Impact of Systemic Blood Pressure on the Relationship between Intraocular Pressure and Blood Flow in the Optic Nerve Head of Nonhuman Primates

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**PURPOSE.** Studies suggest that reduced ocular perfusion pressure in the optic nerve head (ONH) increases the risk of glaucoma. This study tested a hypothesis that the magnitude of blood flow change in the ONH induced between two same intraocular pressure (IOP) alterations depends on the level of mean systemic blood pressure (BP).

**METHODS.** In eight anesthetized rhesus monkeys, systemic BP was maintained at either a high, medium, or low level (n = 6 each, ranging from 51–113 mm Hg); IOP was rapidly altered from 10 to 30 mm Hg and then to 10 mm Hg manometrically. Blood flow in the ONH (BFONH) was repeatedly measured with a laser speckle flow graph for 10 minutes at each IOP level period. The BFONH and relative changes to the baselines at each measured time point were calculated and compared longitudinally among the three BP groups.

**RESULTS.** There was no statistically significant difference in mean baseline BFONH across the BP groups. In the high-BP group, BFONH had no significant change during the IOP alterations. However, the same IOP alterations caused a significant BFONH change in the two lower BP groups. The duration of the BFONH Changes from baseline to a peak and to a steady state was significantly delayed in the two lower, but not the higher, BP groups.

**CONCLUSIONS.** Systemic BP plays an important role in maintaining the normal autoregulation of the ONH, and it became deficient in the lower BP groups. In patients with glaucoma, a normal, sustained BP may be important to prevent worsening glaucoma. (Invest Ophthalmol Vis Sci. 2009;50:2154 –2160) DOI:10.1167/iovs.08-28882

Blood flow (BF) to many organ and tissue beds is regulated to maintain relatively constant flow despite fluctuations in perfusion pressure, the difference between the arterial pressure entering the organ and the venous pressure leaving the organ.1–3 This is known as autoregulation and occurs in several tissues, including the eye. A typical autoregulation pattern follows a nonlinear relationship between BF and perfusion pressure, whereby a plateau is observed across the range in which autoregulation remains effective. Beyond the upper and lower limits of this plateau, the vasmotor adjustments are exhausted and BF becomes more linearly related to perfusion pressure (Fig. 1).

In the eye, the ocular perfusion pressure (OPP) can be estimated as the difference between the mean arterial blood pressure (BP) and intraocular pressure (IOP), because the IOP is close to the pressure in the veins leaving the eye.4 This unique feature has enabled autoregulation to be studied by describing the relationship between BF and IOP in ocular tissues in the retina, the optic nerve head (ONH), and the choroid in both humans and animals.5–19 However, the autoregulation curve obtained by altering the IOP typically includes an OPP range that is only around the lower limit (the left portion of the autoregulation curve in Fig. 1), because the range of perfusion pressure that can be achieved by manipulating IOP is limited.20,21

The autoregulatory capacity of tissue beds within the eye is of clinical interest, because both the IOP and the BP fluctuate widely. Also, both elevated IOP and insufficient optic nerve BF have been cited as risk factors for primary open-angle glaucoma (POAG). The theory of autoregulatory dysfunction has been proposed to explain the complex interplay of IOP, BP, and BF and their potential roles in POAG.22–30 According to this theory, the ocular vascular beds fail to maintain adequate BF during decreased perfusion pressure or even during otherwise "normal" perfusion pressure, due to autoregulation dysfunction.

Since OPP is determined by both IOP and BP, the physiological response to reduced BP is expected to cause effects on ocular BF similar to those caused by increased IOP. Indeed, several clinical studies in patients with glaucoma, particularly those with "normal" IOP, often have lower systemic BP.31,32 In addition, patients with glaucoma may demonstrate larger and more frequent diurnal BP fluctuations, which again underscores the potential importance of autoregulation to the pathophysiology of glaucoma.33–36 In a longitudinal study of patients with glaucoma,37 reduced systolic BP was identified as one of the risk factors for the progression of glaucoma. This finding evoked a caution against overzealous use of antihypertension treatment in patients with both glaucoma and systemic hypertension. These clinical observations highlight the importance of BP and its relationship with IOP and BF in both normal and diseased conditions.

The effect of varying BP on the anterior optic nerve BF (BFONH) and on the peripheral vasculature has been investigated previously in both animals19 and humans38–41 by means of physical exercise or postural changes while maintaining constant IOP. However, these studies were limited, in that BP can be altered within only a limited range in humans. Moreover, the lower OPP range is likely to have direct relevance to the theory that inadequate BF plays a role in the pathogenesis of glaucoma.

This study was designed to further clarify the relationships between systemic BP, IOP, and BFONH in a group of nonhuman primates. Our purpose was to test the hypothesis that the amplitude of the BFONH change depends as much on systemic...
FIGURE 1. An autoregulation curve that describes the relationship between BF and perfusion pressure. The general pattern of the curve includes a plateau known as autoregulation range (the solid fraction of the curve), across which the range of the plateau the autoregulation remains effective. Beyond this plateau, the relationship becomes more linear (BF/MBR) and is known as the upper and lower limits. In the eye, the relationship between the BF and perfusion pressure is investigated mostly around the lower limit of the autoregulation curve because of its clinical importance.

BP as on the absolute level of IOP at an equivalent position on the autoregulatory curve.

MATERIAL AND METHODS

Subjects

Eight adult rhesus monkeys (*Macaca mulatta*; five male and three female; 14 ± 5 years old) without observable eye diseases were included in this study. All experimental methods and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local Institutional Animal Care and Use Committee.

Anesthesia and General Preparation

Immediately before testing, the animal was sedated initially by an intramuscular injection of ketamine/xylazine (15 and 0.8 mg/kg); anesthesia was maintained thereafter by administration of pentobarbital (6–9 mg/kg, IV) every 30 to 40 minutes. The animal was placed on a separate test, the intra- and intersession repeatability of LSFG measures of BF in the ONH was evaluated. In the test, the measurement was repeated in one eye each of 11 monkeys three times during each visit (intrasession) for three different visits within a 4-month period (intersession), as follows. One eye of each animal was randomly chosen as the study eye.

BF Measured with Laser Speckle Flowgraphy

A laser speckle flowgraphy (LSFG; Softcare, Iizuka, Japan) device was used to measure the BF of the ONH based on a laser speckle phenomenon. This instrument consists of a fundus camera equipped with a halogen lamp and a diode laser. The halogen lamp is used to define the area to be examined; the laser (λ = 830 nm, maximum output power, 1.2 mW) is switched on at the time of measurement. The measured fundus area is approximately 3.8 × 3 mm (width × height), with an estimated depth of tissue penetration of 0.5 to 1 mm, based on estimation in human eyes. The scattered laser light from the illuminated target area is captured by a CCD camera with 700 × 480 pixel resolution; the light intensity of each pixel is converted into an electric signal. Each imaging session lasts 4 seconds, and 120 frames (30/sec) in total are captured by a frame grabber and transferred to a computer file.

Offline analysis software combines all captured images over the 4 seconds into a composite perfusion map, with each pixel being assigned the computed mean blurring rate (MBR), which is closely associated with the BF velocity. The large blood vessels and areas outside the optic disc were excluded from analysis (Fig. 2). The MBR within the ONH was then extracted with a software tool. For OPPs from 0 to 100 mm Hg, studies show that the blurring rate is closely correlated to the BF in the retina and choroid when validated against measures using the microsphere technique, which measures the actual volume of blood in these tissues. A significant correlation was also found between the blurring rate and BF measured with a hydrogen clearance method in the ONH, although the BF was altered in a smaller range (±20% of normal). Thus, this blurring rate, estimated as a quantitative index of BF within a volume of the ONH or retina, was used in this study after the large vessels were excluded to minimize the impact of the diameter change of the large vessels on the BF estimate.

Experimental Protocols

Intra- and Intersession Repeatability Assessment. In a separate test, the intra- and inter-session repeatability of LSFG measures of BF in the ONH was evaluated. In the test, the measurement was repeated in one eye each of 11 monkeys three times during each visit (intrasession) for three different visits within a 4-month period (intersession), as follows. One eye of each animal was randomly chosen as the study eye.

BF as on the absolute level of IOP at an equivalent position on the autoregulatory curve.
The anterior chamber was cannulated with a 27-gauge needle connected to a bottle filled with sterile saline. The IOP was set to 15 mm Hg by placing the bottle at a corresponding height. The BF was measured with the LSFG at least 30 minutes after the IOP was set.

The BFONH for each of the three measurements was averaged (mean ± SD) from all three visits and compared to determine intrasession variability. Intrasession variability was evaluated by comparing the mean BFONH of the three visits. The variability was evaluated by the coefficient of variance, the ratio of SD over the mean.

Assessment of the Impact of Systemic BP on IOP-Related BF Changes. For each test, one eye of the eight rhesus monkeys was randomly chosen in any given session. The IOP in the eye was manometrically set first at 10 mm Hg and after equilibrating for at least 10 minutes, the baseline BFONH was measured three times with LSFG. The saline reservoir was then raised to a height calibrated to be equivalent to 30 mm Hg in less than 2 seconds. Based on data from a separate, previous test, it took approximately 7 to 9 seconds to reach at least 90% of the desired 20 mm Hg IOP change. At the same time, the BFONH was measured every 20 seconds beginning 2 seconds after the saline reservoir was raised for the first minute and then once approximately every minute for at least 5 minutes. Henceforth, this condition is referred to as IOP10-30. The IOP was maintained at 30 mm Hg for at least another 5 minutes, and the BFONH was measured again. The saline reservoir was rapidly lowered from 30 to 10 mm Hg (henceforth referred to as IOP30-10). When lowering IOP, it took approximately 7 to 9 seconds for it to reach 90% of the 20-mm Hg change. The BFONH was measured in a similar manner as described during the period of IOP10-30. During both IOP10-30 and IOP30-10 changes, all but the first BF measurement were acquired when the IOP had reached the new desired level (Fig. 3).

Optimally, a study of the effects of systemic BP on the amplitude of the BFONH change for an otherwise equivalent change in IOP would be performed across a wide range of systemic BP. However, precise control of systemic BP within a narrow range predefined for each experiment or animal is technically difficult, and pharmaceutical intervention to maintain a particular BP could introduce additional factors that could confound BF results. Hence, systemic BP varied across different testing days and individual animals such that the total range of BP levels observed after initial anesthesia for 18 total test sessions in eight animals was from 51 to 113 mm Hg. Therefore, analysis was performed by post hoc grouping (tertiles) according to the mean systemic BP observed for each test of an eye as follows: Eyes with BP between 82 and 113 mm Hg were grouped into the high-BP category; those with BP between 65 and 74 mm Hg were grouped into the medium-BP category; and those with BP between 50 and 61 mm Hg were grouped into the low-BP category. Consequently, there were six tests of six different eyes for each BP group. Although only one eye was tested during any given experiment, some of the animals contributed both eyes to one or more BP groups depending on the systemic BP observed during repeat testing (Table 1). The average ages of the monkeys in each BP group (high, medium, and low) were 11 ± 2, 17 ± 7, and 11 ± 2, respectively.

Data Analysis and Statistics

We used raw values of BFONH for statistical analysis (see below); however, to visualize results graphically, we normalized the percentage change of BFONH at each time point from the IOP10-30 and IOP30-10 transitions against the initial baseline BFONH values in each experiment. Two-way, repeated-measures analysis of variance (ANOVA) was used to evaluate the effect of BP on the BFONH changes across the BP groups. Post hoc analysis (Scheffé test) was used to evaluate the significance of each BFONH change during IOP10-30 and IOP30-10 against the baseline values in each BP group (six eyes for each BP group).

RESULTS

Intra- and Intersession Repeatability Assessment

The coefficients of variance of BF measurement (SD/mean) were 7.2% for intrasession and 13.2% for intersession, which demonstrates excellent intra- and intersession repeatability in the LSFG measurements.

Assessment of the Impact of Systemic BP on IOP-Related BF Changes

Table 1 lists the average BP (±SD) for each BP group, along with the BFONH measured under each of the three main conditions: baseline (IOP = 10 mm Hg), after an IOP increase from 10 to 30 mm Hg (IOP10-30), and after an IOP recovery from 30 to 10 mm Hg (IOP30-10). There are also two phases for each of the two latter conditions: the initial state and the steady state. The initial state denotes the period from the onset of IOP alteration to the time immediately after a maximum BFONH change. The BFONH response was dynamic in most eyes during this initial state. The steady state denotes the period after the initial dynamic state, sampled 5 to 6 minutes from the onset of IOP alteration. Based on the post hoc grouping, the BP in the high-BP group is significantly higher than in the other two groups (P < 0.001, ANOVA) and the medium-BP group is significantly higher than in the low-BP group (P = 0.03, ANOVA). The BP during the period of the BFONH testing varied by 6 ± 1, 5 ± 2, and 4 ± 2 mm Hg in the high-, medium-, and low-BP groups, respectively. There was no statistically signifi-

### Table 1. BP, Baseline BFONH, and Relative Change of BFONH during IOP10-30 and IOP30-10

<table>
<thead>
<tr>
<th>BP Group</th>
<th>Animal/Eye (n)</th>
<th>Mean BP (SD)</th>
<th>BFONH Baseline</th>
<th>BFONH (IOP10-30)</th>
<th>BFONH (IOP30-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean BP (SD)</td>
<td>BFONH Baseline</td>
<td>Initial State (%)</td>
<td>Steady State (%)</td>
</tr>
<tr>
<td>High</td>
<td>4/6</td>
<td>102.2 (12.1)</td>
<td>11.1 ± 3.1</td>
<td>9.6 ± 2.5 (14%)</td>
<td>11.1 ± 3.3 (0%)</td>
</tr>
<tr>
<td>Medium</td>
<td>5/6</td>
<td>69.7 (4.7)</td>
<td>11.0 ± 2.3</td>
<td>6.7 ± 1.9 (19%)</td>
<td>8.2 ± 2.9 (25%)</td>
</tr>
<tr>
<td>Low</td>
<td>3/6</td>
<td>55.7 (4.1)</td>
<td>13.9 ± 3.4</td>
<td>7.1 ± 2.3 (49%)</td>
<td>7.5 ± 2.4 (46%)</td>
</tr>
</tbody>
</table>

The results were based on six individually treated eyes in each group. The same conclusion was drawn if the average for both eyes was used for the analysis.

* P < 0.001 (Scheffe post hoc test) compared with baseline.
† P ≤ 0.01 (Scheffe post hoc test) compared with baseline.
In the high-BP group (Fig. 4A), the BFONH responded rapidly to IOP changes in both directions: on average, BFONH decreased 14% during IOP10-30 and increased with an overshoot of 16% above baseline during IOP30-10; both BFONH changes occurred within approximately 10 seconds from the start of the IOP changes. In the high-BP group, BFONH returned from maximum to baseline levels within approximately 20 seconds.

In the medium-BP group (Fig. 4B), the IOP 10-30 change induced a larger BFONH decrease below baseline (−39%, \( P < 0.001 \), ANOVA, post hoc) compared with the high-BP group. In addition, although the BFONH returned toward baseline level 10 minutes after the IOP increase (steady state), it remained 25% below baseline (\( P = 0.14 \), ANOVA, post hoc). In the IOP 30-10 change, the BFONH overshot was larger, such that the peak value was 22% above baseline. The BFONH then gradually returned to baseline. In both IOP 10-30 and IOP 30-10, it took approximately 50 and 30 seconds, respectively, for the BFONH to reach their peak values and 250 and 160 seconds to return to their steady state (Table 2).

In the low-BP group (Fig. 4C), the BFONH at the initial state decreased by 49% from baseline at IOP10-30 and remained depressed (46% below baseline) during the subsequent 10 minutes (\( P < 0.01 \) for both states). In the IOP 30-10 condition, BFONH returned to baseline without the obvious overshoot observed in the medium-BP group (\( P > 0.05 \)). In both the IOP 10-30 and IOP 30-10 conditions, it took more than 60 and 40 seconds, respectively, for the BFONH change to reach peak values (Table 2).

### DISCUSSION

Based on the concept that OPP is determined by both IOP and BP, it was expected that if IOP is controlled at a constant level, the systemic BP would be a determining factor for BFONH. It was also expected that under the experimental conditions in this study, the relationship between the BFONH and the OPP should be similar to the general pattern of autoregulation as derived in other studies by changing the IOP. The results show that the BP in the three respective BP groups (high, medium, and low) were 102, 70, and 56 mm Hg, respectively; the corresponding OPP then can be estimated as approximately 85, 55, and 40 mm Hg, where the IOP was 10 mm Hg and the height from the eye to the heart was 7 to 8 cm, which corresponds to approximately 5-mm Hg pressure (BP–IOP–5 mm Hg). Despite this wide range of estimated OPP, the baseline BFONH showed no significant differences across the three groups. However, when the OPP was acutely reduced from baseline by 20 mm Hg in each group (IOP10-30), the BFONH in the medium- and low-BP groups decreased significantly, but not in the high-BP group. The OPP levels in these two groups during the IOP 30-mm Hg phase were approximately 35 mm Hg for the medium-BP group (70–30–5) and even lower for the low-BP group, which would represent the lower limit of the normal autoregulation range in monkeys. This estimate for the lower limit of normal autoregulation is close to those in the ONH reported previously for cats (between 37 and 48 mm Hg) and monkeys (30 mm Hg) and in one study of humans (34 mm Hg), but slightly higher than in another study of humans (30 mm Hg). This comparison confirms that the estimated range of OPP achieved through this approach produced a pattern of autoregulation similar to that of the human eye.
variation and greater reductions in nocturnal BP than relative BFONH changes and OPP observed in our study follows parameter was altered. Nonetheless, the relationship between autoregulation capacity. In our study, OPP was altered in part otherwise equivalent change in IOP can have a dramatically dif-
ferent impact on BF, depending on perfusion pressure and changes.

optic neuropathy include both IOP and systemic coma, and particularly in normal-tension glaucoma, the vascul-
do healthy people. The combination of low systemic BP
has never been verified in monkeys.73 In addition, the maxi-
mum temporal resolution of LSFG for the BFONH measure-
ment by the LSFG is limited. Though the penetration other laser-based flowmetry techniques, the depth of flow
measurements at low OPP, may deviate from the true values. Like most of the

BFONH change induced by rapid IOP changes may have important clinical implications. In glau-
coma, and particularly in normal-tension glaucoma, the vascu-
lar factors associated with the development of glaucomatous optic neuropathy include both IOP and systemic BP.51,52,57,61 These patients often have significant diurnal IOP variation62–64 and greater reductions in nocturnal BP than do healthy people.54–56 The combination of low systemic BP and increased IOP may result in a significant OPP decrease. Without a capable autoregulatory mechanism, such a reduction could result in decreased BFONH. For instance, in a recent study by Choi et al.,66 the mean systemic BP in patients with glaucoma was 92 mm Hg, which we can estimate to be approximately 67 mm Hg in the ophthalmic artery.57 These patients exhibited diurnal fluctuation of 22.8 mm Hg; whereas the mean IOP was 14 mm Hg and fluctuated over a 5.3-mm Hg range. Thus, it is quite possible that the OPP in glaucoma can be 40 mm Hg or lower. At this marginally low OPP level, the BFONH can be significantly reduced by BP and/or IOP fluctua-
tion, as demonstrated in the present study. BFONH would be reduced further if the capacity of autoregulation is impaired. In agreement with clinical observations,31,52,57,61,65 the results of the present study suggest that the systemic BP is at least an equal or even a greater risk factor for BF deficiency in the glaucomatous ONH when compared with IOP based on the net pressure changes.

It is not clear how the BF autoregulation in the ONH is affected by BP. There are at least two theories proposed for autoregulation: myogenic and metabolic mechanisms. Myo-
genic mechanism invoke the immediate vascular smooth muscle contraction and relaxation in response to transmural pressure change in resistance vessels to maintain stable BF.1–3,66–68 Metabolic mechanism proposes that BF adapts to the existing metabolic activity of the tissue in response to metabolites with vasodilator activity.69–71 For the myogenic mechanism, one of the basic requirements for the proper autoregulation function is background vascular tone, or vascular reserve.72 This reserve allows the resistance vessel to further contract or dilate, by the myogenic response of vascular smooth muscles, to control vascular resistance. There are multiple factors, including intra-
vascular pressure, that maintain and affect the background vascular tone. In the present study, this background vascular tone was most likely diminished when the BP was very low, as was the myogenic response. Thus, when IOP was altered by 20 mm Hg, a near passive BFONH response after the OPP decline resulted.

In both the high- and medium-BP groups, an overshoot in the BF response was induced during the initial state of IOP30–10. It should be noted that this response of BFONH may be related to the rapidity of change in IOP. For example, in this and previous studies by Riva et al.56 and by Geijer and Bill,20 after the IOP was rapidly reduced from a pre-elevated level to normal in seconds, the BFONH was actually increased above previous normal values by 16% to 22%, 44%, and even more than 200%, despite of different techniques used for the BF measurement and different magnitude of the IOP reduc-
tion that initiates the responses. However, this response was absent when the IOP changes took a longer time (~20 seconds) in rabbits,53 suggesting again that it is the myo-
genic regulation that dominates the initial state of vascular smooth muscle response to the rapid shear pressure changes.

Several limitations of this study should also be noted. First, the effect of anesthetia on the BFONH response during low-BP cannot be excluded, although the dose difference of the anesthetie between the BP groups was negligible and depends largely on the response of individual animals. Second, despite excellent repeatability of LSFG measurement of BFONH, the technique has limitations. The LSFG does not measure the BF directly but relies on a linear calibration curve, the estimated BF at certain extreme experimental conditions, such as very low OPP, may deviate from the true values. Like most of the other laser-based flowmetry techniques, the depth of flow measurements by the LSFG is limited. Though the penetration depth is said to reach the lamina or even deeper, the depth has never been verified in monkeys.73 In addition, the maximum temporal resolution of LSFG for the BFONH measurement is once every 20 seconds, 4 seconds for each measurement. Thus, the measurement during the initial state after the OPP change may not capture the maximum BFONH; however, the actual value, if missed, should be no less than the measured ones.

In summary, the present study demonstrated that systemic BP plays an important role in maintaining the normal autoreg-
ulation of the BFONH, although the detailed mechanisms are yet to be fully understood. The results suggest that in patients with glaucoma with significant IOP and BP variation, to achieve a normal and sustained BP is equally or even more important than controlling IOP to maintain normal BFONH.
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