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Impact of aging and diet restriction on retinal function during and after acute intraocular pressure injury

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Abstract

Advancing age is a major risk factor for many neurodegenerative diseases but the underlying pathophysiology is not clear. We hypothesize that aging impairs the ability of neurons in the central nervous system to recover functionally after injury. To test this in retinal ganglion cells in vivo, we developed an optic nerve "stress test" which monitors the functional capacity of the optic nerve and retina, during and after a subischemic injury induced by intraocular pressure elevation. We report that older (18-month) C57BL/6J mice suffered greater loss of inner retinal function compared with younger adult mice following intraocular pressure (IOP) challenge. To investigate whether age-related vulnerability to IOP challenge can be modified, we subjected 12-month-old mice to dietary restriction (DR) (alternate-day fasting) for 6 months. Compared with age-matched ad libitum fed controls, DR mice showed greater recovery in inner retinal function following IOP challenge. DR was associated with reduced oxidative stress level following injury and improved mitochondrial oxidative phosphorylation enzyme activity compared with ad libitum controls. Taken together, this study provides in vivo evidence that DR improves functional recovery of the retina following injury and points to the potential benefits of therapies that target mitochondria for the protection of the aging retina and optic nerve against injury.

Keywords: Aging; Optic nerve; Neurodegeneration; Mitochondria

1. Introduction

Advancing age is a major risk factor for many neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and glaucoma (Drachman, 2006; Lin and Beal, 2006; Quigley and Broman, 2006; Wallace, 1999). An emerging theory is that aging increases the vulnerability of central nervous system neurons to injury (Mattson and Magnus, 2006). The retina is a specialized extension of the central nervous system and has the advantage in that accurate assessment of functional activity can be assessed in vivo using full-field electroretinography (He et al., 2006; Kong et al., 2009; Saszik et al., 2002; Weymouth and Vingrys, 2008).

In vitro studies on cultured optic nerve from older mice showed greater vulnerability to oxygen-glucose deprivation injury and lower adenosine triphosphate (ATP) levels compared with that of young mice (Baltan et al., 2008, 2010). The association of nerve vulnerability with reduced ATP levels highlights the possibility that age-related decline in mitochondrial function can play a role in increasing vulnerability of nerves to injury. During normal aging, the frequency of mitochondrial DNA (mtDNA) mutations increases by several fold over that seen in young individuals, particularly in postmitotic tissues such as the brain, retina, and heart (Cortopassi et al., 1992; Wang et al., 2010). Thus the ability of neurons to sustain normal function and to

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withstand external stress could be influenced by defective production of cellular energy due to mitochondrial impairment (Kann and Kovács, 2007; Yadava and Nicholls, 2007). Impairment of mitochondrial function has been associated with neurodegenerative disorders (Lin and Beal, 2006) and optic neuropathies (Abu-Amero et al., 2006; Carelli et al., 2004). Indeed, recently our laboratory identified retinal functional impairment in a transgenic mouse with age-related mitochondrial deficiency, which is further exacerbated by stress during acute intraocular pressure elevation (Kong et al., 2011).

A robust method of modifying the detrimental effects of aging is dietary restriction (DR). DR performed either by restricting calorie intake or by alternate-day fasting, has been found to delay aging processes in many organisms (Colman et al., 2009; Sohal and Weindruch, 1996). DR has been shown to improve mitochondrial function and increase mitochondrial biogenesis in various tissues (Cohen et al., 2004; López-Lluch et al., 2006; Nisoli et al., 2005). DR has also been shown to protect against ischemic injury to the retina in rats (Kawai et al., 2001); however prior studies have largely used structural instead of functional end points and it is unclear whether DR improves mitochondrial function in the retina and how this influences the ability of the retina to recover from injury.

The optical properties of the eye permit accurate assessment of retinal function by full-field electroretinography (ERG), which measures the neuronal response to light (Saszik et al., 2002; Weymouth and Vingrys, 2008). The ERG can be measured in rodents during a period of acute intraocular pressure (IOP) elevation (IOP challenge), and thus provides a direct measure of the retina's capacity to maintain normal function under stress in vivo (He et al., 2006; Kong et al., 2009). Such IOP challenges have been shown previously to induce reproducible oxidative (Liu et al., 2007) and metabolic (Baltan et al., 2010; Novack et al., 1990) stress in the retina and optic nerve. Repeated exposure to IOP challenge has also been shown to induce structural changes in retinal ganglion cells of rats (He et al., 2008). The response to a single insult therefore models the very early and potentially reversible stages of injury that may ultimately contribute to a neurodegenerative disorder. We demonstrate here that compared with young animals, older animals suffered greater decline in retinal function and increased oxidative stress following IOP challenge. These effects of aging were significantly reduced by DR such that diet-restricted older mice (18 months old) demonstrated greater recovery of inner retinal function following IOP injury compared with ad libitum-fed controls. Associated with this protection, DR also inhibited the age-related decline in mitochondrial enzyme expression and activity in the retina. These results highlight the potential of therapies that target the mitochondria for the protection of the aging retina and optic nerve against injury.

2. Methods

2.1. Animals and dietary restriction

All experimental methods and animal care procedures complied with National Institutes of Health guidelines for the care and use of laboratory animals and were approved by our Institutional Animal Research and Ethics Committee (Royal Victorian Eye and Ear Hospital and The University of Melbourne, Australia). Wild-type female C57BL/6J mice (Royal Victorian Eye and Ear Hospital, Victoria, Australia) were maintained in a 22 ± 1 °C, 12-hour light (approximately 40 lux, on at 8 AM)/12-hour dark environment. Mice in ad libitum (AL)-fed groups were provided with murine chow (WEHI mix, Barastoc, Victoria, Australia) and water ad libitum. Mice in DR groups were littermates of AL 18-month-old animals. These mice ($n = 30$) were provided with food ad libitum until 12 months of age; from then on they were assigned to a dietary restriction feeding regime for a further 6 months. The DR regime involved limiting access to chow for 24 hours every other day, with chow removed at 9:00 AM on Monday, Wednesday, and Friday and returned at 9:00 AM on subsequent days. Animals were weighed weekly throughout this period at the end of a feeding day. Body weights for AL mice were 25 ± 1 g at 3 months, 29 ± 1 g at 12 months, and 35 ± 1 g at 18 months. DR mice at 18 months of age weighed 31 ± 1 g ($p < 0.01$ compared with AL controls). Intraocular pressure measurements made prior to IOP elevation performed using a non-invasive rebound tonometer (Icare, Finland; Espoo, Finland) were not statistically different between cohorts (11 ± 2 mm Hg for 3-month-old, 14 ± 2 mm Hg for 12-month-old, 11 ± 2 mm Hg for AL 18-month-old, 12 ± 2 mm Hg for DR 18-month-old).

2.2. Electroretinography recording

The retina is a specialized extension of the central nervous system where the functional activity of neurons can be can be assessed accurately in vivo using full-field electroretinography (He et al., 2006; Kong et al., 2009; Saszik et al., 2002; Weymouth and Vingrys, 2008). Full-field scotopic ERGs were recorded simultaneously from both eyes as described in (Kong et al., 2009). Briefly, animals were dark-adapted overnight (> 12 hours) and prepared for ERG recording and IOP challenge in the dark using head mounted night vision goggles (Scout2, Trivisio Prototyping, GmbH; Dreieich, Germany). Animals were anesthetized with intraperitoneal injection of ketamine:xylazine (70 mg/kg:7 mg/kg; Troy Laboratories, Pty Ltd, Smithfield, NSW, Australia) (Saszik et al., 2002). Mydriasis and topical anesthesia were achieved with 1 drop of tropicamide (0.5%, Alcon Laboratories, Inc., Fort Worth, TX, USA), phenylepherine (2.5%, Minims, Chauvin Pharmaceuticals, Surrey, UK) and Proxymetacaine hydrochloride (0.5%, Alcon Laboratories, Inc.). A circulating warm water heating pad was used to maintain body temperature (37 °C). Systolic