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Chronic delivery of low-level exogenous current preserves retinal function in pigmented P23H rat

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ABSTRACT

Diffuse electrical currents delivered to the eye were investigated in a rat model of retinitis pigmentosa for potentially therapeutic effects. Low-level currents were passed between electrodes placed on the cornea and in the mouth during 30-min sessions two times per week from 4 to 16 weeks of age. Single-flash electroretinograms (ERG) were recorded and analyzed for amplitude and measures of sensitivity, and basic histology was performed. ERG a-wave amplitudes were slightly greater in treated vs. age-matched controls at 16 weeks of age, but the combined thicknesses of the outer nuclear layer and outer segment layer were similar at this age. Treated animals exhibited a significant preservation of b-wave amplitudes, and a striking preservation of rod sensitivity, measured as the stimulus strength required to reach half-saturation of the a-wave. Analysis of the leading edge of the a-wave using a delayed Gaussian function revealed a decrease in the parameter reflecting gain of the phototransduction cascade over the 12-week course of treatment, and no significant change in control animals over the same period. These results suggest that while the exogenous currents failed to preserve the number or gross structure of rods, the responsivity of individual photoreceptors was relatively preserved, perhaps via an increase in efficiency of photon capture (R^*/photon). This preserved functionality may delay the retraction of bipolar cell dendrites from degenerating photoreceptors.

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1. Introduction

Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are two classes of eye diseases characterized by progressive cell loss and network remodeling in the retina, resulting in partial or total blindness (Marc et al., 2003). Principal therapies under investigation include pharmacology, gene therapy, and transplantation. Here we investigate the use of exogenous electrical currents delivered to the eye for potentially therapeutic effects. A transgenic animal model that exhibits a mutation and retinal degeneration similar to autosomal dominant retinitis pigmentosa (adRP,) the pigmented line 1 P23H rat, was used.

1.1. Precedent for electrical stimulation therapy

The effects of applied "sub-threshold" electric fields and currents on cells and tissues are relatively understudied. Fundamental studies have investigated directed growth of several cell types *in vitro* (e.g. PC12 cells; Cork et al., 1994). Subsequently, others investigated the ability of applied electric fields to direct regenerating spinal neurons, especially into artificial guidance structures (Borgens, 1999; Cheng & Lin, 2004). The application of exogenous electric fields has been shown to have significant effects on healing and regeneration in several different tissues, including periodontal bone (Kubota et al., 1995), skin (Ojingwa & Isseroff, 2002), peripheral motor nerves (Brushart et al., 2002), muscle afferents (Marqueste et al., 2004), spinal neurons (Borgens, Blight, & McGinnis, 1987; Borgens & Bohnert, 1997), and spiral ganglion cells (Leake et al., 1991). Al-Majed, Tam, and Gordon (2004) demonstrated that electrical stimulation accelerated and enhanced the expression of many regeneration-associated genes in rat femoral motor neurons. Leake, Hradek, and Snyder (1999) demonstrated that chronic electrical stimulation by a cochlear implant promoted survival of spiral ganglion neurons after neonatal deafness (in cats).

Positive effects of exogenous currents have been elicited under a wide range of experimental conditions. The *in vivo* studies listed above involved excitable as well as non-excitable tissues. In cases of excitable tissue, the effective applied electric fields were both above threshold for activating the target tissue or below threshold. The wave shape of the applied voltage (or current) varied from near DC (pulses of >1 s) to biphasic square pulses on the order of tens of microseconds in duration. The predominant guiding factors for selection of stimulus parameters have been empirical evidence bounded by safe limits (avoidance of heat, electrochemical, or electroporation injury due to application of an electric field). Recently, the parameter space of electrical stimulation (pulse duration,

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stimulation frequency, duration of stimulation sessions, pulse kinetics) was explored systematically to find optimal values to promote survival of axotomized retinal ganglion cells (Morimoto et al., 2010).

Cho and colleagues have done extensive work to characterize the effect of exogenous electric fields on cell motility and modulation of intracellular calcium concentration *in vitro* (Cho et al., 2000, 2002; Khatib, Golan, & Cho, 2004; Sun & Cho, 2004; Sun, Wise, & Cho, 2004). For a variety of cell types (fibroblasts, macrophages, osteoblasts), the measured response to stimulation showed a strong dependence on frequency of the sinusoidal electric field. The greatest effects were elicited at frequencies between 1 and 10 Hz; increasing the frequency only as high as 20 Hz resulted in a near complete elimination of the measured effects. In cultured retinal Muller cells exposed to 20 Hz biphasic square pulses, increased IGF-1 transcription, intracellular calcium concentration, and BDNF expression were observed (Sato, Fujikado, Lee et al., 2008; Sato, Fujikado, Morimoto et al., 2008). Calcium plays an extensive and critical role in retinal physiology, including modulating signaling pathways in cell death and the release of protective neurotrophic factors. To make use of the above mentioned findings, a 5 Hz sinusoidal stimulation waveform was chosen for the present study.

Similar to the work presented here, several investigations focus on the retina itself. Politis, Zanakis, and Albala (1988) showed that chronic DC currents promoted a regenerative response in mammalian optic nerve. Morimoto, Fujikado, and Fukuda (2002) and Morimoto et al. (2010) presented extensive work on the survival rates of axotomized retinal ganglion cells after chronic pulsed biphasic electrical stimulation. Morimoto's group found that transcorneal electrical stimulation (TES) rescued the axotomized retinal ganglion cells, likely by activating endogenous retinal IGF-1 system (Morimoto et al., 2005; Tagami et al., 2009). In a related study, TES was associated with a preservation of outer nuclear layer thickness in RCS rats up to 9 weeks of age (end of study period), but the functional benefit observed in this study was transient (Morimoto et al., 2007). Preservation of the inner nuclear layer in isolated RCS rat retina was associated with delivery of monophasic transretinal stimulation (20 Hz, 2.5–10.7 nC per phase) (Schmid et al., 2009). The TES system was also investigated in patients with ischemic optic neuropathy and traumatic optic neuropathy, and was reported to benefit visual acuity and visual field responses (Fujikado et al., 2006).

Chow et al. (2004) demonstrated clinical evidence that chronic electrical stimulation of the retina in patients blinded by retinitis pigmentosa (RP) results in improved vision. This particular study involved six patients with RP who received a silicon-based chip containing micro-photodiodes placed in the subretinal space. The currents were generated in response to natural illumination of the back of the eye, and were below threshold for directly activating second-order neurons of the diseased retina. The frequency spectrum of the currents were determined by the temporal variations in natural retinal illuminance, which is determined by temporal changes in the visual scene; this can be estimated to be significantly less than 30 Hz, the frequency at which flicker-fusion occurs, and probably close to 1 Hz, a typical frequency for saccadic eye movements. More recently, TES was explored for safety and efficacy in a small population of RP patients that were treated 30 min per week for six weeks (biphasic 20 Hz pulses, amplitudes above and below phosphene threshold), with positive results in visual field and *b*-wave amplitude.

The early results in human patients described above motivated an animal study using subretinal implants based on the same photodiode technology (Pardue et al., 2005). Here, RCS rats received an active implant in one eye, with the opposite eye serving as one of three control types (inactive implant, sham surgery, no surgery). Animals receiving active implants demonstrated a temporary (4–6 weeks post surgery) improvement in function, measured by the ERG b-wave amplitude, which disappeared by 8 weeks post surgery, relative to all three control groups. Animals receiving either an active or passive device demonstrated a relative preservation of photoreceptor cells in the vicinity of the implant, relative to the sham surgery and no surgery control eyes. The currents generated by the photoelectric Chow-type implant were reported to be approximately 0.01–1 $\mu A\,cm^{-2}$ modulated (by the investigators) at 120 Hz under ambient fluorescent light levels, but these numbers are estimates made without consideration of actual retinal illuminance or *in vivo* load impedance seen by the implant. More recently, Ciavatta et al. (2009) found increased FGF2 expression and larger ERG b-waves in RCS rats with subretinal implants, relative to control animals similar to those described above. An extensive micro-array gene expression profiling study was reported for wild-type rats following a single TES session (1 h, 20 Hz biphasic pulses, 200 μA), demonstrating differential expression of several potentially neuroactive genes, in particular the down regulation of proapoptotic *Bax* (Willmann et al., 2011).

2. Methods

2.1. Animals

The P23H transgenic rat mimics autosomal dominant retinitis pigmentosa (adRP) retinal degeneration characteristics (Machida et al., 2000; Hafezi et al., 2000; Marc & Jones, 2003). P23H is a point mutation in the opsin gene in which cysteine is replaced by adenine, leading to the substitution of histodine for proline, at the 23rd position, on the rhodopsin protein. The specific line of animals used in this study, line 1 homozygous P23H rat (on an albino background, provided by Dr. Mathew LaVail, UCSF), were bred with pigmented WT Long Evans rats to produce a pigmented P23H heterozygous model that has a single P23H transgenic allele in addition to the normal two wild-type opsin alleles. In albino heterozygotes, the outer nuclear layer (ONL) degenerates rapidly between 4 and 8 weeks of age, has degenerated to less than 30% of normal counts by 16 weeks, and by 29 weeks the ONL has fewer than two rows of cells remaining, representing less than 10% of normal ONL thickness (Machida et al., 2000). Degeneration rates are somewhat slower for the pigmented animals used in this work, with photoreceptor loss of 40–60% by 16 weeks of age (Sekirnjak et al., 2009).

2.2. Exogenous currents

Transcorneal electrical stimulation (TES) was delivered to the left eye between sintered Ag/AgCl pellet electrodes, one placed centrally on the cornea, and a second oral electrode was placed between the left cheek and gum. Care was taken to ensure that the corneal electrode did not directly contact the cornea surface, but instead made electrical contact through a film of artificial tears (Baush and Lomb).

The exogenous current source was fabricated by adding a voltage-to-current conversion circuit (precision op amp BB OPA27GP) to a standard function generator (BK Precision, model 4011A), which then supplied a controlled 5 Hz sinusoidal current to the range of expected loads, up to the limit of the operational amplifier (4.7 mA). The load is the impedance between the electrodes contacting the rat. This impedance was measured in three rats under conditions of varying degree of wetness of the eye (artificial tears), which influenced the impedance of the electrode-cornea interface, and over a range of frequencies. The impedance was found to vary

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