The Gradient of Retinal Functional Changes during Acute Intraocular Pressure Elevation

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PURPOSE. To characterize retinal function during a period of acutely elevated intraocular pressure (IOP) across a wide range of IOPs, including those typically observed in animals with experimental glaucoma.

METHODS. Unilateral elevation of IOP was achieved manually in adult Brown Norway rats (nine experimental groups; n = 4–7 in each; 10–100 mm Hg and sham control). Full-field ERGs were recorded simultaneously from treated and control eyes, beginning 75 minutes after IOP elevation. Scotopic ERG stimuli were brief white flashes (−6.1 to 2.7 log cd-s/m²). Photopic ERGs were recorded (1.2–2.7 log cd-s/m²) after 15 minutes of light adaptation (150 cd/m²). Relative amplitude (treated/control, %) of ERG components versus IOP was described with a cumulative normal function.

RESULTS. Resting IOP was 12.1 ± 2.8 mm Hg and mean femoral artery pressure was 97.6 ± 10.7 mm Hg. ERG components showed a graded effect dependent on IOP. Systematic delays in the timing of the scotopic threshold response (STR) and photopic b-wave were observed between IOPs of 30 and 40 mm Hg. Analysis of amplitudes revealed that the negative STR component (nSTR) and the photopic OPs were the most sensitive to acute IOP elevation. These components were first significantly affected at 50 mm Hg, whereas all parameters of middle and outer retinal function (scotopic P2 and P3) remained normal. The nSTR and photopic OPs declined by 50% at IOP < 61 mm Hg. The scotopic P2, OPs, and positive STR (pSTR) had intermediate sensitivity, such that they were reduced by 50% at IOPs between 61 and 66 mm Hg. Scotopic P2 amplitude, but not sensitivity, was significantly reduced by 60 mm Hg. At 60 and 70 mm Hg, the decline in P2 amplitude was not attributable to changes in photoreceptor response (P3) amplitude or sensitivity. The least sensitive component was the scotopic a-wave (RM50) showing a 50% reduction at an IOP of 71 mm Hg.

CONCLUSIONS. During acute IOP elevation, functional changes progress from the proximal to the distal retina. Alterations in ganglion-cell–related ERG potentials occurred at IOPs (30–50 mm Hg) commonly observed in rat experimental glaucoma models. Nonspecific functional changes were observed at acute IOP above 50 mm Hg, suggesting that IOP should be maintained below this level in experimental glaucoma models if selective ganglion cell injury is to be sought. Repeated IOP spikes above this level may cause permanent, nonspecific dam-

Elevated intraocular pressure (IOP) is one of the most important risk factors for development of glaucomatous optic neuropathy (GON). In fact, reduction of IOP by medical and/or surgical means continues to be the mainstay of therapy for glaucoma. Despite successful treatment to lower IOP, some individuals still experience continued progression of GON and associated visual field loss. One plausible hypothesis is that acute IOP spikes or transient elevations that go undetected during routine clinical examination lead to disease progression. It is known that IOP has diurnal fluctuations; higher IOPs are typically recorded during late night or early morning hours. Moreover, IOP fluctuations are thought to be even larger in patients with glaucoma. Some studies have even suggested that large diurnal IOP fluctuations may be an independent risk factor for the development of glaucoma.

Variability of IOP is also a problem in both primate and rodent models of experimental glaucoma. Greater variability in IOP has been observed in primates and rat models of chronically elevated IOP. For example, Jia et al. have reported that after episcleral hypertonic saline injection, 6 of 17 rats had persistent, large IOP elevations during the nocturnal phase, but normal pressures during the light phase. More importantly, optic nerve injury was observed in four of these six rats, demonstrating that repeated IOP spikes could result in optic nerve damage, but remain undetected if assessment is limited to the light phase. Jia et al. have stressed the importance of frequent IOP measurements to monitor this critical experimental variable more accurately. Further, they have shown that diurnal IOP fluctuations can be substantially reduced by housing animals in a constant low-light environment.

It is difficult to discriminate between the effects of chronic mild-to-moderate elevation of IOP and those that may be due specifically to intermittent overlying acute IOP spikes. Consequently, few investigators in studies of experimental glaucoma in rats have attempted to do so. Chauhan et al. have shown in a rat model of chronic IOP elevation that structural and functional changes share a closer relationship with peak IOP than the number of days of IOP elevation or cumulative time-IOP integral. This suggests that IOP spikes may be more significant to the development of neuropathy. However, the effect of an acute IOP spike on retinal function is not known at the IOPs commonly observed in experimental models.

In contrast, the functional consequences of short-term complete retinal ischemia have been thoroughly documented by investigators studying the ischemia-reperfusion model. However, these models generally involve levels of IOP that completely suppress ocular blood flow. Under these circumstances, retinal function, as measured with the electroretinogram (ERG), is typically reduced to zero during the period of ischemia. In experimental rodent models of glaucoma, the IOPs observed, even during acute spikes, are rarely (if ever) high enough to occlude retinal blood flow completely. Therefore, the purpose of this study was to evaluate retinal function during a period of acutely elevated IOP and to evaluate a wide...
range of IOPs, including those typically observed in animals with experimental glaucoma, up through those that cause complete ischemia.

**MATERIALS AND METHODS**

**Subjects**

All experimental methods and animal care procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Legacy Institutional Animal Care and Use Committee. Adult Brown-Norway rats between 10 and 12 weeks of age (180–260 g, Charles River Laboratories Inc., Wilmington, MA) were maintained in a 22°C, 12-hour light (<40 lux)/12-hour dark environment. Normal rat chow and water were available ad libitum.

**Acute IOP Elevation**

Animals were dark-adapted overnight (>12 hours) and prepared for cannulation and ERG recording under dim red light (λ > 600 nm). Animals were anesthetized with an intramuscular thigh injection of a mixture of ketamine (55 mg/kg−1, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA), xylazine (5 mg/kg−1, X-ject E; Phoenix Scientific, Inc., St. Joseph, MO), and acepromazine maleate (1 mg/kg−1, Acepet; Phoenix Scientific, Inc.). Additional anesthesia was provided via the same route at 45-minute intervals (ketamine-xylazine-acepromazine 30:2:1 mg/kg). Sufficient depth of anesthesia was sensitively monitored online by inspection of the ERG signal baseline.

**IOP and Blood Pressure under Anesthesia**

IOP was measured in a group of seven naïve adult Brown-Norway rats under the anesthesia conditions described earlier, using a calibrated hand-held tonometer (TonoPen 2; Mentor, Norwell, MA) after 1 drop of topical proparacaine hydrochloride (0.5%; Alcon Laboratories, Inc.). The IOP for each eye was taken as the average of 10 sequential measures. The mean IOP (± SD) in this group was 12.1 ± 2.8 mm Hg (range, 8.5–15.6). This is consistent with prior findings in adult Brown-Norway rats under ketamine anesthesia.21

Mean arterial blood pressure was measured in 12 naïve, anesthetized rats by cannulation of the femoral artery with a section of polyethylene tubing (0.38 mm inner diameter) connected to a pressure transducer (MX860) and monitoring system (ProPaq Encore, model 206EL; Protocol Systems, Inc.). After 5 minutes of stabilization, mean arterial pressure was sampled every 4 seconds for 5 minutes. The data over this period were averaged. We found that the average (± SD) femoral artery blood pressure was 97.6 ± 10.7 mm Hg (range, 80–120).

**Electroretinography**

Full-field ERGs were recorded simultaneously from both eyes, as previously described22 (UTAS-E3000 system; LKC Technologies, Gaithersburg, MD). Simultaneous recording allowed ERGs to be obtained from the control and treated eyes under identical states of anesthesia and adaptation. Eyes were lubricated after electrode placement and periodically throughout the session with 1.0% carboxymethylcellulose sodium (Celluvisc; Allergan, Irvine, CA). Signals were recorded with band-pass settings of 0.3 to 30 Hz for the STR responses and 0.3 to 500 Hz for all other responses. Signals were digitized at 1 and 2 kHz for STRs and all other responses, respectively.

Stimuli were bright flashes (xenon arc discharge, x = 0.32, y = 0.53) delivered via a Ganzfeld integrating sphere. Stimulus intensities were measured using a calibrated photometer (Spectra Pritchard PR-1980B; Photograph Research, Chatsworth, CA) with a (human) scotopic luminosity filter in place.

After a 10-minute stabilization period, a pair of baseline ERG responses was recorded that included a scotopic threshold response (STR, ~5.55 log cd·s/m²) and a scotopic response to a single flash of slightly brighter intensity (~0.78 log cd·s/m²). The IOP was then increased to the target level, and the same pair of ERG responses was recorded every 5 minutes for 75 minutes. After 75 minutes, ERG responses for a more extensive protocol were recorded: STR responses were obtained for flash intensities ranging from ~6.04 to ~5.36 log cd·s/m² in 0.24 log unit increments, by averaging 40 to 60 responses per intensity (60 for the dimmest and 40 for the higher intensities), with an interstimulus interval of 2 seconds. Scotopic ERGs obtained for intensities between ~3.30 and 2.72 log cd·s/m² were recorded as single-
flash responses. At these stimulus intensities, the interval between flashes was progressively lengthened from 10 to 120 seconds to allow complete recovery of the b-wave. After completion of the scotopic ERG intensity series, animals were light adapted for 15 minutes to a steady white background (150 cd/m²). Light-adapted flash responses were recorded for intensities between 1.22 and 2.72 log cd-s/m² in 0.5-log-unit increments. Each record was an average of 20 responses obtained with a 2-second interstimulus interval.

Data Analysis

Scotopic Threshold Response. Analysis of the STR is shown in Figure 1A. The amplitude of the positive scotopic threshold response (pSTR) was measured from baseline to the initial peak, and the amplitude of the negative (n)STR was measured from the baseline to the trough after the pSTR. For comparison between treated and control eyes, the average relative amplitude (treated/control, %) between /H11002 5.71 and /H11002 5.36 log cd-s/m² was evaluated for both the pSTR and nSTR.

Photoreceptor Response. The time course of the initial portion of the photoreceptor a-wave is well described with a delayed Gaussian function as given by equation 1.23–25

In equation 1, P₃ is the sum of the photocurrent from the massed light response of the rod photoreceptors. It is expressed as a function of stimulus intensity (I, cd-s/m²) and time after the stimulus flash (t, in seconds). With this model, the P₃ response at a given time (t) after a stimulus flash of intensity (I) is determined by two parameters: the maximum response amplitude (Rₘ₃, in microvolts) and a gain parameter (S, m² cd⁻¹/s). The delay term (t₃, in seconds), which incorporates both biochemical and other recording latencies, was fixed at 3.33 ms, which represents the average delay determined by floating all parameters in 12 control eyes. For each eye in this study, these two parameters of rod photoreceptor function were estimated by fitting the P₃ model (Fig. 1B, solid curves) to the family of responses obtained for the 4 brightest flash intensities (0.10 to 2.72 log cd-s/m²). An example is shown in Figure 1B (raw data, symbols; P₃ model, solid curves). Optimization of each fit was achieved by minimization of the root mean square (RMS) error term on computer (Solver module in Excel; Microsoft Corp., Redmond, WA).

Rod Bipolar Cell Response: Scotopic P₂. Prior studies have shown that the scotopic b-wave of the rat is primarily driven by the rod bipolar cells.26 It has also been shown that derived P₂ responses provide a better indication of inner nuclear layer (INL) activity than do b-wave measures.27 Thus, the scotopic P₂ was isolated for each eye in this study to quantify the effects of acutely elevated IOP on the rod bipolar cells.27 First, the P₃ component, estimated by the method just described, was subtracted from the raw ERG responses.27 The result was a family of scotopic P₂ responses with OPs intact but a-waves removed (Fig. 1C, see the Appendix). The OPs were then isolated (Fig. 1D) using a band-pass filter (−3 dB at 50 and 280 Hz) and

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933230/)
subtracted from the P2-OP complex. The peak amplitude of the resultant P2 component, isolated for each stimulus intensity between -3.3 and 2.72 log cd·s/m² was measured from the prestimulus baseline, as shown by the open symbols in Figure 1E. The P2 amplitude was plotted versus stimulus intensity and fitted with a hyperbolic function (equation 2), as shown in Figure 1F.26,29

\[ V = V_{\text{max}} \cdot \frac{1}{1 + (I^n + K^n)} \]  

(2)

In equation 2, the P2 voltage response \( V \) for a given stimulus intensity \( I \) is determined by three parameters: the maximum response amplitude \( V_{\text{max}} \) (µV), a sensitivity parameter \( K \) (log cd·s/m²) that indicates the intensity of the stimulus at half \( V_{\text{max}} \), and an exponent \( n \) that determines the slope of the linear portion of the function (see the Appendix). Estimation of these three parameters for each eye in the study was achieved by minimization of the RMS error (Solver, Excel; Microsoft Corp.). There are other positive potentials arising from the proximal retina, including the pSTR, whose contributions to the rod-ERG are very small relative to P2 over this intensity range.22,30,31 Their effect on the derived P2 parameters \( V_{\text{max}} \) and \( K \) is negligible when the fit of equation 2 is confined to this intensity range.35

Photopic Response. As in previous studies, the rat photopic ERGs contained a very small a-wave component. Thus, the photopic b-wave was isolated simply by removing the band-pass filtered OPs (−3 dB at 50 and 280 Hz). The amplitudes were then evaluated from baseline to the peak. Average relative amplitude between treated and control eyes was assessed for all intensities from 1.72 to 2.72 log cd·s/m².

Oscillatory Potentials. Figure 1D shows an example of a band-pass-filtered, raw ERG (−3 dB at 50 and 280 Hz) to isolate the scotopic OPs. The amplitudes of both scotopic and photopic OPs were measured by summing the RMS amplitude of the entire OP complex, beginning at the trough preceding the first OP and ending at the trough after the last OP. For comparison between treated and control eyes, the average relative amplitude (treated/control, %) was averaged over a range of intensities for photopic (0.72–2.72 log cd·s/m²) and photopic OPs (1.72–2.72 log cd·s/m²).

Retinal Function Versus IOP. The amplitude of each ERG component (Rhodopsin, P2, \( V_{\text{max}} \), scotopic OPs, photopic b-wave, photopic OPs, pSTR, and nSTR) was expressed as a percentage of that in the fellow control eye. The mean relative amplitude was calculated for each IOP group and plotted as a function of IOP for each ERG parameter. An example is shown in Figure 1G. The open symbols plot the mean relative amplitude for each IOP group. The solid curve through the data represents the best fit (least RMS error) of an inverse cumulative normal function (equation 3). The parameter \( \mu \) dictates the position of the inverse cumulative normal function and thus provides a measure of sensitivity to IOP elevation. The parameter \( \sigma \) describes the steepness of the transition portion of the function.36

\[ f(x) = 1 - \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{(x - \mu)^2}{2\sigma^2}} \]  

(3)

Sensitivity to IOP Elevation: Comparisons between ERG Components. To compare the sensitivity of the various ERG components to acutely elevated IOP, a bootstrap procedure35 was implemented (in Excel, using Visual Basic: Microsoft Office X; Microsoft Corp.). The bootstrap technique was used to generate estimates of the mean and 95% limits of agreement for both the sensitivity parameter \( \mu \) and the slope parameter \( \sigma \) for each ERG component. Similar bootstrap techniques have been applied to the estimation of the parameters of transition functions such as in equation 3 and their variability.34

In the current implementation, each iterative bootstrap sample consisted of \( n \) random selections taken (with replacement) from the available data in each IOP group (whereby \( n = n \) for each group). The sample mean was then calculated for each group and plotted as a function of IOP. The sample data were fitted with the inverse cumulative normal, as described earlier, and the parameters \( \mu \) and \( \sigma \) were thus obtained for each iteration. After 200 iterations, the average value for each parameter was taken as the bootstrap estimate of its mean and the 2.5th and 97.5th percentiles were taken as the bounds of the 95% CI for that mean. Pilot work showed that results of 200 and 1000 iterations returned nearly identical estimates. As expected, the bootstrap estimates of \( \mu \) and \( \sigma \) were very similar to the values derived from the best fit to the actual data for each parameter. However, the statistical limits of confidence associated with the former allowed us to compare more rigorously the relative sensitivity of various ERG components to elevated IOP.

Other Statistical Methods. Analysis-of-variance (ANOVA; Prism, ver. 3.02; GraphPad Software Inc., San Diego, CA) was applied to test the significance of the treatment effect, whereby the null hypothesis was no effect of IOP elevation. Tukey's ANOVA (two-treatment vs. ERG stimulus intensity) was applied to the data for each of the seven ERG components independently. Therefore, the α-level was adjusted to 0.01 to correct for multiple comparisons.

Results

The Effect of Acute IOP Elevation on the Rat ERG

Figure 2 provides individual examples of ERG responses from four select IOP groups. These groups were chosen because they illustrate the range of IOPs at which retinal function progresses from selective to nonselective deficits. These data were collected according to the protocol described earlier, beginning 75 minutes after the onset of elevated IOP. The ERG responses shown span a range of stimulus intensities (indicated at left of Fig. 2A). In each case, responses from the experimental eye are represented by the bold traces, and responses from the fellow control eye are represented by the thin traces. Figure 2A shows results for an animal in the sham group, Figures 2B, 2C, and 2D show results for animals from the 50-, 60-, and 70-mm Hg groups, respectively. As found previously,20,22 control responses to the dimmest flashes (lower four pairs of each column) consisted of a small positive component (pSTR), followed by a larger negative component (nSTR). Scotopic responses to intermediate flash intensities (−3.04 to −1.60 log cd·s/m²) were dominated by the corneal positive b-wave. More intense stimuli (≥−0.89 log cd·s/m²) evoked responses that revealed a more prominent photoreceptor a-wave and OPs. The OPs were apparent on the rising edge of the b-wave, but the isolated OPs were also shown to the right of each raw ERG waveform. The light-adapted (photopic) ERG responses for two intensities are also shown at the top of each column. These responses were also dominated by a corneal positive b-wave and OPs.

The individual example shown in Figure 2A demonstrates that ERG responses from experimental eyes of the sham group were indistinguishable from those recorded in the control fellow control eye. The small amplitude differences occasionally noted between responses of eyes in the sham group and their fellow control eyes were always well within the range of normal interocular variability established previously in a group of 16 naive animals.20

The results at IOP elevated to 50 mm Hg (Fig. 2B) were characterized by reduction of the nSTR amplitude (e.g., bottom four responses) and a decrease in the negative slope of the descending portion of the photopic b-wave (e.g., top two responses). The pSTR amplitude appeared larger than control responses. The most likely explanation for this paradoxically larger pSTR is that the reduction of the nSTR component may have been relatively selective at this IOP, thus exposing more of the underlying positive component. At intermediate intensities (−3.04 to −1.60 log cd·s/m²), a subtle delay in the rising
slope of the scotopic b-wave was occasionally noted, and the timing of the scotopic OPs was altered; however, there were no changes in ERG component amplitudes. With brighter intensities \( (> -1.60 \text{ log cd-s/m}^2) \), all components of the scotopic ERG (a-wave, b-wave, and OPs) were very similar between the experimental and control eyes. Higher IOPs resulted in more widespread ERG changes. At 60 mm Hg (Fig. 2C), reduction of the nSTR became even more pronounced. The pSTR component was also reduced and delayed. Scotopic b-waves were reduced slightly more than a-waves, and the OPs were also smaller. At 60 mm Hg, the photopic b-wave and OP amplitudes were also smaller, in addition to the shallow b-wave recovery noted for an IOP of 50 mm Hg.

At an even higher IOP (Fig. 2D; 70 mm Hg), the ERG changes were more dramatic: the STR, scotopic b-wave, and OPs as well as photopic b-wave and OP amplitudes were also smaller, in addition to the shallow b-wave recovery noted for an IOP of 50 mm Hg. The complete results for all animals and all stimulus intensities are presented in Figure 4 for the scotopic a-wave (top row), P2 (second row), nSTR (third row), photopic b-wave (fourth row), and photopic OPs (bottom row). The group mean amplitudes \( \pm \text{SEM} \) in the experimental eyes (filled circles) and fellow control eyes (open circles) are plotted as a function of stimulus intensity within each panel. Each column of five panels represents the results in one IOP group, beginning with the sham group at the left and ending with the 100-mm Hg group at the right. Figure 4 shows that at IOP settings below 50 mm Hg, there were no significant differences in amplitude between treated and control eyes for any of these ERG components. However, at 50 mm Hg, the amplitudes of the nSTR \( (P < 0.0001, \text{ANOVA}) \) and photopic OPs \( (P < 0.0001, \text{ANOVA}) \) were significantly reduced, whereas the amplitudes of the scotopic a-wave, the P2 \( V_{\text{max}} \) and the photopic b-wave were not significantly affected. At 50 mm Hg, the scotopic OP amplitudes were not significantly reduced (data

**Figure 2.** Representative individual examples of ERG findings for selected IOP groups. ERG responses for experimental eyes (bold traces) and their fellow control eyes (thin traces) are shown for the following groups: sham (A) and 50 (B), 60 (C), and 70 (D) mm Hg. Stimulus flash intensities are listed at left for scotopic (bottom) and photopic (top) responses. Isolated OPs are shown to the right of corresponding waveforms.
Significant effects on the scotopic a-wave and P2 intensity–response function were observed only in the groups with an IOP of \( \geq 60 \) mm Hg, a level that reduced the nSTR, photopic b-wave and photopic OP amplitudes by \( >50\% \). In the group with IOP elevated to 70 mm Hg, the nSTR was reduced to near noise levels, whereas the scotopic P2 and a-wave were reduced by only \( \sim 50\% \). The effects on all components grew as IOP increased above 70 mm Hg, up to the 100-mm Hg group, in which no ERG component was ever detected.

**Quantifying the Relative Sensitivity of ERG Components to Acute IOP Elevation**

The relative sensitivity of all the various ERG components to acutely elevated IOP was formally evaluated using the analysis presented in Figure 5. As described in the Methods section, the group mean relative amplitude (treated/control, \%) was plotted versus IOP. For clarity, the results for different components are presented in different panels. Figure 5A shows that a reduction in the amplitude of the nSTR occurred before the pSTR. In fact, at 50 mm Hg, the pSTR amplitude was increased (145\% \pm 17\%), whereas the nSTR was reduced to 78\% \pm 5\% of the mean control eye amplitude. Similarly, the data presented in Figure 5B show that the scotopic P2 (\( V_{\text{max}} \)) is more sensitive to IOP than the a-wave (\( R_{\text{mP3}} \)), whereas the sensitivity of the scotopic OPs lay somewhere between these other two components. The photopic ERG b-wave and OPs (Fig. 5C) appeared to be more sensitive to IOP than their scotopic counterparts. The data for each of these components was fit with the inverse cumulative normal function to obtain a quantitative estimate of sensitivity (position parameter, \( \beta \)). Figure 5D illustrates the results of this procedure for the nSTR, scotopic P2, and a-wave (\( R_{\text{mP3}} \)). For clarity, the other components were omitted from Figure 5D, and the IOP axis was expanded.

The results of this sensitivity analysis are presented in Table 1. For all ERG components, the parameters derived from the best fit to the raw data closely match the bootstrap estimates. The results reveal that the ERG components generally fall into three categories: the nSTR, photopic b-wave, and photopic OPs have the highest sensitivity to acutely elevated IOP, as they were reduced by 50\% for IOP < 61 mm Hg. The scotopic P2, OPs, and pSTR have intermediate sensitivity, such that they were reduced by 50\% for IOPs between 61 and 66 mm Hg. The scotopic a-wave (\( R_{\text{mP3}} \)) is the least sensitive component showing a 50\% reduction at an IOP of 71 mm Hg.

The slope parameter (\( \omega \)) for the pSTR was significantly steeper than the nSTR, which is probably a reflection of the...
apparent increase in the amplitude of the pSTR at an IOP of 50 mm Hg (e.g., see Figs. 2, 5). It is possible that the gradient of effects due to increasing acute IOP vary among these seven ERG components. However, detailed comparison of the slope parameter is beyond the scope of this study. Indeed, it is also likely that interactions ensue at higher IOPs, because of the cascade of effects that arise as more distal retinal elements become affected. The apparent dysfunction of the more proximal retinal elements will be compounded by altered inputs once the more distal elements, such as the photoreceptor (P3) response, become affected. Hood and Birch showed that in the absence of changes in P3 (sensitivity, $S$, or maximum response amplitude, $R_{\text{mP3}}$), a decrease in the maximum amplitude of P2 ($V_{\text{max}}$) must indicate decreased function of the INL (most likely the rod bipolar cells). In Figure 6, the relative changes of maximum amplitude ($R_{\text{mP3}}$ versus $V_{\text{max}}$ P2, Figure 6A) and sensitivity ($S$ versus $K$, Fig. 6B) are compared for the P3 (photoreceptor) and P2 (rod bipolar) components. The reference lines are used to determine significance ($P < 0.05$) and indicate the 95% limits of agreement for interocular amplitude, calculated for the P3 (dashed lines) and P2 (dotted lines) parameters, based on data collected separately in 16 naive animals. The data in Figure 6A were replotted from Figure 5 on a relative log scale and reiterate that P2 amplitude began to decline at a lower IOP than that of P3. Figure 6B shows that the sensitivity of P2 was unaffected at 60 mm Hg, a pressure that caused a twofold decline in P2 maximum amplitude. The data in 6B also show that a small (but statistically significant) decrease in photoreceptor sensitivity ($S_{\text{P3}}$) accounted for a very small proportion of the decrease in P2 maximum amplitude ($V_{\text{max}}$) in the 60-mm Hg group. Figure 6 also demonstrates that all aspects of photoreceptor and INL/rod bipolar function were normal in the 50-mm Hg group. In particular, the maximum amplitude and sensitivity measures of both P3 and P2 components were all normal in this group, indicating that the significant changes observed for the nSTR and photopic OPs were not due to the upstream effects of increased IOP.

**DISCUSSION**

The results of this study show that among the full-field flash ERG components evaluated, the nSTR and photopic OPs were the most sensitive to acutely elevated IOP. These two components were the first to show decreased amplitudes at elevated IOPs. Their amplitudes were significantly reduced at 50 mm Hg, and they declined to half their normal values at IOPs of 59 and 56 mm Hg, respectively. The photopic b-wave was the third most sensitive component by amplitude analysis, as it was reduced by 50% at IOP of ~60 mm Hg. The photopic b-wave
Implicit time, however, was significantly delayed at 30, 40, and 50 mm Hg. Similarly, the pSTR implicit time first manifested significant delays in the 40-mm Hg group, a finding we attribute to reduction of the nSTR. All three of these components (STR, photopic b-wave, and photopic OPs) have recently been shown to be dependent on intact ganglion cell function in the rat. When intravitreal injections of tetrodotoxin (TTX) were used to block action potentials in the rat retina, the effects were most pronounced on these same three components. These components were all significantly affected at 50 mm Hg or below, whereas the P2 and P3 components maintained normal amplitude and sensitivity. Taken together, these results suggest that the retinal elements most sensitive to acutely elevated IOP in the rat are the ganglion (and possibly AII amacrine) cells. In particular, the results suggest that one of the first functional deficits to develop as IOP is progressively elevated is a reduction of spiking activity.

The slope of the descending limb of the photopic b-wave revealed effects at lower IOPs (<30 mm Hg) than did the peak amplitude (between 50 and 60 mm Hg). This observation is consistent with loss of a slow negative component in the photopic ERG that may be analogous to the photopic negative response (PhNR) first described by Viswanathan et al. The PhNR is reduced in human glaucoma, as well as by experimental glaucoma and intravitreal injections of TTX in non-human primates. In our previous studies in the rat, we observed a similar change in the slope of the descending limb of the rat photopic b-wave after intravitreal TTX, but not after optic nerve transection, suggesting that changes observed in the rat PhNR equivalent do not necessarily indicate abnormalities of ganglion cells per se. Perhaps, glial cell functional abnormalities are partly responsible for changes in the rat PhNR equivalent during acutely elevated IOP and/or after TTX application. Why the pSTR, another ganglion cell–dependent component of the rat ERG, was less sensitive to acute IOP elevation than the nSTR remains to be determined. It may be that the ERG near threshold is even more complex and consists of more than two components. It is possible that some portion of the slow negative components unaffected by optic nerve transection and/or TTX are mediated by glial cells and that they are most sensitive to acutely elevated IOP.

In addition to the pSTR, the scotopic P2 and OPs were among the group of components that displayed intermediate sensitivity to acutely elevated IOP. It is thought that the OPs are generated in the inner plexiform layer, where the components most sensitive to elevated IOP arise. Although the summed (RMS) amplitude of the scotopic OPs did not decline to 50% of control values at IOP < 65 mm Hg, subtle changes in the size and timing of individual OP wavelets were observed at lower IOPs. However, the summed amplitude of scotopic OPs was the most variable measure among control eyes. Measurement of individual OP wavelets proved to be even more variable. Thus, the occasional changes observed for individual OPs did not significantly depart from the range of variability across controls.

The scotopic P2 of the rat is thought to be generated primarily by the rod bipolar cells, whose cell bodies are located in the INL. The source and sink of the current underlying the P2 are also thought to lie more distal to the ganglion cell layer, in the inner and outer plexiform layers, respectively. Significant P2 amplitude reduction first occurred while the P3 amplitude was still normal in the 60 mm Hg IOP group. A small decline in the P3 sensitivity parameter in this group may account for a minority of the P2 amplitude change. However, the absence of a concurrent decrease in P2 sensitivity (K) argues that the reduction of P2 maximum amplitude is a more direct reflection of decreased INL (rod bipolar) function.

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**Figure 5.** Relative change in ERG amplitude versus IOP. (A) pSTR and nSTR. (B) Photoreceptor saturated amplitude (RmP3), P2 amplitude (Vmax), and scotopic OP amplitude. (C) Photopic b-wave amplitude and photopic OP amplitude. (D) For comparison, relative amplitude changes of the nSTR (thin solid curve), P2 (Vmax, dashed curve), and a-wave (RmP3, bold curve) are replotted together with their corresponding best-fit cumulative normal functions. Symbols indicate group mean (±SEM) for the ratio of treated-to-control eyes.

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Retinal Function during IOP Elevation

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933230/)
that is due to any change in photoreceptor function. Similar to the 70 mm Hg IOP group, the relatively large effect on P2 maximum amplitude ($V_{max}$) cannot be explained by changes in photoreceptor (P3) function alone (Fig. 6), although the decrease in P2 sensitivity ($K$) could be. The proportional effects of acutely elevated IOP on P2 amplitude relative to the parameters of photoreceptor function (P3) indicate that the function of the INL is more negatively affected at 60 and 70 mm Hg. Therefore, the ERG component least sensitive to acutely elevated IOP was the rod photoreceptor P3. The scotopic P3 is generated by the closure of cGMP channels in the rod outer segment membranes and the resultant decrease in the sodium dark current. Thus, the P3 represents the most distal retinal signal evaluated in this study and the least sensitive to acutely elevated IOP.

In this study, ERG component amplitude changes were not observed at IOPs lower than 50 mm Hg; however, delays in the leading edge of the nSTR and the declining portion of the photopic b-wave were consistently observed at IOPs as low as 30 mm Hg (e.g., Fig. 3). Thus, acute retinal functional changes consistent with reduced ganglion cell activity were apparent throughout the range of IOPs (30−50 mm Hg) that persist chronically in most rats with experimental glaucoma. Generally, a longer duration of ischemia is necessary to cause permanent functional loss as measured with the scotopic b-wave. Generally, a longer duration of ischemia is necessary to cause permanent histologic damage. The present study also found that the photoreceptor P3 was the least sensitive component to acutely elevated IOP.

Most previous studies of acute IOP elevation that have used the ERG as a functional measure have focused on the scotopic b-wave. In these studies, a significant reduction in b-wave amplitude generally occurred when the IOP reached a critical level. For a number of mammalian species, including monkeys, cats, dogs, and rabbits, the critical IOP for b-wave loss is approximately 30 mm Hg below the mean femoral artery blood pressure (i.e., perfusion pressure of +30 mm Hg). In our study, P2 was significantly affected by an IOP of ≥60 mm Hg. This corresponds to a perfusion pressure of approximately 40 mm Hg (i.e., an IOP of 60 was ~ 40 mm Hg below the measured mean femoral artery pressure). Thus, the current results for the scotopic P2, a reflection of rod bipolar cell function, are consistent with those in many previous studies.
bipolar and/or photoreceptor function. Feghali et al.\textsuperscript{46} showed that the rabbit pattern (p)ERG and flash ERG OPs are immediately reduced when IOP is raised to 35 to 50 mm Hg, whereas the b-wave is not affected. Hamor et al.\textsuperscript{67} also found that the PERG is more sensitive than the flash ERG during acute IOP elevation in dogs. Colotto et al.\textsuperscript{68} showed that the PERG is reduced by acute IOP elevation to as low as 30 mm Hg in human subjects, although they did not directly compare the effects on the PERG with outer retinal responses. Kothe and Lovasik\textsuperscript{69} also found that reduction of PERG amplitudes is directly related to the magnitude of acute IOP increase, irrespective of retinal vascular perfusion pressure. Taken together, these results suggest that ganglion cell function is more sensitive to acute IOP elevation than bipolar cell function and that ganglion cell function is affected by acute pressure elevation to levels well below mean arterial pressure.

One important question is whether the acute functional losses observed in this study, at IOPs commonly observed in human and/or experimental glaucoma, are due to ischemic versus direct, pressure-related mechanisms. At much higher IOPs, direct comparisons between ligation and acutely elevated IOP models of ischemia suggest that damage caused by high IOP is more extensive than that caused by ligation alone.\textsuperscript{70,71} However, other investigators have suggested that most effects of acute IOP elevation could be explained on the basis of decreased blood flow and/or ischemia. In the cat, Siliprandi et al.\textsuperscript{72} found that both PERG and flash ERG amplitudes are unaffected by very high levels of IOP (up to \textasciitilde 80 mm Hg) as long as perfusion pressure is maintained at least above \textasciitilde 25 mm Hg. Gerstle et al.\textsuperscript{41} also showed that the ERG b-wave of the owl monkey is unaffected until perfusion pressure is reduced below approximately 30 mm Hg. Grehn and Prost\textsuperscript{73} reported that at a constant perfusion pressure in cats, ganglion cell field potentials are maintained at normal levels, whether the IOP is set to 40 or 135 mm Hg. Recently, Trible and Anderson\textsuperscript{74} suggested that the acute loss of visual sensitivity (visual field depression), in both patients with glaucoma and normal subjects during acute periods of elevated IOP (to 30 or 40 mm Hg), is primarily dependent on ocular perfusion pressure.

It seems that only a study in which \textit{retinal blood flow} is measured directly, along with the ERG components most sensitive to acute IOP elevation, such as the STR or the PERG, will be able to answer the critical question of whether acutely elevated IOP can cause ganglion cell dysfunction independent of a reduction in retinal blood flow.

In summary, this study demonstrated that acutely elevated IOP results in a gradient of functional deficits in the rat retina, progressing from inner to outer layers as IOP is increased. Alterations in ganglion-cell-related ERG potentials occurred at IOPs (30–50 mm Hg) commonly observed in experimental rat models of chronic glaucoma. Changes in bipolar and photoreceptor cell function (outer retina) were observed at acute IOPs \textgreater 50 mm Hg, suggesting that IOP should be maintained below this level in experimental glaucoma models if selective ganglion cell injury is to be sought. Repeated IOP spikes above this level may cause permanent damage to bipolar and/or photoreceptor cells, perhaps via ischemic mechanisms. Thus, IOP should be monitored frequently in these models.

**APPENDIX**

**Choice of the Computational Model**

Hood and Birch\textsuperscript{25} demonstrated that a computational model of the form described by Lamb and Pugh\textsuperscript{24} provides a superior description of the leading edge of the ERG a-wave response to bright stimulus flashes, particularly soon after the flash, compared with the class of models that consist of an \( n \)-stage nonlinear low-pass filter followed by a saturating, nonlinear function. The potential advantage of the latter, however, is that it describes more of the rod response—that is, beyond the leading edge. Hood and Birch\textsuperscript{25} suggested that the nonlinear low-pass \( n \)-stage model may be more appropriate when estimation of the entire P3 waveform is required, such as in isolation of the P2 response by subtraction of the P3 estimate from the ERG.\textsuperscript{77} We also found that the Lamb and Pugh\textsuperscript{24} model provided a much more accurate fit to the four brightest flash ERG a-wave responses compared with the \( n \)-stage model (for the latter, time to peak, \( t_p \) was set to 189 ms and the number of stages, \( n \), was set to four). Thus, we chose the Lamb and Pugh model to characterize photoreceptor function in this study. Along with the anonymous reviewers, we were concerned that our estimates of P2 would be less accurate than if we had used the \( n \)-stage model to estimate the P3 for later times after the stimulus. However, our analyses revealed that the two P3 models returned nearly identical results for the rod response to the dimmest flashes in the P2 intensity-response range out to times beyond the b-wave peak. Further, because the P3 response is much smaller than the P2 in response to dim flashes, the P2 estimate is negligibly affected by the choice of the P3 model. The responses of both models to the four brightest flashes reach saturation before the b-wave peak, so the estimates for the P2 peak amplitude differ only by the amount of discrepancy between the two estimates of P3-saturated amplitude. The \( n \)-stage model systematically underestimated the saturated a-wave amplitude by \textasciitilde 15\%, and it also substantially overestimated the bright-flash responses along the leading edge—that is, at the earliest times after the flash, as others have reported. The Lamb and Pugh model provided a much better fit to the bright responses, including the saturated amplitude. On average, \( P2 V_{\text{max}} = 4.6\% \pm 0.7\% \) larger when the Lamb and Pugh model was used to estimate P3. The sensitivity parameter (or semisaturation constant, \( K \)) of the P2 function was essentially identical for both P3 models. Sensitivity was only 0.06 \pm 0.01 log units lower (i.e., \( K \) was slightly larger) when the Lamb and Pugh model was used. The slope, \( n \), of the P2 intensity response function was allowed to vary in this study, or else the P2 fits would have been poor, especially at the higher IOPs. The value of \( n \) was approximately 5\% larger when the \( n \)-stage model was used instead of the Lamb and Pugh model—0.64 vs. 0.67 in control eyes for the two models respectively. Because the Lamb and Pugh model provided a vastly superior characterization of the ERG a-wave and because the choice of the P3 model had a minimal effect on the parameters derived from the P2 intensity-response function, we choose to use the Lamb and Pugh type model throughout the study.

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