The Dynamic Response of Intraocular Pressure and Ocular Pulse Amplitude to Acute Hemodynamic Changes in Normal and Glaucomatous Eyes

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Citation: Li JC, Gupta VK, You Y, Ng KW, Graham SL. The dynamic response of intraocular pressure and ocular pulse amplitude to acute hemodynamic changes in normal and glaucomatous eyes. Invest Ophthalmol Vis Sci. 2013;54:6960–6967. DOI:10.1167/iovs.13-12405

PURPOSE. To evaluate the effects of acute arterial blood pressure (ABP) and venous pressure changes on IOP in rats with experimental glaucoma.

METHODS. Unilateral experimental ocular hypertension was established in Sprague-Dawley rats by weekly intracameral injection of microbeads. Ocular pulse amplitude (OPA) and IOP were recorded from the anterior chamber using 1.2-Fr microsensors under urethane anesthesia. The effects on IOP during hemodynamic challenges using phenylephrine (PE) (50 μg/kg/min IV) and rapid saline loading (20 mL/kg/min IV) were studied.

RESULTS. Over an 8-week period, IOP was significantly elevated by 60% in the unilateral ocular hypertensive eyes. Both ABP and IOP were significantly increased by PE infusion. A significantly greater IOP increase was found in the experimental eyes compared with control eyes (1.32 ± 0.18 mm Hg vs. 0.90 ± 0.09 mm Hg). Correspondingly, higher OPAs and an amplification of the OPA during arterial hypertension were found in the experimental eyes. A sustained rise in IOP secondary to IV saline loading was observed, with a greater rise observed among experimental eyes (0.74 ± 0.13 mm Hg vs. 0.37 ± 0.005 mm Hg).

CONCLUSIONS. Sympathetic acceleration of ABP using PE resulted in surges in IOP and OPA. In contrast, increased venous pressure resulted in a more sustained rise in IOP but to a lesser extent. These responses were more pronounced in eyes with experimental glaucoma compared with control eyes, which may reflect the higher starting IOP contributing to a reduced ocular compliance. Our results suggest that eyes with ocular hypertension are more susceptible to IOP variability induced by hemodynamic fluctuations.

Keywords: phenylephrine, intraocular pressure, blood pressure, ocular pulse amplitude, ocular hypertension

Intraocular pressure is a well-known risk factor in the development of glaucomatous optic neuropathy (GON), characterized by the irreversible loss of retinal ganglion cells with the natural history of progressive visual field loss.

The mechanism by which raised IOP results in GON is unclear, but a linear correlation between arterial blood pressure and IOP and IOP has been well established both by cross-sectional population studies1-4 and by animal models.1-6 It has been estimated in humans that for approximately every 10 mm Hg rise in systolic blood pressure, there is an associated 0.2 to 0.3 mm Hg rise in IOP.7 Real-time analyses have also revealed that IOP can rise and fall in synchrony with acute hemodynamic changes that occur during physical exercise as well as during pharmacologic stimulation by vasoactive agents.8,9

Despite the growing speculation of a vascular basis of POAG,10,11 the relationship between POAG and hypertension is not well defined, with epidemiologic studies showing disparate results with an overall suggestion of a weak association between hypertension and POAG.1,12,13 Using a rodent model, we aimed to evaluate the dynamic relationship between IOP and acute hemodynamic fluctuations and to show how their relationship is altered in the context of reduced facility of aqueous outflow.

MATERIALS AND METHODS

Animals

All animal procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Macquarie University Animal Ethics Committee. Male Sprague-Dawley rats of 8 weeks old (220-250 g) were sourced from the Animal Resource Center, Perth, Australia. The animals were housed in an environmentally controlled room at 25°C and 12-hour light/12-hour dark cycle with ad libitum access to food and water. Animals were quarantined and kept until the age of 16 weeks prior to the commencement of the experiments and were later killed at the age of 24 weeks upon completion.

Microbead Injection and Glaucoma Induction

The technique of intracameral injection of microbeads to induce ocular hypertension and glaucoma has been adapted from previous reports.14-17 Polystyrene microspheres of 10 μm in size were used, with a concentration of 3.6 × 10⁶ beads/mL (FluoSpheres; Invitrogen, Carlsbad, CA). They were injected using a 25-μL Hamilton syringe connected to a disposable 33-
gauge needle (TSK Laboratory, Tochigi, Japan). All intracameral procedures were performed under magnification using an operating microscope (OPMI Vario S88, Carl Zeiss, Oberkochen, Germany) with care taken to avoid needle contact with the iris or lens.

To simulate a model of chronic ocular hypertension and glaucoma, the injections were performed weekly over 8 weeks. Injections were performed under anesthesia from an intraperitoneal mixture of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg). Animals were placed on a heating pad during the procedure, and both pupils were dilated with topical tropicamide 1% and anesthetized with proparacaine 0.5% drops. During anesthesia and prior to each weekly injection, IOP was measured by using a handheld electronic tonometer (Icare Tonovet, Helsinki, Finland). The IOP displayed on the tonometer was the mean of six consecutive measurements. The average of three consecutive IOP readings was recorded. The right eye was used as the experimental eye, and the left eye served as a paired control. The needle was inserted bevel down, tangentially beneath the corneal surface, to facilitate self-sealing of the puncture wound. Once the needle tip was visualized within the anterior chamber, 5 μL microbead solution was injected. At the end of the procedure, anesthesia was reversed using atipamazole (0.75 mg/kg subcutaneous injection [SCI]), and 0.3% ciprofloxacin drops (Ciloxan; Alcon Laboratories, Frenchs Forest, NSW, Australia) and 0.1% dexamethasone eye drops (Maxidex, Alcon Laboratories) were instilled in both eyes. An ointment (Lacri-lube; Allergan, Gordon, NSW, Australia) was also applied to protect against corneal drying until the animal recovered.

Simultaneous Arterial and Intraocular Pressure Recording

Following the 8 weeks, rats were anesthetized with an intraperitoneal injection of 10% urethane (1.3 g/kg) for invasive ABP and IOP recording. Depth of anesthesia was assessed by periodical assessment of withdrawal and corneal reflexes. Animals were ventilated spontaneously without the use of paralyzing agents. Body temperature was monitored using a rectal temperature probe and maintained at 37°C using a heating pad.

Solid state microsensor catheters (Scisense, London, ON, Canada) with a pressure resolution of 10 μV/V/mm Hg were used to record the ABP and IOP. Prior to each use, the catheters were cleaned and then stabilized by immersion in saline and calibrated using electronic calibration as per manufacturer’s recommendations. Continuous ABP recording was measured using a 1.9-Fr catheter inserted into the femoral artery. Intravenous access was via the femoral vein using polyethylene tubing (PE-50).

After the initial surgical preparation, animals were immobilized in the sphinx position with the head secured in a rodent stereotaxic frame. The 1.2-Fr catheters were inserted into the anterior chambers of both eyes. Dilation and anesthetic drops were applied to both eyes prior to sensor insertion. A small self-sealing incision through the cornea was made by a stab incision superiorly and lateral to the limbus at the 12 o’clock position using an ophthalmic blade (Alcon A-Ok 15° Angle Blade; Alcon Laboratories). The catheter tip was manually advanced through the cornea and positioned to sit freely in the anterior chamber (Fig. 1). Eye lubricant was applied after catheter insertion to prevent drying of the cornea.

Hemodynamic Manipulation

Hemodynamic manipulations were performed once stability of the recording parameters heart rate (HR), ABP, and IOP were confirmed. Simulations of an acute ABP rise were achieved using IV infusion of phenylephrine (PE) 50 μg/kg/min (Sigma-Aldrich, St. Louis, MO) delivered over 2 minutes. Simulations of acute central venous pressure expansion were performed by the infusion of isotonic saline (20 mL/kg) over 1 minute. Infusions were delivered using an infusion pump (Harvard Pump 11 Plus; Harvard Apparatus, Holliston, MA).

Data Acquisition and Statistical Analysis

Data recorded from the intracameral sensors were analyzed for changes in IOP from baseline. The absolute value of IOP was not used for analysis because of the known variable negative
FIGURE 2. (A) Weekly IOP readings measured from experimental eyes and contralateral control eyes. Measurements were obtained using Icare tonometer during ketamine and medetomidine anesthesia immediately before the intracameral injection of microbeads. Values are mean ± SEM; n = 28; unpaired t test; *P < 0.005. (B) Simultaneous increase in ABP (red) and IOP (blue) during PE infusion in a single rat. Top recording in black represents heart rate derived from the pressure waveform frequency, illustrating the response of the baroreceptor reflex. (C) Simultaneous recording of the ABP and IOP pressure waveforms indicating a real-time synchronous relationship between ABP and IOP.
offset of several millimeters of mercury that occurs upon immersion and re-immersion of the microsensor catheters.\textsuperscript{18} Output from the catheters was sampled at 2 kHz and recorded using a data acquisition system (Power1401-2 interface with Spike2 Software version 6; Cambridge Electronic Design, Cambridge, UK). Data were analyzed in 5-second bins. Ocular pulse amplitude (OPA) was calculated using automatic detection of the waveform peaks and trough by the software. The mean OPA during baseline and during raised IOP was averaged over 20 seconds. Statistical analysis was performed using commercially available statistical software (Prism 6; GraphPad Software, La Jolla, CA). Student’s \textit{t}-test and one-way ANOVA were used for comparison of means. Differences with \(P < 0.05\) were considered statistically significant.

**RESULTS**

A total of 30 rats were used in this study. The weekly injections were well tolerated, with animals gaining weight during the study period. After the 8-week time point, the rats weighed 500 to 550 g. One rat developed cataract and another developed corneal neovascularization in the experimental eye and were excluded from the study. The remaining 28 rats were divided evenly between PE and saline infusion groups.

**Microbead Injection and IOP**

A sustained increase in IOP can be achieved using repeated microbead injections (Fig. 2A). A single intracameral injection was sufficient to induce a sustained increase in IOP as shown by the higher IOP amongst experimental eyes compared with control eyes after week one (12.5 ± 0.53 mm Hg for experimental eye compared with 10.2 ± 0.2 mm Hg for control; mean ± SEM, \(P < 0.001\), \(n = 28\)). With subsequent weekly injections, ocular hypertension was maintained among the experimental eyes with a maximal increase of 1.52-fold over the 8-week period (16.0 ± 0.75 mm Hg for experimental eye compared with 10.56 ± 0.24 mm Hg for control; mean ± SEM, \(P < 0.001\), \(n = 28\)). No significant change in IOP was observed in the control eyes.

**Ocular Hypertension During Surges in ABP**

The simultaneous sampling of ABP and IOP allowed the tracing of IOP fluctuations in response to changes in ABP (Fig. 2B). The high frequency sampling also allowed pressure recording to the resolution of their pulse waveforms (Fig. 2C).

Prior to hemodynamic manipulation with PE, the baseline mean arterial pressure (MAP) was 94.1 ± 2.3 mm Hg (mean ± SEM). Phenylephrine infusion resulted in a transient but statistically significant rise in MAP that peaked at the completion of infusion (157.1 ± 5.5 mm Hg, mean ± SEM, \(P < 0.0001\)). Mean arterial pressure reverted to baseline level 2 minutes following the PE infusion (95.1 ± 2.3 mm Hg, mean ± SEM, \(P = 0.92\)).

Similarly, the IOP rose and fell in synchrony with ABP, in both the experimental and control eyes during the PE infusion (Fig.
3) A plot of the mean change in IOP with the mean changes in MAP showed that a linear relationship was present between IOP and ABP during both the upward and downward movement of ABP in both the experimental glaucoma and control eyes. (Figs. 4A, 4B).

Ocular Hypertensive Response in Eyes With Experimental Glaucoma

In experimental eyes, the amplification of IOP during the acute hypertension was greater than the rise recorded in the control eyes (Fig. 3B). The maximal gain in IOP observed in experimental eyes was approximately 1.5 times the maximal IOP in control eyes (1.52 ± 0.18 mm Hg, compared with 0.90 ± 0.09 mm Hg; mean ± SEM; \( P = 0.04; n = 14 \)). Comparing the slope of the change in IOP versus the change in ABP also showed that the rate of change in IOP to ABP was greater in the experimental eyes (comparing Fig. 4A with 4B).

Ocular Pulse Amplitude

The ocular pulse amplitude is the difference in IOP during cardiac systole and diastole. Ocular pulse amplitude was examined using data recorded from five rats before and during PE infusion with data expressed as mean ± SD. In the anesthetized rat, the baseline IOP oscillated at approximately 0.1 mm Hg with each cardiac cycle (Fig. 2C). The baseline OPA was greater in experimental eyes, with a difference that was statistically significant (0.137 ± 0.008 mm Hg in experimental eyes versus 0.085 ± 0.005 mm Hg in the control eye; one-way ANOVA; \( P \leq 0.0001; n = 5 \)). Upon acute ABP stimulation with intravenous PE, there was a substantial gain in OPA amongst the experimental eyes with over 50 percent increase from baseline OPA (OPA of experimental eyes = 0.21 ± 0.02 mm Hg during ABP surge) (Fig. 6). The transient systemic hypertension induced by PE had no significant effect on the OPA amongst the control eyes (Fig. 6).

IOP Response During IV Saline Challenge

In both the experimental and control eyes, despite the substantial fluid loading, the IOP responded with a small but statistically significant rise and plateauing from baseline for both the experimental and control eyes (0.366 ± 0.005 mm Hg, \( P \leq 0.001 \) for control eyes; 0.742 ± 0.130 mm Hg, for experimental eyes, mean ± SEM, \( n = 14 \), unpaired t-test, \( P \leq 0.001 \)). Paired t-test analysis between the two samples yielded a significant difference in IOP between the experimental and control eyes.
control eyes during the postinfusion period ($P < 0.0001$) (Fig. 7B). The saline infusion also yielded a persistent elevation in ABP during the 5-minute postinfusion period (Fig. 7A). A plot of the mean IOP and mean MAP during the rapid saline challenge showed that in both the control and experimental eyes, the rate of IOP increase was initially proportionally slower than the rate of the increase in ABP (Figs. 5A, 5B).

**DISCUSSION**

Our present study reports three important findings:

1. In the outflow obstruction model of glaucoma, there is an amplification of the IOP during acute elevations in blood pressure (Fig. 3);
2. During acute increases in blood pressure, there is an associated increase in the OPA in the eyes with experimental glaucoma (Fig. 6); and
3. Intravenous volume loading results in a mild but sustained IOP elevation in eyes, with a greater response in eyes with experimental glaucoma (Fig. 7).

Our study confirmed the previously reported real-time linear relationship between IOP and ABP$^8,9,19$ (Figs. 2B, 2C). Of interest, the plot of ABP versus IOP during the PE infusion showed a hysteresis relationship between ABP and IOP as shown by the difference in the rate of change in IOP during the rise of ABP compared with the fall in ABP (Fig. 4). The limited outflow facility of the experimental ocular hypertension eyes may have prevented a compensatory increase in outflow filtration in response to the IOP elevation thus resulting in a greater change in IOP per unit change in ABP. However, this mechanism does not explain the marked increase in the baseline OPA observed among eyes with experimental ocular hypertension (Fig. 6). Although the mechanism is beyond the scope of this study, we hypothesize that the higher OPA may be related to the higher baseline IOP in the experimental eyes. Higher baseline pressures would tend to decrease the compliance property of the eye owing to increased scleral wall tension, which in turn translates to a greater rise in IOP during the expansion of choroid blood flow resulting in an increase in both the magnitude of IOP as well as OPA.

The greater OPA in eyes with experimental ocular hypertension observed during baseline as well as during the blood pressure–induced rise of IOP suggests there is both a static and dynamic component of reduced ocular compliance in eyes with ocular hypertension. Our result of elevated OPA in the experimental eyes with ocular hypertension agrees with the findings from studies that have shown that a direct relationship exists between OPA and IOP.$^{20,21}$ Studies have also reported patients with glaucoma to have a normal or reduced OPA and that the highest OPAs are found in patients with ocular hypertension.$^{20–22}$ Of interest, it has also been reported that a relatively higher OPA was correlated to patients with less severe disease.$^{23}$

Our study also found that the OPA magnitude of the control eyes was unaffected by the transient elevation in blood pressure, suggesting that the ability for ABP to increase OPA may be dependent on the presence of elevated IOP or reduced aqueous humor outflow.

The real-time mirrored fluctuations of IOP with changes to ABP may be explained by the ocular pressure-volume relationship model.$^{24}$ Given external energy is required to further pressurize the ocular compartment, the transmission of pulsatile energy from the arterial system into the intraocular space is likely owing to the sudden rise in ocular blood flow.
that occurs during systole or during an acute hypertensive challenge. Pulsatile energy is predominately transmitted by the choroid given it represents the majority of the ophthalmic blood flow. It is likely that the observed synchronous rise in IOP results from an expansion of choroidal volume brought on by an increase in ocular blood flow. The expansion of the choroid against the rigid sclera would therefore explain the transient rise in IOP, with the amount of IOP increase determined by both the magnitude of ABP rise as well the compliance property of the sclera.

In rats that received the rapid saline infusion protocol, the magnitude of IOP increase was not up to the same extent as observed during PE infusion. The pattern of elevation following the saline infusion differed by eliciting a sustained form of IOP elevation that persisted following the saline infusion (Fig. 7B). Since changes in central venous pressure tend to affect cardiac preload and cardiac output in vivo, it is possible that an increase in blood pressure during the rapid saline infusion contributed to the rise in IOP (Fig. 7A). However, the ABP–IOP relationship obtained during the saline infusion (Fig. 5) differed from the relationship observed for the PE infusion (Fig. 4). Despite the overall smaller maximal IOP response observed during the saline infusion, there was a greater IOP elevation per unit MAP elevation during the saline infusion compared with the PE infusion. This finding suggests that other mechanisms, apart from the induced change in blood pressure, may be contributing to the IOP rise during the saline infusion.

A sustained increase in IOP and OPA has also been linked to the expansion of the choroid in glaucoma patients undergoing the water-drinking test. During the water-drinking test in humans, IOP typically peaks by 2.5 mm Hg in the normal eye and approximately 4 mm Hg for glaucomatous eyes 30 minutes after the ingestion of water. The mechanism behind the ocular response to the water-drinking test remains unclear. Our saline-infusion test is not equivalent to the water-drinking, but our findings suggest that hemodynamic changes from the expansion of central venous pressure can contribute partly to the IOP rise during the saline infusion in glaucoma.

The effectiveness of intracameral injection of microbeads to induce ocular hypertension with subsequent GON has been validated by previous studies. We acknowledge that mechanical obstruction with microbeads is not directly representative of the aqueous outflow obstruction seen in human glaucoma; however, it provides a characterization of restricted filtration that does not involve destruction of tissue as seen in other laser or cautery models.

A limitation of our study is the potential for pressure measurements to be confounded by general anesthesia. The weekly IOP readings were obtained whilst animals were under the effect of ketamine anesthesia. It has been reported that ketamine-based anesthesia can reduce IOP by 25% to 40% in rats (Chen B, et al. IOVS 2002;43:ARVO E-Abstract 4074), suggesting that our animals may have had higher IOP during their unanaesthetized state. All our invasive pressure measurements were obtained from our animals using a standardized dosage of urethane anesthesia. Urethane delivered by intraperitoneal injection has not been shown to affect the IOP of rats (Chen B, et al. IOVS 2002;43:ARVO E-Abstract 4074). However, urethane anesthesia has been shown to reduce the resting cardiovascular parameters of MAP and heart rate; still this is unlikely to confound our results since both the vasopressor response to PE and the bradycardic response from the baroreceptor reflex are preserved during urethane anesthesia.

The steady-state aqueous humor dynamics described by the Goldmann equation associates IOP with the difference between aqueous humor formation and aqueous outflow. In rodents, the normal aqueous formation rate, also known as turnover rate, has been estimated to be 2% to 5% of total aqueous volume per minute. A rapid formation of aqueous humor during transient increases in ABP would therefore be unlikely to contribute to the simultaneous rise in IOP given the rate of aqueous production is limited by metabolic dependent processes with ciliary perfusion pressure being unaffected by hypertension.

Last, although we did not specifically investigate the direct pharmacologic influence of PE on IOP, we believe the IOP rise observed during the intravenous PE infusion is a result of arterial hypertension. The pharmacologic action of PE is mediated by α-adrenergoreceptors and their activation will tend to lower IOP through the reduction of aqueous humor production by its action on the ciliary body and also through its vasoconstrictive action on the ophthalmic arteries owing to the artery’s rich sympathetic innervation. The nonselective activation of α2 selective adrenergic receptor by PE may also have the effect of lowering IOP, similar to the action of the selective α2 adrenergic agonists such as apraclonidine by decreasing aqueous production. In effect, the stimulation of arterial hypertension by PE in our study is analogous to the sympathoadrenal activation of arterial hypertension observed during the stress response as well as during isometric exercises.

CONCLUSIONS

Our study showed that there is an amplification of the IOP that accompanies a sympathomimetic acceleration of ABP. Using a rodent model, we have shown for the first time that the IOP and OPA response to acute systemic blood-pressure surges is heightened in the context of ocular hypertension. A less prominent but prolonged increase in IOP was achieved in our attempt to rapidly alter venous pressure by rapid saline infusion. Further studies are required to validate the dynamic real-time relationship between hemodynamic fluxes and IOP in humans as our results suggest that individuals with ocular hypertension are at a greater risk of having ocular pressures that are more susceptible to blood pressure variations, which over a long period may be of clinical significance.

Acknowledgments

Supported by Ophthalmic Research Institute of Australia (ORIA), Allergan Australia, and Novartis Australia.

Disclosure: J.C. Li, None; V.K. Gupta, None; Y. You, None; K.W. Ng, None; S.L. Graham, None

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