Published in final edited form as:

Growth Horm IGF Res. 2011 August ; 21(4): 219–227. doi:10.1016/j.ghir.2011.05.008.

Selective inner retinal dysfunction in growth hormone transgenic mice

Brent T. Martin^a, Edward O. List^b, John J. Kopchick^b, Yves Sauvé^{a,c}, and Steve Harvey^{a,*}

^aDepartment of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

^bEdison Biotechnology Institute, Ohio University, Athens, Ohio 45701, USA

^cDepartment of Ophthalmology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

Abstract

Objective—The discovery of locally produced growth hormone (GH) and its receptor in the retina of rodents raises the possibility that GH might modulate retinal function. To test this hypothesis, we determined the retinal electroretinogram (ERG) of bovine GH (bGH) transgenic mice.

Design—ERGs were recorded from 11 wild type (WT) and 9 bGH mice, at 2 months of age in response to a series of light flashes at increasing intensity. Three ERG components were assessed for their amplitude and timing: a-wave, b-wave and oscillatory potentials (OPs). OPs were isolated with a 75–300 Hz digital filter. Retina layer sizes, nuclei number and vascularisation were assessed by respectively staining cross sections with DAPI and Bandeiraea simplicifolia.

Results—OPs were selectively affected in the bGH mouse compared to WT. When OP amplitude values were normalized to the a-wave amplitude (to account for inter-animal variability in WT and bGH groups), OP2, OP3, and OP4 showed amplitude reductions (of 65%, 72%, and 68%, respectively) in the bGH mouse compared to the WT. This was accompanied by a prolongation of the implicit time for the peak of OP3 (28.1 vs 31.1 ms, WT vs bGH) and OP4 (37.8 vs 41.6 ms), while the implicit time of a- and b-waves were unaffected. Fast Fourier transform analysis revealed that the OPs' dominant frequency was significantly reduced ($P < 0.05$) in the bGH mice (100 Hz) compared to WT (108 Hz). There was no significant change in retinal histology except for a significant increase in the axial length of the eye in bGH mice.

Conclusions—Mice expressing bGH display a selective inner retinal defect as demonstrated using ERG recordings. The specific OP defect observed in these mice is similar to the ERG results obtained in patients with diabetic retinopathy and in related animal models.

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding author. Tel.: +1 780 492 2809; fax: +1 780 492 8915. steve.harvey@ualberta.ca (S. Harvey).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

growth hormone; retina; electroretinography; growth factors; diabetic retinopathy; retinopathy of prematurity

1. Introduction

There is accumulating evidence for the role of growth hormone (GH) in the nervous system. Several labs have reported that GH, and its receptor (R), are present throughout the central and peripheral nervous systems [1,2]. Some of the functions attributed to neural GH include developmental alterations [3] and increased cell survival [4]. Additionally, GH can affect neural signalling systems and has neuromodulatory effects in the hippocampus [5]. GH can also be packaged into vesicles as a neurotransmitter and released upon stimulation [6]. To further these studies on the roles of GH in neural signalling, the mouse retina was selected as an extension of the central nervous system. In the retina, neural signalling can be studied by recording mass electrical potentials as a function of light stimulation, with the electroretinogram (ERG).

Like other areas of the CNS, the retina is an acknowledged site of GH production in chicks [7], rats [8], and humans [9]. The retina is also likely to be a site of GH action, since the GHR gene is expressed in retinal tissue, in which GH- and GHR-immunoreactivity are colocalized [10,11]. Actions of GH in retinal function are indicated by comparisons of the retinal proteome in wild-type (WT) and GHR null ($-/-$) mice, which showed alterations in the level of 7 proteins involved in retinal vascularization, neural proliferation and neurite outgrowth [12]. Other evidence for roles of GH in retinal activity comes from clinical studies that indicate GH and insulin-like growth factor-1 (IGF-1) can contribute to the development of diabetic retinopathy [13]. Some of the most compelling evidence for GH activity in the retina comes from Hattori and Fukuchi [14]. They reported that seven 0.5mg doses of ovine GH, administered i.p. over 2 weeks, led to a significant decrease in the amplitude of the ERG a- and b-waves and decreased the implicit time of the oscillatory potentials (OPs). These actions of exogenous GH could therefore reflect neural actions of GH within the neural retina or GH actions on the retinal vasculature, as changes in neural signalling or in retinal perfusion can alter the ERG [15, 16].

To further study possible effects of GH on retinal function we have characterized the ERG in transgenic mice that systemically expresses bovine GH (the bGH mouse) [17].

2. Methods

2.1 Animals

The bGH mice were produced at Ohio Univeristy and the method is described elsewhere [17]. Briefly, the bGH mouse was created by microinjection of a bGH mini gene consisting of a mouse metallothionein transcriptional regulatory element coupled to the first exon and first intron of bGH followed by the remaining cDNA sequence into the pronucleus of C57BL/6J embryos. As the bGH females have poor fertility, heterozygous males were harem bred with 3 normal C57BL/6 female mice from outside the colony (Charles River

Laboratories, Wilmington, MA, USA). Mice with the transgene were identified by PCR analysis of ear-notch biopsies (Forward primer-TGGACAGCTCACCAGCTATG; Reverse – AAGCAGGAGAGCAGACCGTA) and confirmed by having a mass at 2 months of age at least 10 grams greater than wild-type littermates. These mice have chronically high serum levels of bGH, IGF-1 and insulin compared to age-matched littermates [18, 19]. Transgenic male founder mice were bred to C57BL/6J females to generate the offspring used in this study. All animals were housed and handled in accordance with the University of Alberta Animal Policy and Welfare Committee protocols. The animals were maintained on a 12:12 light–dark cycle (lights on at 5 AM; ambient temperature 21°C; relative humidity = 50%) and supplied ad libitum with water and standard rodent diet. 11 WT and 9 bGH animals were studied at 2 months of age for all electroretinogram recordings. For histological studies and blood vessel analysis, 5 WT and 5 bGH mice were utilized. To confirm the expression of the bGH gene, blood samples were obtained from nine 2 month old bGH mice and nine WT mice by cardiac puncture and collected into heparinised tubes. After separation, the plasmas were stored at –80°C analysed for bGH content. The neural retinas were also dissected from the eyes of three 2 month old bGH mice and from three 2 month old WT mice and homogenized in a commercial buffer containing a cocktail of protease inhibitors. After separation, the extracts were also stored at –80°C to await bGH analysis. bGH concentrations in the plasma and tissues samples were determined by a specific radioimmunoassay, using a monkey antibody against bGH (AFP B555), National Hormone and Peptide Program, Torrance, CA, USA). The analysis was performed by MS Susan Cook in the Endocrine Laboratory of the Western College of Veterinary Medicine (WCVN), University of Saskatchewan, Saskatchewan.

2.2 Electroretinogram Recordings

Electroretinogram recordings were performed as previously established in this laboratory [20]. In brief, animals were dark adapted for two hours before being anaesthetized with ketamine (62.5 mg/kg i.p.) and xylazine (12.5 mg/kg i.p.) under dim red lighting. Mouse body temperature was maintained at 38 °C using an electric heating blanket. Pupils were dilated using 1% Tropicamide and then one drop of methylcellulose was added to prevent corneal dehydration and allow electric contact between the gold recording loop and the eye. Reference electrodes were platinum needles (25G, Grass) implanted subcutaneously 2–3mm posterior to the eye, while the ground electrode was a platinum needle inserted subcutaneously in the nape of the neck. Stimuli were white flashes of light (xenon, < 2ms) presented in a ColorDome full field ganzfeld (Diagnosys LLC, Lowell, MA). Responses were recorded at 5000 Hz sampling frequency and amplified from 1-700 Hz with no notch filtering using the Espion E² system (Diagnosys). Recordings lasted for 250 ms after the stimulus was presented, with a baseline of 40 ms before the flash. The scotopic intensity response was recorded in response to flashes ranging from –5.2 to 2.8 log cd s/m², with each flash repeated 3–5 times and the responses averaged together. To allow for rod recovery, inter-stimulus interval ranged from 4 seconds at the dimmest flash to 60 seconds at the brightest flash.

2.3 Electroretinogram Analysis

The b wave amplitude was measured from the a-wave negative peak to the positive peak of the b-wave. If the peak of the b-wave coincided with the oscillatory potentials, the point halfway between the trough and peak of the highest OP was used as the b-wave amplitude. Oscillatory potentials were isolated from the averaged recording traces using a 75-300 Hz digital filter in the Espion E² system. The peaks were numbered following Rousseau and LaChapelle [21] for OPs 2 through 5. Amplitude of the oscillatory potentials was measured from bottom of the trough preceding each OP peak, to the top of that peak. Criterion amplitudes were set as follows, to separate signal from noise: OPs and a-wave: 10 μ V, b-wave: 20 μ V. The response threshold was defined as the minimum stimulus intensity that elicits a response greater than the criterion amplitude. The frequency spectra and dominant frequency of isolated OPs was analyzed by performing a fast Fourier transform (ScopeDSP, Iowegian International) on traces recorded in response to a 2.4 log cd s/m² flash on scotopic background as described in Yu and Peachey [22].

Group data are specified as the mean $\pm$ SEM. Statistical comparison of responses across a range of intensities was performed using repeated-measures one-way analysis of variance (RM-ANOVA) with the General Linear Module of SPSS (SPSS Inc, Chicago, IL). The Greenhouse-Geisser epsilon [22] was applied to correct for violations of sphericity. Group comparisons were made using the student's t-test.

2.4 Histology

Immediately after pentobarbital-induced euthanasia, the eyes of 5WT and 5bGH mice were excised, measured for axial length using a dial calliper, and then fixed overnight in a solution of 4% paraformaldehyde (pH 7.4) at 4°C. Following removal of the cornea and lens, tissues were dehydrated in graduated ethanol steps (50, 70, 95, 100%), cleared (Citri-solv), and embedded (Paraplast). Sections were cut at 6 μ m and only those that included the optic nerve head were used. To stain nuclei, slides were dewaxed (Citri-solv), rehydrated, and then cover slipped using a DAPI containing mounting reagent (ProLong Gold). Images were taken on a fluorescent microscope with appropriate filters and a 40X objective from three areas on the retina. The central window was immediately adjacent to the optic nerve head, the peripheral window was immediately adjacent to the ora serrata, and a middle window was equidistant between the two previous windows. DAPI stained nuclei were counted by hand and measurements of the retina were taken in ImageJ

2.5 Visualization and analysis of retinal vasculature

Additional retinas from each group (5 from WT and 5 from the bGH group) were also processed as flat mounts. Animals were sacrificed as above, enucleated, and the cornea and lens dissected out. The eye cup had four radial incisions made into it before the retina was carefully detached and fixed overnight in a solution of 4% paraformaldehyde (pH 7.4) at 4°C. Retinas were blocked with a mixture of 5% normal goat serum, 5% non-fat milk powder, and 0.1% Triton X-100 for 2 hours at room temperature. Following blocking, retinas were incubated with 10 μ g/mL *Bandeiraea simplicifolia* (Griffonia simplicifolia) TRITC-conjugate Lectin (Sigma) for 72 hours at 4°C to visualize . After extensive washing, retinas were mounted, coverslipped, and imaged on a Zeiss LSM 510 confocal microscope

with a motorized stage. Images were analyzed using a methodology based on that of Kociok *et al* (2009) [23]. Images were opened in ImageJ and a background subtraction operation was performed (rolling ball 50 pixels). A threshold level of fluorescence was then set to distinguish the blood vessels from the background. Total surface area of the retina was measured by creating a section in the image that included the retina but had a border 100 μm from the ora serrata to exclude edge effects. The percentage of the retina covered by vasculature is then defined as the percentage of pixels above the intensity threshold compared to the number of pixels in the total area.

2.6 Statistics

Group data are specified as the mean $\pm$ SEM. Statistical comparison of responses across a range of intensities in the ERG was performed using repeated-measures one-way analysis of variance (RM-ANOVA) with the General Linear Module of SPSS (SPSS Inc, Chicago, IL). The Greenhouse-Geisser epsilon [22] was applied to correct for violations of sphericity. Group comparisons were made using Student's t-test.

3. Results

3.1 bGH Concentrations

While bGH immunoreactivity was not measurable in protein extracts of WT retinas ($<0.01\text{ug/g}$ protein) it was readily measurable at a concentration of $1.07 \pm 0.08\text{ ug/g}$ protein in the retinas of the bGH mice. Similarly, while bGH immunoreactivity was not measurable in the plasma of the 9 WT mice ($<0.6\text{ug/l}$) it was readily measurable at a mean concentrations of $48.9 \pm 8.0\text{ ug/l}$ in the 9 bGH mice (range 28.5 – 105.4 ug/l).

3.2 Morphology

The feature most commonly associated with growth hormone excess is gigantism, and this can be seen in the eyes of bGH mice. When the axial length of the eye was measured, bGH mice were observed to have significantly larger eyes ($3.75 \pm 0.05\text{ mm}$) in comparison to WT animals ($3.04 \pm 0.03\text{mm}$, $P < 0.001$, $n=5$). In contrast, no significant differences were found between bGH and WT mice in measures of retinal width or cell number in the different layers of the retina (Table 1, Fig. 1). Similarly, there was no significant difference in the percentage of the retina occupied by blood vessels between bGH and WT mice (21.6 ± 2.4 versus 19.0 ± 0.7 , respectively, $P > 0.05$).

3.2 ERG a- and b-waves

Representative ERGs under scotopic conditions from a 2 month old bGH mouse (dashed) and WT littermate (solid line) are shown in Fig. 2. The negative a-wave, positive b-wave and OPs became visible at different flash intensities as this value increased from -4.7 to 2.4 log cd s/m^2 . The average luminance required to elicit a detectable a-wave was not significantly different ($P > 0.05$, t-test) between WT (-1.46 ± 0.11) and bGH ($-1.61 \pm 0.13\text{ log cd s/m}^2$) mice (Fig. 3A). Similarly, there was no difference between WT and bGH mice in the luminance threshold for the b-wave (-5.42 ± 0.33 vs $-6.20 \pm 0.43\text{ log cd s/m}^2$, respectively, $P > 0.05$) (Fig. 3B). As flash luminance increased beyond response threshold, the amplitude of the a- and b-waves increased until reaching a plateau with no significant

difference between WT and bGH mice for either wave. Implicit time for the a- and b-waves decreased as a function of stimulus luminance (Fig. 3C,D), again with no significant difference between WT and bGH mice.

3.3 OPs of bGH mice are reduced in amplitude and delayed

The scotopic OPs were distinguished by numbering the peaks 2 through 5 (Fig. 2B). OP1 was not analyzed as it is superimposed upon the a-wave and is small in amplitude [21]. OP3 and OP4 consistently exhibited the largest amplitudes at higher stimulus luminance (Fig. 4B,C), while OP5 was present over the widest range of luminance (Fig. 4D).

None of the OPs displayed a significantly different response threshold between WT and bGH mice ($P > 0.05$). The first OP to be elicited as stimulus luminance increased through the recording session was OP5 (WT; -3.07 ± 0.08 vs bGH; -3.18 ± 0.06 log cd s/m²) followed by OP4 (WT -2.64 ± 0.05 vs bGH -2.64 ± 0.05), OP3 (WT 2.06 ± 0.06 vs bGH -2.12 ± 0.04), and finally OP2 (WT -1.58 ± 0.09 vs bGH -1.44 ± 0.14).

The OPs exhibited different maximum amplitudes as stimulus luminance increased. There was a significant decline in the maximum amplitude of OP2 (WT; 34.0 ± 2.8 vs bGH; 24.2 ± 2.2 μ V, $P < 0.01$), OP3 (WT 100.5 ± 9.9 vs bGH 72.8 ± 6.5 , $P < 0.05$), and OP4 (WT 116.8 ± 10.0 vs bGH 87.7 ± 8.3 , $P < 0.05$) in bGH mice. OP5, however, showed no difference ($P > 0.05$) between WT and bGH animals (73.9 ± 6.6 vs 57.8 ± 6.9 μ V, respectively). Only OP2 showed a significant ($P < 0.05$, RM-ANOVA) difference in mean response over the entire luminance range between WT and bGH mice. Both OP2 ($P < 0.01$, RM-ANOVA) and OP3 ($P < 0.05$) showed a significant interaction between genotype, amplitude, and luminance.

There was no significant difference ($P > 0.05$) in the minimum implicit time for OP2 (WT; 17.6 ± 1.5 vs bGH; 20.6 ± 0.7 ms), OP3 (25.8 ± 2.2 vs 30.8 ± 0.9 ms), OP4 (34.6 ± 3.0 vs 40.6 ± 1.1) or OP5 (42.8 ± 3.7 vs 50.2 ± 1.3) (Fig. 5). In contrast to the minimum implicit time data, there was a significant difference ($P < 0.05$, RM-ANOVA) between bGH and WT mice for the mean implicit time over the entire luminance range for OP3, OP4, and OP5, but not OP2 ($P > 0.05$). None of the OPs showed any significant interaction between genotype, implicit time, and luminance (RM-ANOVA, $P > 0.05$).

3.4 Alterations in the ERG are specific to the OPs and occur following the a wave peak

As the bGH mice have significantly larger eyes than their WT littermates there may be a uniform deviation in all components of the ERG due to changes in resistance across the ERG recording circuit. To account for this, all components of the ERG were normalized against the a-wave amplitude in each animal, as the a-wave appeared to be the least affected and most reliable component. Following normalization, there is no difference in b-wave amplitude between WT (1.51 ± 0.01 , arbitrary values) and bGH (1.53 ± 0.02) mice, however, a significant ($P < 0.05$) reduction in the amplitude of OPs 2, 3, and 4 is observed in bGH mice at a flash intensity of 2.4 log cd s/m² (Fig. 6). The alterations in OP amplitude and timing are therefore not an artifact of a size difference in the eyes of bGH mice.

To analyze the timing of the ERG between bGH and WT mice, any differences in amplitude were removed by normalizing the raw ERG trace data against the a-wave maximum, and

defining this as -1 (Fig. 7). There was no significant difference ($P > 0.05$) between the WT and bGH mice in normalized amplitude between 0 and 18.6ms post-flash. At 18.8 ms post-flash, the WT and bGH traces became significantly different ($P < 0.05$) and remained separate until 20.2 ms, when the lines crossed while going in separate directions. As phototransduction leads to the activation of post-synaptic cells with strict temporal regulation, the point in time where bGH and WT mice showed a significant difference in synchronicity is informative as to where the retinal dysfunction is occurring.

3.5 The dominant OP Frequency is reduced in bGH mice

OPs oscillate within a stereotypical range of frequencies. These frequencies were analyzed using fast Fourier transform algorithms on averaged group data for bGH and WT mice (Fig. 8A). The hill between 80 and 130 Hz is the frequency domain of the OPs. There was a significant reduction ($P < 0.05$) in the peak (arrows, Fig. 8A) of this OP frequency domain for bGH ($96.3 \pm 2.4\text{Hz}$) mice when compared to WT (104.7 ± 2.6) mice (Fig. 8B).

4. Discussion

These results demonstrate that transgenic bGH mice have a selective defect in the oscillatory potentials of the scotopic ERG. The ERGs of WT mice in this study are consistent with recordings of normal C56BL/6 mice by other investigators [24], indicating that the colony does not have any strain specific, inherited retinal abnormalities. The observed reduction in OP amplitudes and slower timing is therefore a consequence of the high bGH levels in mice carrying the bGH transgene. The selectivity of this alteration is evidenced by the lack of any significant difference in the a-wave or b-wave of bGH mice when compared to WT littermates. The OP deficiency is also not a result of a larger eye size in bGH mice, as normalization of ERG amplitude to the a-wave only exaggerates the defect. These data therefore suggest that GH can affect retinal function, either directly through GHR's in the retina, or indirectly through actions on other body systems outside the retina.

4.1 Physiological basis of the OPs

The components of the ERG were first described by Granit [25] and labelled as PI through PIII, but the more recent terminology used in this paper identifies the a-wave, b-wave, and the OPs [Reviewed in 24]. The a-wave is generated by a reduction of the photoreceptor dark current when they hyperpolarize in response to light [25, 26]. The slower b-wave is the result of ON-bipolar cells, post-synaptic to the photoreceptors, activating as the dark current diminishes [27]. The generation of OPs is a complex subject, and efforts to investigate their source have used varying methods. Intraretinal recordings have localized the OPs to the level of the inner plexiform layer [28], so likely cellular candidates are the axons of bipolar cells, the amacrine cells, and the dendrites of ganglion cells. Studies using pharmacological interventions have found that OPs may result from network firing patterns from cells of the inner retina belonging to the ON-pathway [29] with modulation by amacrine cells [30].

4.2 Disease and abnormal OPs

The phenotype of depressed and delayed OPs observed in the bGH mouse is similar to that observed in several clinical conditions. In retinopathy of prematurity, the retinal vasculature

is damaged by exposure to high levels of oxygen and all of the OPs show depressed amplitude [31]. Experimental models for glaucoma, with elevated intraocular pressure and disruption in blood flow, also show marked amplitude reductions in oscillatory potentials [32]. In diabetic retinopathy, abnormalities in the OPs often precede vascular abnormalities and predict the progression of the disease [15, 33]. The commonality between these disease states is the sensitivity of the OPs to retinal vascular damage, to the point that some authors use OPs as indicators of inner vascular function [16].

4.3 GH and diabetic retinopathy

For many years, GH has been suspected as a causative agent in the development of diabetic retinopathy [13]. Diabetic retinopathy is the progressive retinal damage associated with the complications of diabetes, leading to blindness [Reviewed in 34]. The relationship between GH and diabetic retinopathy was first noted by the observation that pituitary ablation correlated with remission of diabetic retinopathy [35]. Retinopathy-like symptoms also occur in GH deficient patients after GH replacement therapy [36]. Conversely, in the absence of GH or in the presence of GH antagonism, retinopathy-like symptoms do not occur [37,38]. Many of these symptoms may result from GH induction of insulin-like growth factor 1 (IGF-1) at the liver or other tissues [39], as IGF-1 is known to induce vascular leakage [40] and neovascularisation in the eye [41]. In addition to the induction of IGF-1, peripheral actions of GH that might contribute to the etiology of diabetic retinopathy include its induction of hyperglycemia [13]. The effect of GH on blood glucose levels is complex, but most reports indicate that GH stimulates an increase in glucose release from the liver which is counter-balanced by increased insulin secretion [42]. This scenario holds true in the bGH mouse, which is normoglycemic but hyperinsulinemic [19]. Thus, despite the normal glucose levels, the raised insulin levels could lead to VEGF induction in the retina and neovascularisation [43].

In addition to actions of GH in the periphery that could induce an inner retinal defect, the diabetic retinopathy-like dysfunction seen in the bGH mice could result from bGH binding GHRs in the retina. The presence of GHRs in the retina [10,12,44] supports the possibility that GH has direct actions within the retina, especially as the GHRs are mostly located in the likely site of OP generation, the inner retina [10, 12].

The GHRs expressed in the retina are likely to have a physiological role, as cells of the inner retina also synthesize GH in neonatal and adult rats [8], and it is thus likely to be an autocrine or paracrine site of GH action. In the bGH mouse, bGH is likely to follow the expression of the metallothionein transcriptional regulatory sequence, which localizes to the nerve fiber layer and the inner plexiform layer [45]. It is unlikely that bGH produced outside the eye has direct actions on the retina, as the permeability of the retinal blood vessels to systemic GH is poor [46] and the vitreal concentration of GH does not correlate well with the serum concentration [47]. It is, however, possible that GHRs are also expressed in the blood vessels of the inner retina, as in some other areas of the cardiovascular system [44], which would provide a location for serum bGH to act.

4.4 GH and the ERG

It is now well established that GH has neural actions in the central nervous system [2,4,48] and GHRs are expressed in tissues of the bGH mouse [49]. It is therefore possible that GH may influence the ERG in the bGH mouse by direct actions mediated through retinal GHRs. Indeed, within the brain, GH is known to modulate the synthesis, release or degradation of adrenergic, serotonergic and cholinergic neurotransmitters [2]. Further, the presence of GHRs in the rodent inner retina [1,12], in sites in which NMDA [50] or AMPA [51] receptors are expressed supports the possibility that GH may directly modulate the signalling of glutamate in the retina [52], as GH does in the mouse hippocampus [5]. This is strengthened by the finding that glutamate signalling is very likely to be involved in the generation of the OPs [53]. GH may also act directly as a neurotransmitter within the nervous system, since it can be packaged into vesicles and released with the same characteristics as neurotransmitters in some rat neuronal cell lines [6]. Moreover, in addition to neurotransmitter or neuromodulatory actions, GH also has neurotrophic actions that may contribute to its modulation of the OPs. It does, for instance, induce axon elongation [54] and is neuroprotective for retinal ganglion cells during embryogenesis and senescence [55, 56]. These neurotrophic effects could have led to developmental alterations in the bGH mouse, as the retina is exposed to high levels of GH from conception onwards.

Our study is not the first to report that GH affects the ERG. A previous study by Hattori and Fukuchi [14] also found changes in the OPs after giving adult mice repeated i.p. GH injections for 2 weeks. In their study, high systemic levels of GH caused a significant depression in the amplitude of the a- and b-wave, which contrasts with our finding of no significant alteration in any aspect of the a- or b-wave in bGH mice. Hattori and Fukuchi also found no change in OP amplitude, while we found a significant decrease in the maximum amplitude of OPs 2,3, and 4. Lastly, Hattori and Fukuchi found that the implicit time of the OPs was decreased in response to GH, while we found the exact opposite in that the implicit time of our OPs increased. These results are difficult to reconcile, but perhaps relate to the time course and route of administration. In our study with transgenic animals, bGH mice were exposed to high levels of GH in both the eye and the serum from conception onwards, as opposed to the study of Hattori and Fukuchi, in which mouse development was in the absence of exogenous GH. The duration of the study also differed, as bGH mice were exposed to high levels of GH for 2 months after birth, while mice treated by Hattori and Fukuchi only experienced high GH levels for 2 weeks and only in the serum, suggesting it would be unlikely that GH had any direct action on the retina. Moreover, our preliminary study with normal mice demonstrated that exogenous GH has no acute ERG response after the intravitreal injection of mouse GH [57]. It is thus likely that over the short term (weeks), the metabolic and hyperglycaemic effects of exogenous GH stimulates the OPs and shortens their implicit time. However, persistent GH stimulation, coupled with heightened levels of insulin and IGF-1, may lead to retinal damage and the observed slowing of the OPs in bGH mice.

In summary, bGH mice show a selective defect in the OPs of the scotopic ERG. This defect may result from direct effects of GH within the neural retina or from indirect effects mediated peripherally.

Acknowledgments

Research supported by NSERC and CIHR; BTM was supported by NSERC and AHFMR scholarships; JJK is supported in part by the State of Ohio's Eminent Scholars Program that includes a gift from Milton and Lawrence Goll and by NIH R15DK075436, R01AG019899, World Anti Doping Agency, and Ohio University's Diabetes Research Initiative; YS is supported by AHFMR Senior Scholarship. The authors would like to thank Susan Cook (Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan) for the bGH analysis of the mouse tissue and blood plasmas.

References

1. Lobie PE, García-Aragón J, Lincoln DT, Barnard R, Wilcox JN, Waters MJ. Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. *Brain Res Dev Brain Res*. 1993; 74:225–233.
2. Harvey S, Hull K. Neural growth hormone: an update. *J Mol Neurosci*. 2003; 20:1–14. [PubMed: 12663929]
3. Noguchi T. Effects of growth hormone on cerebral development: morphological studies. *Horm Res*. 1996; 45:5–17. [PubMed: 8742112]
4. Scheepens, Sirimanne ES, Breier BH, Clark RG, Gluckman PD, Williams CE. Growth hormone as a neuronal rescue factor during recovery from CNS injury. *Neuroscience*. 2001; 104:677–687. [PubMed: 11440801]
5. Mahmoud GS, Grover LM. Growth hormone enhances excitatory synaptic transmission in area CA1 of rat hippocampus. *J Neurophysiol*. 2006; 95:2962–2974. [PubMed: 16481459]
6. Schweitzer ES, Kelly RB. Selective packaging of human growth hormone into synaptic vesicles in a rat neuronal (PC12) cell line. *J Cell Biol*. 1985; 101:667–676. [PubMed: 4019588]
7. Baudet ML, Sanders EJ, Harvey S. Retinal growth hormone in the chick embryo. *Endocrinology*. 2003; 144:5459–5468. [PubMed: 12960021]
8. Harvey S, Baudet ML, Sanders EJ. Retinal growth hormone in perinatal and adult rats. *J Mol Neurosci*. 2006; 28:257–264. [PubMed: 16691013]
9. Harvey S, Parker E, Macdonald I, Sanders EJ. Growth hormone is present in the human retina and vitreous fluid. *Neurosci Lett*. 2009b; 455:199–202. [PubMed: 19429121]
10. Baudet ML, Rattray D, Harvey S. Growth hormone and its receptor in projection neurons of the chick visual system: retinofugal and tectobulbar tracts. *Neuroscience*. 2007; 148:151–163. [PubMed: 17618059]
11. Harvey S, Martin BT, Baudet ML, Davis P, Sauve Y, Sanders EJ. Growth hormone in the visual system: comparative endocrinology. *Gen Comp Endocrinol*. 2007; 153:124–131. [PubMed: 17303134]
12. Baudet ML, Hassanali Z, Sawicki G, List EO, Kopchick JJ, Harvey S. Growth hormone action in the developing neural retina: a proteomic analysis. *Proteomics*. 2008; 8:389–401. [PubMed: 18203262]
13. Frystyk J. The growth hormone hypothesis - 2005 revision. *Horm Metab Res*. 2005; 37(Suppl 1): 44–48. [PubMed: 15918110]
14. Hattori M, Fukuchi S. Studies on ERG of KK mice especially on oscillatory potentials. 3. Influence of ovine growth hormone. *Nippon Ganka Gakkai Zasshi*. 1974; 78:705–714. [PubMed: 4472649]
15. Bresnick GH, Korth K, Groo A, Palta M. Electroretinographic oscillatory potentials predict progression of diabetic retinopathy. preliminary report. *Arch Ophthalmol*. 1984; 102:1307–1311. [PubMed: 6383303]
16. Vadalà M, Anastasi M, Lodato G. Transient reduction of the ocular perfusion pressure and the oscillatory potentials of the ERG. *Vision Res*. 2005; 45:1341–1348. [PubMed: 15733966]
17. Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ. Comparing adiposity profiles in three mouse models with altered GH signalling. *Growth Horm IGF Res*. 2004; 14:309–318. [PubMed: 15231300]

18. McGrane MM, de Vente J, Yun J, Bloom J, Park E, Wynshaw-Boris A, Wagner T, Rottman FM, Hanson RW. Tissue-specific expression and dietary regulation of a chimeric phosphoenolpyruvate carboxykinase/bovine growth hormone gene in transgenic mice. *J Biol Chem.* 1988; 263:11443–11451. [PubMed: 2841327]
19. Balbis, Dellacha JM, Calandra RS, Bartke A, Turyn D. Down regulation of masked and unmasked insulin receptors in the liver of transgenic mice expressing bovine growth hormone gene. *Life Sci.* 1992; 51:771–778. [PubMed: 1513204]
20. Suh M, Sauvé Y, Merrells KJ, Kang JX, Ma DW. Supranormal electroretinogram in fat-1 mice with retinas enriched in docosahexaenoic acid and n-3 very long chain fatty acids (C24-C36). *Invest Ophthalmol Vis Sci.* 2009; 50:4394–4401. [PubMed: 19264893]
21. Rousseau S, Lachapelle P. The electroretinogram recorded at the onset of dark-adaptation: understanding the origin of the scotopic oscillatory potentials. *Doc Ophthalmol.* 1999; 99:135–150. [PubMed: 11097118]
22. Yu M, Peachey NS. Attenuation of oscillatory potentials in nob2 mice. *Doc Ophthalmol.* 2007; 115:173–186. [PubMed: 17479213]
23. Kociok N, Radetzky S, Krohne TU, Gavranic C, Liang Y, Semkova I, Jousen AM. ICAM-1 depletion does not alter retinal vascular development in a mouse model of oxygen-mediated neovascularisation. *Exp Eye Res.* 2009; 89:503–510. [PubMed: 19482023]
24. Weymouth AE, Vingrys AJ. Rodent electroretinography: methods for extraction and interpretation of rod and cone responses. *Prog Retin Eye Res.* 2008; 27:1–44. [PubMed: 18042420]
25. Granit R. The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *J Physiol.* 1933; 77:207–239. [PubMed: 16994385]
26. Kang Derwent JJ, Linsenmeier RA. Intraretinal analysis of the a-wave of the electroretinogram (ERG) in dark-adapted intact cat retina. *Vis Neurosci.* 2001; 18:353–363. [PubMed: 11497412]
27. Stockton RA, Slaughter MM. B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol.* 1989; 93:101–122. [PubMed: 2915211]
28. Ogden TE, Wylie RM. Avian retina I. Microelectrode depth and marking studies of local ERG. *J Neurophysiol.* 1971; 34:357–366. [PubMed: 5560037]
29. Dong CJ, Agey P, Hare WA. Origins of the electroretinogram oscillatory potentials in the rabbit retina. *Vis Neurosci.* 2004; 21:533–543. [PubMed: 15579219]
30. Möller, T Eysteinnsson. Modulation of the components of the rat dark-adapted electroretinogram by the three subtypes of GABA receptors. *Vis Neurosci.* 2003; 20:535–542. [PubMed: 14977332]
31. Liu K, Akula JD, Hansen RM, Moskowitz A, Kleinman MS, Fulton AB. Development of the electroretinographic oscillatory potentials in normal and ROP rats. *Invest Ophthalmol Vis Sci.* 2006; 47:5447–5452. [PubMed: 17122135]
32. Grozdanic SD, Betts DM, Sakaguchi DS, Allbaugh RA, Kwon YH, Kardon RH. Laser-induced mouse model of chronic ocular hypertension. *Invest Ophthalmol Vis Sci.* 2003; 44:4337–4346. [PubMed: 14507878]
33. Vadalà M, Anastasi M, Lodato G, Cillino S. Electroretinographic oscillatory potentials in insulin-dependent diabetes patients: A long-term follow-up. *Acta Ophthalmol Scand.* 2002; 80:305–309. [PubMed: 12059871]
34. Wilkinson-Berka JL, Wraight C, Werther G. The role of growth hormone, insulin-like growth factor and somatostatin in diabetic retinopathy. *Curr Med Chem.* 2006; 13:3307–3317. [PubMed: 17168853]
35. Lundbaek K, Jensen VA, Olsen TS, Orskov H, Christensen NJ, Johansen K, Hansen AP, Osterby R. Growth hormone and diabetic angiopathy. *Lancet.* 1970; 2:472. [PubMed: 4195153]
36. Hansen R, Koller EA, Malozowski S. Full remission of growth hormone (GH)-induced retinopathy after GH treatment discontinuation: long-term follow-up. *J Clin Endocrinol Metab.* 2000; 85:2627. [PubMed: 10902819]
37. Merimee TJ. A follow-up study of vascular disease in growth-hormone-deficient dwarfs with diabetes. *N Engl J Med.* 1978; 298:1217–1222. [PubMed: 651962]
38. Smith LE, Kopchick JJ, Chen W, Knapp J, Kinose F, Daley D, Foley E, Smith RG, Schaeffer JM. Essential role of growth hormone in ischemia-induced retinal neovascularisation. *Science.* 1997; 276:1706–1709. [PubMed: 9180082]

39. Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. *Endocr Rev.* 2001; 22:53–74. [PubMed: 11159816]
40. Saishin Y, Saishin Y, Takahashi K, Melia M, Vinore SA, Campochiaro PA. Inhibition of protein kinase C decreases prostaglandin-induced breakdown of the blood-retinal barrier. *J Cell Physiol.* 2003; 195:210–219. [PubMed: 12652648]
41. Grant MB, Mames RN, Fitzgerald C, Ellis EA, Aboufrikha M, Guy J. Insulin-like growth factor I acts as an angiogenic agent in rabbit cornea and retina: comparative studies with basic fibroblast growth factor. *Diabetologia.* 1993; 36:282–291. [PubMed: 7682976]
42. Scanes, CG. *Growth Hormone*. CRC Press; Boca Raton, Florida: 1995. p. 371
43. Chantelau E, Kimmerle R, Meyer-Schwickerath R. Insulin, insulin analogues and diabetic retinopathy. *Arch Physiol Biochem.* 2008; 114:54–62. [PubMed: 18465359]
44. Lobie PE, García-Aragón J, Wang BS, Baumbach WR, Waters MJ. Cellular localization of the growth hormone binding protein in the rat. *Endocrinology.* 1992; 130:3057–3065. [PubMed: 1374020]
45. Suemori S, Shimazawa M, Kawase K, Satoh M, Nagase H, Yamamoto T, Hara H. Metallothionein, an endogenous antioxidant, protects against retinal neuron damage in mice. *Invest Ophthalmol Vis Sci.* 2006; 47:3975–3982. [PubMed: 16936113]
46. James CR, Cotlier E. Fate of insulin in the retina: an autoradiographic study. *Br J Ophthalmol.* 1983; 67:80–88. [PubMed: 6336953]
47. Modanlou HD, Gharraee Z, Hasan J, Waltzman J, Nageotte S, Beharry KD. Ontogeny of VEGF, IGF-I, and GH in neonatal rat serum, vitreous fluid, and retina from birth to weaning. *Invest Ophthalmol Vis Sci.* 2006; 47:738–744. [PubMed: 16431975]
48. Aberg ND, Carlsson B, Rosengren L, Oscarsson J, Isaksson OG, Rönnbäck L, Eriksson PS. Growth hormone increases connexin-43 expression in the cerebral cortex and hypothalamus. *Endocrinology.* 2000; 141:3879–3886. [PubMed: 11014245]
49. Iida K, Del Rincon JP, Kim DS, Itoh E, Nass R, Coschqano KT, Kopchick JJ, Thorner MO. Tissue-specific regulation of growth hormone (GH) receptor and insulin-like growth factor-1 gene expression in the pituitary and liver of GH-deficient (Lit/Cit) mice and transgenic mice that overexpress bovine GH (bGH) or a bGH antagonist. *Endocrinology.* 2004; 145:1564–1570. [PubMed: 14726438]
50. Watanabe M, Mishina M, Inoue Y. Differential distributions of the NMDA receptor channel subunit mRNAs in the mouse retina. *Brain Res.* 1994; 634:328–332. [PubMed: 7510577]
51. Zhang C, Hammassaki-Britto DE, Britto LR, Duvoisin RM. Expression of glutamate receptor subunit genes during development of the mouse retina. *Neuroreport.* 1996; 8:335–340. [PubMed: 9051806]
52. Brandstätter JH, Koulen P, Wässle H. Diversity of glutamate receptors in the mammalian retina. *Vision Res.* 1998; 38:1385–1397. [PubMed: 9667006]
53. Matthews GP, Crane WG, Sandberg MA. Effects of 2-amino-4-phosphonobutyric acid (APB) and l-cysteine on the oscillatory potentials of the rat electroretinogram. *Exp Eye Res.* 1989; 49:777–787. [PubMed: 2591494]
54. Baudet ML, Rattray D, Martin BT, Harvey S. Growth hormone promotes axon growth in the developing nervous system. *Endocrinology.* 2009; 150:2758–2766. [PubMed: 19213842]
55. Harvey S, Baudet ML, Sanders EJ. Growth hormone-induced neuroprotection in the neural retina during chick embryogenesis. *Ann N Y Acad Sci.* 2009a; 1163:414–416. [PubMed: 19456374]
56. Sanders EJ, Parker E, Harvey S. Endogenous growth hormone in human retinal ganglion cells correlates with cell survival. *Mol Vis.* 2009; 15:920–926. [PubMed: 19421410]
57. Martin, BT.; Mema, S.; Sauve, Y.; Harvey, S. Functional Roles for Growth Hormone in the Mouse Retina [abstract]. Proceedings of the 2008 Annual Meeting of the Association for Research in Vision and Ophthalmology; Ft. Lauderdale, FL. p. Abstract nr 2004

Fig 1. Representative DAPI-stained sections of neural retina from 2 month old WT and bGH mice. The sections reflect central (cent) areas adjacent to the optic nerve, peripheral (per) areas at the edge of the retina and a mid point (mid) between these two areas. Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer.

Fig 2.

A. Representative ERG traces from WT (solid line) and bGH (dashed line) mice at 2 months of age in response to increasing flash luminance (log cd s/m²). The left panel filters the trace from 0.3 to 300 Hz to include all components of the ERG, while the right panel is band-pass filtered from 75 to 300 Hz to isolate the oscillatory potentials (OPs). B. Inset showing the oscillatory potentials (2–5) with a delay and reduction of amplitude apparent in bGH mice compared to WT. Bold numbers are OP peaks of WT mice, while italic numbers are OP peaks of bGE mice

Fig 3.

Scotopic intensity response of the ERG showing the averaged peak amplitude (A,B) and implicit time (C,D) of the a- and b-wave as flash luminance increases. Data for 11 WT (filled points) and 9 bGH mice (open points). bGH mice showed no significant alterations in the maximum amplitude, mean amplitude over the entire stimulus range, or in the implicit time of the a- and b-wave. The a-wave is reported as an absolute value, rather than a negative value. Data points indicate mean $\pm$ SEM.

Fig 4. Scotopic intensity response of the ERG showing the amplitude of the oscillatory potentials in response to flash luminance. The oscillatory potentials 2-5 (A-D) were analyzed individually for amplitude by measuring from the preceding trough to the peak of that OP. Data points indicate mean $\pm$ SEM. * indicates a significant ($P < 0.05$) reduction in maximum amplitude.

Fig 5.

Scotopic intensity response of the EERG showing the implicit time of the oscillatory potentials in response to flash luminance. The oscillatory potentials 2-5 (A-D) were analyzed individually for implicit time by measuring the delay between the flash and the peak of that specific OP. Data points indicate mean $\pm$ SEM. * indicates a significant (P < 0.05) difference in the mean implicit time over the range of the line.

Fig 6. Amplitude of ERG components normalized to the a-wave amplitude within each animal. Filled bars represent WT mice, open bars are bGH mice. Flash intensity was 2.4 log cd s /m². * P < 0.05. Means $\pm$ SEM.

Fig 7.

Averaged ERG traces where amplitude is normalized to the negative amplitude of the a-wave peak. Thick solid and dashed lines represent mean values for WT and bGH animals, respectively. Thin lines indicate SEM. * indicates sample points in the shaded region are significantly different ($P < 0.05$) between WT and bGH animals.

Fig 8.

Comparing WT and bGH frequency domains. A. Average frequency spectra obtained in response to a 2.4 log cd s/m² flash on scotopic background for WT (solid line) and bGH mice (dashed line). Arrow (WT) and arrowhead (bGH) show the dominant OP frequency. B. Graph quantifying the dominant OP frequency in WT (filled) and bGH (open) mice. Means $\pm$ SEM.

Table 1

Similarity in morphological features between the ssretinas of WT and bGH mice.

	Central*		Middle*		Peripheral*	
	WT	bGH	WT	bGH	WT	bGH
Rows of PR Nuclei	10.5 ± 0.4	10.8 ± 0.4	10 ± 0.3	9.8 ± 0.5	7.3 ± 0.6	7.5 ± 0.4
INL Nuclei ^a	85.5 ± 3.1	79.8 ± 2.1	71 ± 4.6	74 ± 5.6	56 ± 7.3	55 ± 4.5
GCL Nuclei ^a	17.8 ± 1.9	17 ± 0.6	10.5 ± 2.2	12 ± 0.4	10.5 ± 1.0	10.3 ± 1.9
IPL Width (µm)	45.5 ± 1.4	47.6 ± 2.8	38.6 ± 2.7	39.8 ± 1.7	32.5 ± 2.8	30.4 ± 2.4
Retinal Width (µm) ^b	153.4 ± 7.2	150.7 ± 5.1	132 ± 7.4	129 ± 6.0	103.7 ± 10.1	102.5 ± 7.1

* measurements were made in three areas of each retina, a central window adjacent to the optic nerve, a peripheral area near the end of the retina, and a middle area that was at the mid-point between these two windows

^a number of cells in a 100 µm² window

^b measured from inner limiting membrane to external limiting membrane

Abbreviations: Photoreceptors (PR), inner nuclear layer (INL), ganglion cell layer (GCL), inner plexiform layer (IPL). Values represent mean ± SEM (n=5).