Glaucoma

Chronic Hypertension Increases Susceptibility to Acute IOP Challenge in Rats

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PURPOSE. To consider the effect of chronic arterial hypertension on the susceptibility of the retina to acute IOP challenge.

METHODS. Anesthetized adult Long-Evans rats with normal (n = 5, receiving saline subcutaneously), chronic high blood pressure (BP) for 4 weeks (n = 15, Angiotensin II subcutaneously), and acute high BP for 1 hour (n = 10, Angiotensin II intravenously) underwent IOP elevation (10–120 mmHg, 5 mmHg steps each 3 minutes). During IOP elevation, retinal function and ocular blood flow were monitored with electroretinogram (ERG) and laser-Doppler flowmetry (LDF), respectively. Blood pressure was monitored via a femoral artery cannula. Electroretinogram and LDF responses are expressed as a percentage of baseline and compared between groups. The left ventricle and the aorta were dissected to assess the morphologic changes associated with chronic hypertension.

RESULTS. Four weeks of hypertension (systolic BP 192 ± 4 mmHg) produced cardiac hypertrophy and thickened aortic arterial walls compared with controls (systolic BP 112 ± 3 mmHg). Retinal function was unaltered with chronic hypertension compared with normotensive animals. During acute IOP elevation, ERG and LDF were reduced in a dose-dependent manner in all BP groups. Both chronic and acute hypertension made the ERG and LDF less susceptible to IOP elevation. However, the degree of resistance to IOP elevation was greater in acute hypertension compared with chronic hypertension (P < 0.05).

CONCLUSIONS. Acute BP elevation makes retinal function and blood flow less susceptible to IOP elevation. The reduced susceptibility afforded by improved ocular perfusion pressure is compromised after 4 weeks of chronic hypertension.

Keywords: IOP, chronic high blood pressure, electroretinogram, retinal blood flow

Ocular perfusion pressure (OPP) represents the balance between the opposing forces of blood pressure (BP) and IOP (OPP = BP − IOP). Studies have shown that short-term BP elevation increases the IOP threshold needed to induce retinal dysfunction and blood flow attenuation.1−4 Whether this is also the case in chronic arterial hypertension remains unclear.

Chronic hypertension is commonly associated with structural changes in the cardiovascular system, such as cardiac muscle remodeling and atherosclerosis. These changes reduce cardiac output and increase the resistance in peripheral arteries, therefore leading to greater risk of ischemic heart disease and stroke, which is contrary to the expectation that better organ perfusion pressure should be beneficial. In the eye, whether chronic hypertension affords protection against, or increases the vulnerability to IOP elevation has clinical relevance for glaucoma. As evidenced by the end organ damage (i.e., to the retina, kidney, heart, and brain) that can occur in chronic hypertension, it seems that higher BP does not always equate to better perfusion pressure.

Studies have shown that low BP, including greater nocturnal BP dipping5,6 and overly aggressive treatment of systemic hypertension,7,8 is associated with a greater risk of glaucoma development. These outcomes are consistent with beneficial effects of a high OPP. A review of epidemiologic studies found conflicting outcomes regarding associations between systemic hypertension and glaucoma.9 The Baltimore Eye Survey found that systemic hypertension appears to be protective against glaucoma in younger patients but a risk factor in older patients.10 Therefore, the authors proposed that in early hypertension, prior to blood vessel compromise secondary to atherosclerosis, patients are likely to benefit from improved OPP and blood flow to the eye. This protective effect may become negated later in the course of disease when vascular damage becomes more dominant.10,11 One way to test this hypothesis would be to compare the effect of acute and chronic hypertension on the susceptibility to IOP elevation. As yet there is no compelling experimental evidence that chronic hypertension is less protective than is an equivalent acute hypertensive increase in OPP, despite the presence of atherosclerotic changes, which are known to be associated with the chronic state.

In this study, we compared the effect of normal BP with acute (1 hour) and chronic (4 week) BP elevation on the susceptibility of the electroretinogram (ERG) and ocular blood flow in response to acute IOP elevation in rats. The acute IOP elevation protocol is employed to gauge the capacity of the eye to cope with reduced OPP and increased mechanical stress. By comparing the three BP groups, we aimed to differentiate the effect of chronic...
hypertension from acute BP elevation per se. More specifically, we hypothesized that despite an improvement in OPP due to the hypertensive state, the ERG and ocular blood flow will be more susceptible to IOP elevation in chronic hypertension.

**MATERIALS AND METHODS**

**Animals**

All animal experimental procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animal ethics approval was obtained from the Animal Ethics Committee (Ethics ID: 0705168) of the University of Melbourne (Parkville, Victoria, Australia). Adult Long-Evans rats (8–12 weeks, 200–400 g; Monash Animal Services, Clayton, Victoria, Australia) were housed in a 20°C environment with light cycling (12-hour light/12-hour dark, on at 8 AM, 50 lux maximum). Food (Barastoc Rat and mice feed, Ridley Agriproducts, VIC, Australia) and water were available ad libitum.

**Experimental Design**

Chronic high BP was induced in 15 rats by subcutaneous infusion of Angiotensin II (Ang II; 0.7 mg/kg/day, 2.5 μL/h delivered by an Alzet osmotic minipump, model 2ML4; Durect Corporation, CA, USA) for 4 weeks. In the control group, five rats received vehicle (normal saline) infusion for an equivalent volume and duration. Systolic BP and IOP were monitored in conscious animals at baseline and then weekly throughout the 4 weeks.

At the end of 4 weeks, the effect of chronic hypertension induced by Ang II on retinal function was assessed using the scotopic ERG recorded in response to a range of stimulus intensities (see below for details). The effect of chronic hypertension on ocular susceptibility to IOP challenge was considered by raising IOP in 5 mm Hg steps over approximately 1 hour (10–120 mm Hg, 3 minutes/step), during which ERG (retinal function) was recorded in one eye and LDF (blood flow) in the fellow eye. Electrorinogram and LDF response to IOP elevation was compared across the three groups: animals with 4-week hypertension, 1-hour hypertension of the same magnitude, and normal controls. Data for the acute hypertension was compared previoulsy and re-plotted here for comparison (with permission from Plos One).

Following the above experiments, animals were euthanized prior to dissection of the left ventricle and aorta for gross morphology and histology to consider arterial and cardiac morphologic changes associated with chronic hypertension.

**BP Elevation and Monitoring**

In the chronic hypertension group, Ang II was delivered subcutaneously for 4 weeks by implanting an osmotic minipump. For implantation, rats were anesthetized with a ketamine; xylazine mixture (intramuscular, 60:5 mg/kg). Then a 1-cm skin incision was made on the rat's back and an osmotic minipump was inserted into the subcutaneous space. The wound was closed using interrupted sutures and caprofen was injected (subcutaneous 4 mg/kg) for post-surgical analgesia.

Systolic BP was monitored in conscious rats using a tail cuff sphygmanomaneter (ML125; ADInstruments Pty Ltd., NSW, Australia). Basal systolic BP was measured daily for 5 days in conscious rats (n = 20) to return the 95% confidence interval (CI) for mean habitual systolic BP. During experiments, BP was measured 3 days in each week. Daily measurement was, in turn, the average of 10 repeats over 10 minutes (1-minute intervals). To control for diurnal fluctuation, all BP measurements were taken between 10 AM and 12 PM. After gentle immobilization in a custom-made restrainer, animals were allowed to acclimatize for 20 minutes to minimize stress-related changes in BP (Supplementary Fig. S1). Following the 20-minute acclimatization, systolic BP was recorded every minute for 10 minutes.

The effect of chronic hypertension on ERG and blood flow was compared with transient high BP of similar magnitude, induced by intravenous infusion of Ang II for 1 hour (data from previous study). In brief, 1% Ang II was infused via a femoral vein cannula at a rate of 45 to 90 μg/kg/min. The infusion rate was adjusted as needed to maintain BP at a stable level over the duration of the acute IOP elevation.

During acute IOP challenge, BP was measured directly via femoral artery cannulation in all groups. Mean arterial pressure (MAP) was calculated as (MAP = diastolic + 1/3 [systolic – diastolic BP]).

**Acute IOP Challenge**

Intraocular pressure was elevated from 10 to 120 mm Hg in steps of 5 mm Hg, each lasting 3 minutes (total duration of 69 minutes). During LDF measurement, IOP elevation was induced by anterior chamber cannulation using a 30-G needle connected to a height adjustable reservoir containing normal saline. The height of the fluid level was calibrated against a mercury manometer to produce the desired IOP. During ERG measurements, cannulation was performed in the vitreous chamber to avoid interference with ERG electrodes placed on the cornea. We have previously shown that both anterior and posterior chamber cannulations produce the same IOP elevation as verified by an independent measurement cannula (see online supplement of Ref. 3).

**Electroretinography**

Following minipump implantation, the scotopic ERG was measured at the end of 4 weeks under general anesthesia (intramuscular ketamine 60 mg/kg, xylazine 5 mg/kg). Following dark-adaptation for 12 hours, one drop of 0.5% proxymetacaine and one of 0.5% tropicamide was applied for topical anesthesia and for mydriasis, respectively. A Ganzfeld sphere (Photometric Solutions International, Victoria, Australia) was used to delivery luminous energy ranging from –6.26 to 2.07 log cd·m⁻²·s calibrated with an IL1700 integrating photometer (International Light Technologies, Peabody, MA, USA). Responses were recorded using an active electrode (custom-made, chlorided sliver electrode) placed on the corneal apex and referenced to a ring electrode placed behind the limbus on the conjunctiva. A ground needle electrode was placed in the tail. Signals were amplified (×1000) over a bandwidth of 0.3 to 1000 Hz (~3 dB) and digitized using an acquisition rate of 4 kHz.

Electroretinogram waveforms were analyzed using well-established methods described in past publications. In brief, the photoreceptor response was modeled to return amplitude (Rm, μV) and sensitivity (S, cd⁻¹·m²·s⁻³), in terms of a delayed-Gaussian function fit to the leading edge of the a-wave. The rod bipolar cell function was also quantified in terms of its maximal amplitude (Vmax, μV) and sensitivity (1/K, cd⁻¹·m²·s⁻³), from modeling the b-wave amplitudes as a function of stimulus energy with a hyperbolic curve. Ganglion cell response was measured using the peak-to-trough amplitude of the scotopic threshold response (STR) recorded at ~5.25 log cd·m⁻²·s.

During the step-wise IOP elevation, a single ERG b-wave (stimulus energy ~1.12 log cd·m⁻²·s) was serially measured at the end of each IOP step. The signal was quantified as the trough-to-peak amplitude.
Laser-Doppler Flowmetry

During IOP elevation, ocular blood flow was monitored continuously at a sampling rate of 1 kHz using LDF (ML191; ADInstruments Pty Ltd, NSW, Australia). As previously described, a needle-type probe (0.48-mm diameter, MN110XP; ADInstruments) was inserted 2 mm into the vitreous chamber to measure blood flow changes in response to IOP elevation. The backscatter returned by the LDF probe provided an indication of the distance between the probe and the retinal surface. Such backscatter was kept at a fixed level by a micromanipulator throughout blood flow measurement, thereby minimizing any movement-related artifact due to IOP elevation.

Both retinal and choroidal blood circulations contribute to the signal returned by the intravitreal LDF probe used in this study (please see online supplement of Ref. 3). Although the exact proportion of the two components is not precisely known, a further pilot study showed that 50% of the LDF signal is derived from the retinal circulation (Supplementary Fig. S2). Given that photoreceptor oxygenation is derived from the choriocapillaris and inner retinal function is an appropriate index of ocular blood supply. Therefore, throughout this manuscript the term “ocular blood flow” is employed to describe the collective contribution from both circulations.

Morphology of Heart and Aorta

At the end of experiments, animals were euthanized prior to dissection of the heart and aorta. Immediately postmortem, the heart was excised, cleared of blood, and the thickness of the left ventricle was measured at 1 mm from the cardiac apex with a Vernier caliper (Mitutoyo Corporation, Kawasaki, Japan). This procedure eliminates any shrinkage produced by paraffin embedding. Each measurement was an average of four repeats.

The left ventricle-septum complex was dissected under a ×10 microscope at anatomical landmarks to improve consistency. After the tissue was freeze-dried for 2 hours (Micromodulyo 1.5K Vacuum Freeze Dryer; Edwards High Vacuum International, West Sussex, UK), the combined dry weight of the left ventricle and septum was measured and expressed as a percentage of the body weight.

Parameter values were compared with two-tailed Student’s t-tests. The significance level was set at 0.05. Data were analyzed using SPSS software (version 16.0; SPSS, Chicago, IL, USA). All results are expressed as mean ± SEM. Sample size was 30 per group. Group comparison was made with two-way repeated measures ANOVA. 

Sample Size and Data Analysis

When comparing chronic and acute high BP, it is important that both groups have similar BP values (~190 mm Hg) during the IOP challenge protocol. Therefore, only 9 of 15 eyes in the chronic high BP group were used in the ERG assay. Of the six eyes that were excluded, three were due to excessively low BP arising from general anesthesia, and three due to surgical complications (e.g., fluid leakage, cataract formation). Likewise, when LDF was measured in the fellow eyes, 11 of 15 eyes had BP levels under anesthesia that were comparable with those achieved in the acute hypertension group.

RESULTS

BP and IOP

Five daily habitual systolic BP and the 95% CI for mean (108–119 mm Hg) are shown in Figure 1A. In Figure 1B, systolic BP at week 0 represents average baseline measurements for each group prior to treatment. With Ang II infusion, systolic BP increased steadily to levels well above baseline (192 ± 4 mm Hg by week 4, P < 0.05). In controls that received normal saline infusion, systolic BP remained within the 95% CI of (4% paraformaldehyde in phosphate buffer pH 7.2) overnight. Prior to sectioning, the tissue was dehydrated through graded alcohol, transferred to xylene, and infiltrated with molten paraffin wax. Using the standard fast procedure, the tissue was passed through three changes of 100% ethanol, xylene, and paraffin. After embedding in paraffin blocks, aortic cross-sections of 10-μm thickness were cut and mounted on glass slides. Sections were dewaxed, rehydrated, and prepared for Gomori’s Aldehyde-Fuchsin staining. To enhance visibility of elastin fibers, Indigo Carmine-picric Acid was applied as a counterstain. Light microscopy and image capture was performed at ×10 magnification using a DotSlide 2.0 Whole Slide Scanner (Olympus BX4; Olympus, Tokyo, Japan). To avoid bias, image analysis (software Image-Pro Plus 6.0; Media Cybernetics, Warrendale, PA, USA) was conducted by two independent investigators who were masked to the identity of blood pressure status. As described previously, the wall-to-lumen ratio was defined as the area of the media tunica relative to that of the lumen. The sum of Aldehyde-Fuchsin stained area was quantified as elastin content and expressed as a percentage of total tissue area (i.e., elastin + collagen).

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baseline ($P > 0.05$). Despite the marked BP difference, IOP was similar between the two groups (Fig. 1C, two-way repeat measures [RM] ANOVA, interaction term $P = 0.97$, between groups $P = 0.78$). There was significant IOP fluctuation over the 4 weeks in both groups (time effect $P < 0.001$).

### Cardiovascular Consequences of Chronic Hypertension

After 4 weeks, the body weight of the hypertensive rats was similar to controls (Fig. 2A, $310 \pm 8$ vs. $321 \pm 13$ g, $P = 0.481$, $t$-test). Hypertensive rats developed cardiac hypertrophy, as evidenced by the increased left ventricle-septum complex dry weight ($0.15 \pm 0.01$% relative to body weight compared with controls ($0.10 \pm 0.01$, $P = 0.017$, $t$-test). There was also a trend for the left ventricle to be thickened in chronic hypertension, though this difference just failed to reach statistical significance ($1.91 \pm 0.11$ vs. $1.51 \pm 0.06$ mm, $P = 0.058$, Fig. 2C).

In addition to cardiac hypertrophy, hypertensive rats also developed structural change in the aorta. In Figures 2D and 2E, the cross-section of the aorta (Gomori’s Aldehyde stained, purple: elastin; light blue: collagen) shows a thicker layer of tunica media (smooth muscle) in the hypertensive rat. Group analysis confirmed that both aortic wall thickness ($P = 0.017$, Fig. 2F) and wall-to-lumen ratio ($P = 0.018$, Fig. 2G) were greater in chronic hypertensive rats. Elastin content relative to total tissue (elastin/elastin + collagen, %) was not significantly different between the two groups ($P = 0.812$, $t$-test, Fig. 2H).

### Effect of 4 Weeks of Hypertension on Retinal Function

Figure 3 compares retinal function between chronically hypertensive and normotensive rats. Representative ERG waveforms across a range of stimulus intensities suggest little difference between the two groups (Fig. 3A). Quantifying the components of photoreceptor amplitude ($R_m P_{3}$) and sensitivity ($S$), bipolar cell amplitude ($P_{2 V_{max}}$) and sensitivity ($1/k$), as well as ganglion cell amplitude (STR) confirms that after 4 weeks of hypertension, responses in chronic hypertension were comparable with control animals (Fig. 3B, two-tail unpaired $t$-test, $P = 0.962$, 0.086, 0.919, 0.111, 0.510, respectively).

### Susceptibility to Acute IOP Elevation

As 4-week hypertension did not induce detectable changes in retinal function under normal milieu, retinal susceptibility to a physiological stressor was considered by measuring the ERG and LDF during IOP elevation and has been shown normalized to baseline in Figure 4. For both relative retinal function ($b$-wave amplitude) and blood flow (LDF), the IOP-response curves show a rightward shift in hypertensive rats when compared with controls (two-way RM ANOVA, interaction $P < 0.001$ for Fig. 4A and $P = 0.002$ for Fig. 4B), consistent with a reduced susceptibility to IOP elevation.

The chronicity of hypertension (chronic versus acute) was considered in animals matched for blood pressure (for ERG assay, MAP $161 \pm 13$ vs. $161 \pm 4$ mm Hg, $P = 0.997$; for LDF
assay, 155 ± 13 vs. 156 ± 5 mm Hg, *P* = 0.944, *t*-test). Figures 4A and 4B show that the degree of protection against IOP elevation was reduced in chronically hypertensive rats compared with acute hypertensive animals. A two-way RM ANOVA comparing IOP-response curves between the two forms of hypertension returns a significant interaction term when IOPs greater than 60 mm Hg are considered (Fig. 4A *P* = 0.003, Fig. 4B *P* < 0.001). Thus, 4 weeks of chronic hypertension compromise the amount of protection afforded by the increase in OPP resulting from the higher BP.

**DISCUSSION**

Consistent with our previous studies and other studies of acute hypertension, chronic hypertension of 4-weeks duration makes neuronal function and ocular blood flow less susceptible to acute IOP elevation as indicated by a rightward shift of the IOP-response relationship when compared with normotensive rats (Fig. 4A). By matching the BP levels in acutely and chronically hypertensive rats we showed, for the first time, that 4 weeks of chronic hypertension partially negates this effect.

To date, few experiments have considered the effect of chronic hypertension on the susceptibility to IOP elevation. The neural response was normal under basal conditions (no IOP stress), but was altered when challenged with IOP elevation. In monkeys, Hayreh and colleagues investigated the influence of arterial hypertension on optic nerve head damage induced by chronic IOP elevation. The strength of their study was that long-standing hypertension (40–90 months) and atherosclerosis (62–105 months) was induced before IOP was elevated (4–60 months) by laser photoocoagulation of the trabecular meshwork. Such a long period of experimentation better models the chronicity of human hypertension and the subsequent development of glaucoma. These authors found that systemic hypertension/atherosclerosis produced either no effect or only a marginal exacerbation of optic nerve head and retinal nerve fiber
layer changes in their model of chronic IOP elevation. Given the substantial variability in duration and magnitude of BP and IOP elevations in their cohort, the authors acknowledged that their study might have been underpowered to detect a difference. Nevertheless, their finding is consistent with our observation for the relative detrimental effect in animals with 4-week chronic hypertension.

The reactivity of arterial wall is crucial to mechanisms that preserve blood flow during mild ischemic events (blood flow autoregulation).\textsuperscript{26} We found that 4 weeks of arterial hypertension induces structural changes with thickened and narrowed arterial wall. This data indicates that systemic or local vascular dysfunction in this pharmacologic rodent model (Ang II) is sufficient to compromise autoregulatory mechanisms. We believe that this modification of OPP-related benefits is independent of local effect on elements of the renin-angiotensin system known to exist in the retina.\textsuperscript{27} Our reasoning is based on fact that neither systemic Ang I nor Ang II is able to cross the blood-brain barrier\textsuperscript{28,29} or the blood-retinal barrier.\textsuperscript{30} Additionally, the infusion of Ang II is an often used model for systemic hypertension in rodents.\textsuperscript{31-33} This approach mimics excessive renin-angiotensin system activity, which is known to play an important role in the pathogenesis of essential hypertension. In acutely hypertensive rats, it is understood that Ang II raises BP largely through vasoconstriction and the resultant increased peripheral arterial resistance. In the chronic case, Ang II acts as a vasoconstrictor, but it also stimulates aldosterone production, leading to sodium and water retention by the kidney, with both factors promoting high BP. The time course and the extent of BP elevation in this study shows agreement with previous works using similar Ang II dosing in rats.\textsuperscript{34,35}

Our data suggests that the reduced neural capacity to withstand IOP elevation in the chronic hypertension (Fig. 4A) is associated with a relatively impaired blood flow autoregulation (Fig. 4B). While the exact mechanism by which chronic hypertension impairs retinal autoregulation (reduced blood flow resistance to IOP) is not fully understood, changes in the thickness of vessel walls, altered compliance and rigidity have been implicated.\textsuperscript{36} In addition to its pressor effect to increase BP, Ang II is a potent stimulator for cell growth and remodeling in cardiac myocytes and arteries.\textsuperscript{37} Similar to the pathophysiology in essential hypertension, hypertensive rats in this study developed ventricle hypertrophy and increased arterial wall thickness (Fig. 2). However, vascular remodeling was not evident, as the elastin/collagen ratio in the aorta, an indicator for arterial compliance and rigidity,\textsuperscript{38} was not significantly changed at our 4-week time point (Fig. 2H).

Although cardiac and aortic hypertrophy were observed, it is not clear whether similar changes also occurred at the level of the retina. One limitation of our study is that structural changes in the retinal vasculature were not measured. Previous studies of the same animal model showed that increased vascular thickness and rigidity are present not only in the aorta, but also in the small arteries and arterioles of the rat mesentery.\textsuperscript{35,39} Therefore, it may be reasonable to assume that the smooth muscle hypertrophy in the aorta represents a more widespread change that involves the retinal and choroidal vasculature. Such change may underlie the impairment in ocular blood flow autoregulation we have observed here (Fig. 4B).

Four weeks of chronic hypertension did not completely negate the beneficial effects arising from greater OPP. This may be consistent with the early stage of hypertensive vascular disease as evidenced by the absence of vascular remodeling (aortic elastin/collagen ratio; Fig. 2H). Also consistent with the idea of an early disease model in this study, retinal function was unaltered in the chronically hypertensive rats (Fig. 3). Such finding differs from a number of previous studies where retinal dysfunction has been reported in both patients with chronic hypertension\textsuperscript{40} and after prolonged hypertension in rat models (Ren-2 rat,\textsuperscript{41} spontaneously hypertensive rats\textsuperscript{42}). In those studies ERG changes were associated with retinal vascular alterations. Thus, any structural changes in the retina vasculature in the current model are not severe enough to compromise basal retinal function. We speculate that, with longer periods of hypertension and thus more severe vascular damage, the beneficial effect of high BP might be further reduced.

It is important to rule out that changes in sensitivity to IOP elevation do not arise from altered habitual IOP levels. Previous studies have shown that BP elevation can cause a modest increase in IOP. In humans, every 10-mm Hg rise in systolic BP is associated with an IOP elevation of approximately 0.27 mm Hg.\textsuperscript{10,43-46} A similar association has been reported in spontaneously hypertensive rats at 8 weeks of age.\textsuperscript{47} Based on this association, systolic BP elevation from 110 to 190 mm Hg reported in our study could raise IOP by approximately 2.2 mm Hg, which is of little practical significance. As we found no significant difference in IOP by 4 weeks, it is unlikely that differences in susceptibility to IOP elevation arise from changes to habitual IOP.

A methodological issue in this study is that the precise location of blood flow autoregulation is difficult to pinpoint as the LDF contains contributions from retinal and choroidal circulations, which respond differently to IOP elevation.\textsuperscript{48} Nevertheless, the difference in blood flow response between normotensive and chronic hypertensive is robust, and correlates well with our retinal function measurement. Further studies using techniques such as magnetic resonance imaging,\textsuperscript{49} or optical coherence tomography microangiography\textsuperscript{48} would help differentiate autoregulatory deficiency in retina and choroid.

It is also important to note that any pharmacological approach to manipulate BP has the potential to confound studies of vascular autoregulation. There are alternative, drug-free approaches to elevate BP chronically, such as spontaneous hypertensive rats, or the kidney artery clipping model. However, with these methods, it would not be easy to match BP between acute and chronic hypertension; and each of these models brings with it a new set of potential confounds. While being aware of the limitation of our Ang II model for studying autoregulation, we chose this model as it allows comparison between acute and chronic hypertension of the same BP and mimics excessive renin-angiotensin activity, an important pathological feature of human hypertension.

**SUMMARY**

In this study, we showed that 4 weeks of chronic hypertension compromises the benefit afforded by BP elevation for retinal function against IOP elevation. This effect was partially associated with a reduced capacity for ocular blood flow to autoregulate in response to IOP elevation in chronic hypertension. Structural changes to blood vessels arising from chronic hypertension may underlie some of our observations but longer interventions are needed to evoke the longer-term components (vascular and neural) of this response.

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