converging evidences showed that a small subset of RGCs express melanopsin in their membrane allowing the cells to be intrinsically photosensitive. The mRGCs project to the hypothalamic suprachiasmatic nucleus (SCN) through the retino-hypothalamic tract (RHT) (Gooley et al., 2001; Hannibal and Fahrenkrug, 2002; Hannibal, 2002; Hattar et al., 2002; Berson et al., 2002). The RHT, a monosynaptic anatomical pathway connecting the eye to the SCN (Moore and Lenn, 1972; Sadun et al., 1984; Moore et al., 1995) is now established to originate from mRGCs (Hannibal and Fahrenkrug, 2002; Hannibal, 2002; Hannibal et al., 2004, 2014; Hattar et al., 2002, 2006; Berson et al., 2002). Besides photoentrainment of circadian rhythms, these cells play a role in other non-image forming functions of the eye such as pupil light response (PLR), regulation of melatonin synthesis, mood, sleep, cognition and light-aversion (Legates et al., 2012). In addition, a possible role in visual functions has been proposed (Estevez et al., 2012).

The mRGCs represent less than 1% of the RGCs in humans (Hannibal et al., 2004; Dacey et al., 2005; La Morgia et al., 2010, 2016; Liao et al., 2016;). These cells are characterized by a large soma and in humans approximately equal proportions are located in the retinal ganglion cell and the inner nuclear layer (La Morgia et al.,

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http://dx.doi.org/10.1016/j.mito.2017.04.003
Received 8 August 2016; Received in revised form 14 March 2017; Accepted 7 April 2017
Available online 12 April 2017

2010). Studies primarily in mice, based on the level of melanopsin expression, dendritic field arborization, physiology and central projections, classified the mRGCs into five subtypes (Baver et al., 2008; Ecker et al., 2010, Schmidt et al., 2011a, 2011b). The best characterized are M1, stratifying in the outermost sublamina of the inner plexiform layer (IPL), the M2 stratifying in the innermost sublamina of the IPL and the bistratifying M3 cells with dendrites in both inner and outer sublamina (Schmidt et al., 2011a, 2011b). Melanopsin is expressed primarily in the membrane of the soma and dendrites and even in the axons running within the retinal nerve fiber layer, and the large and imbricated dendritic fields constitute a photoreceptive net in the retina (Provencio et al., 2002; Hannibal et al., 2014, Liao et al., 2016; Hannibal et al., 2017; Nasir-Ahmad et al., 2017).

The mRGCs are characterized by unique photoreceptive properties resembling invertebrate photoreceptors e.g. by depolarization in response to light stimuli and possibly displaying bi-or tristability i.e. not being dependent on other cells for chromophore isomerization (Isoldi et al., 2005; Do and Yau, 2010; Brown, 2016). Melanopsin is maximally sensitive to short wavelength blue light (peak response at 480 nm), and mRGCs present a sustained response to light, which can persist even after the light has been switched off (Do and Yau, 2010). Although mRGCs are independently functioning photoreceptor cells, they receive inputs from rods and cones through amacrine and bipolar cells (Viney et al., 2007; Belenky et al., 2003), thus constituting an “irradiance-detector” system in the eye (Viney et al., 2007; Jusuf et al., 2007; Do and Yau, 2010).

The phylogenic and ontogenic importance of this cellular system is demonstrated by the fact that melanopsin has been discovered in a number of vertebrate organisms, and that in rodents these cells respond to light at post-natal day 0, long before the classic photoreceptors, i.e. rods and cones are functional (Hannibal and Fahrenkrug, 2004; Sekaran et al., 2005; Tu et al., 2005; Koyanagi and Terakita, 2008; Davies et al., 2010; González-Menéndez et al., 2010). The importance of the melanopsin-based system is supported by the observation that in the subterranean blind mole rat, the circadian photoentrainment persists due to residual presence of mRGCs projecting to brain structures deputed to control circadian rhythms (Cooper et al., 1993; Hannibal et al., 2002; Esquiva et al., 2016). Moreover, there is consistent evidence that mRGCs are more resistant to injury such as axotomy and survive different paradigms of stress (Cui et al., 2015; Rovere et al., 2016) and metabolic dysfunction such as in mitochondrial optic neuropathies (La Morgia et al., 2010, 2011). However, in other pathological conditions such as Alzheimer's disease and glaucoma, these cells are affected (La Morgia et al., 2016; Drouyer et al., 2008; Pérez-Rico et al., 2010; Obara et al., 2016; Valiente-Soriano et al., 2015). In this paper, we will review this dichotomy, in addition to present unpublished data on the topic.

2. Models of stress and robustness of mRGCs

2.1. Optic nerve transection

Hollander and colleagues (Holländer et al., 1985) and von Bussman and colleagues (von Bussmann et al., 1993) reported that a small subset of RGCs survived optic nerve transection. In particular, von Bussman and colleagues demonstrated that this subset (about 1%) of cells had large bodies and were intensively stained with cytochrome *c* oxidase (COX) (von Bussmann et al., 1993), which suggests that mRGCs are the surviving cells; subsequent studies have indeed demonstrated increased resistance of mRGCs to optic nerve transection in rodents (Robinson and Madison, 2004; Li et al., 2008; Nadal-Nicolás et al., 2015; Pérez de Sevilla Müller et al., 2014).

2.2. Mitochondrial optic neuropathies

In humans, it has been demonstrated that patients suffering blinding disorders with either extensive damage of rods and cones, as seen in outer retinopathies or certain optic neuropathies, maintained the melatonin suppression response induced by light and the circadian entrainment to the light/dark cycle (Zaidi et al., 2007; Czeisler et al., 1995; Pérez-Rico et al., 2009).

Inherited optic neuropathies due to mitochondrial dysfunction such as Leber's hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA) are both characterized by selective loss of RGCs (Carelli et al., 2004; Yu-Wai-Man et al., 2011). We have studied the mRGC system in these optic neuropathies by evaluating the melatonin suppression response to light in affected LHON and DOA patients. In addition, we have immunohistochemically stained the mRGCs in postmortem retinal and optic nerve specimens from LHON and DOA patients and controls. Both LHON and DOA patients had a melatonin suppression response induced by light comparable to controls, and the immunohistochemistry of retinal and optic nerve specimens revealed that mRGCs were preferentially spared compared to non-mRGCs (La Morgia et al., 2010). The observation that mRGCs are relatively spared in mitochondrial optic neuropathies explains the maintenance of the pupillary light reflex (PLR), a peculiar and previously unexplained clinical feature of LHON (Bremner et al., 1999; La Morgia et al., 2010, 2011). Other studies have subsequently confirmed these findings by assessing the PLR (Kawasaki et al., 2010; Moura et al., 2013; Nissen et al., 2015). Furthermore, a mouse model with rotenone-induced optic neuropathy mimicking LHON was also shown to maintain the PLR (Zhang et al., 2006).

Perganta and colleagues showed preservation of mRGCs in a mouse model of DOA (B6; C3-*Opa1*^{Q285STOP}) (Perganta et al., 2013). Strikingly, the mRGC preservation in another DOA mouse model (B6;C3-*Opa1*$^{329\text{-}355del}$) persisted after breeding with melanopsin deficient mice (*OPN4*$^{-/-}$) proving that mRGC resistance seems to be independent from melanopsin expression (González-Menéndez et al., 2015).

Overall, these observations suggest that mRGCs may have specific properties that make them more resistant to neurodegeneration in mitochondrial optic neuropathies.

2.3. Other injuries

Other evidences of mRGC robustness are provided by studies on cell toxicity to monosodium glutamate (Chambille and Serviere, 1993; Hannibal et al., 2001) and NMDA-induced excitoxicity (DeParis et al., 2012). The robustness of mRGCs to other different stressful insults remains, however, an open question, which needs specifically dedicated studies.

3. Models of mRGC vulnerability and loss

3.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the occurrence of circadian and sleep dysfunction even in the early stages (Mattis and Sehgal, 2016). The presence of neuronal loss and AD pathology has been reported in the SCN of AD patients (Swaab et al., 1985; Stopa et al., 1999; Harper et al., 2008). Moreover, a recent longitudinal study demonstrated that sleep fragmentation is correlated to neuronal loss in the SCN in AD patients (Lim et al., 2014).

Optic neuropathy has also been described in AD, as confirmed by both histological and optical coherence tomography (OCT) studies (Hinton et al., 1986, Coppola et al., 2015). We recently demonstrated that mRGCs are lost and affected by amyloid AD pathology in postmortem retinas of AD patients, possibly contributing to the circadian dysfunction observed in AD (La Morgia et al., 2016). Thus, in AD mRGCs are vulnerable to amyloid pathology. Interestingly, the mRGC loss in AD occurred even with a completely normal count of conventional RGCs, pointing to a primary AD pathology selectively affecting these cells (La Morgia et al., 2016).