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.08-2888

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.1–9 This is known as autoregulation and occurs in several tissue beds, 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 vasomotor 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.6 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.7–19 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...
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 table in prone position. The head position was fixed with a head rest (6–9 mg/kg, IV) every 30 to 40 minutes. The animal was placed on a anesthesia was maintained thereafter by administration of pentobarbital intramuscular injection of ketamine/xylazine (15 and 0.8 mg/kg); an-

Experimental Protocols

Intra- and Intersession Repeatability Assessment. In a separate test, the intra- and inter-session repeatability of LSF measurements 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 estimations 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.

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Relationship of BP, IOP, and Blood Flow in the Optic Nerve

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 (area/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.

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

FIGURE 2. Procedures to extract the MBR in the capillary areas of the ONH. (A) MBR composite map of the ONH and surrounding retina, in which the BF, as estimated by the LSFG, is represented in pseudo colors. (B) A fundus photograph from the same eye was superimposed and colocalized to the MBR composite map by matching the disc margin and visible vessels in both images. (C) The disc margin in the photograph was used to mask out and exclude the area outside the ONH in the MBR map from the analysis. (D) The large blood vessels on the disc were also segmented and excluded from the composite map. Only the capillary areas in the ONH were used for the blood flow estimate: BFONH.
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 63 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 intra session 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>Initial State (%)</td>
<td>Steady State (%)</td>
<td>Initial State (%)</td>
<td>Steady State (%)</td>
<td></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 (−39%)</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 (Scheffé post hoc test) compared with baseline.
† P ≤ 0.01 (Scheffé post hoc test) compared with baseline.
IOP10-30 and IOP30-10 conditions, it took more than 60 and 40 minutes, respectively, for the BFONH to reach their peak values and 250 and 160 seconds to return to their steady state (Table 2).

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 each panel, the down arrows indicate when IOP started to increase from 10 to 30 mm Hg and the up arrows indicate when the IOP started to recover from 30 to 10 mm Hg. Data points to the left of the down arrows were acquired during the baseline period, with IOP set to 10 mm Hg.

In the medium-BP group (Fig. 4B), 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 overshoot was larger, such that the peak value was 22% above baseline. The BFONH then gradually returned to baseline. In both IOP10-30 and IOP30-10, 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 IOP30-10 condition, the BFONH returned to baseline without the obvious overshoot observed in the medium-BP group (P > 0.05). In both the IOP10-30 and IOP30-10 conditions, it took more than 60 and 40 seconds, respectively, for the BFONH to reach peak values (Table 2).

**Table 2.** Average Times from the Start of the IOP Change to the Maximum BFONH Response and the Transition Time for the Return from Maximum BFONH to Steady State

<table>
<thead>
<tr>
<th>Group</th>
<th>IOP10-30</th>
<th>IOP30-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>To Maximum</td>
<td>To Steady State</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Medium</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>Low</td>
<td>50</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>

Data are times in seconds.

**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).55 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.20 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)19 and monkeys (~30 mm Hg)20 and in one study of humans (~43 mm Hg),55 but slightly higher than in another study of humans (<30 mm Hg).36 This comparison confirms that the estimated range of OPP achieved through this approach produced a pattern of autoregulation similar to that observed in the normal monkey ONH.
derived by increasing the IOP. Fig. 5 illustrates the relationship between the relative BF$_{ONH}$ and the estimated OPP during the IOP$_{10-30}$ condition of our study. It demonstrates how an otherwise equivalent change in IOP can have a dramatically different impact on BF, depending on perfusion pressure and autoregulation capacity. In our study, OPP was altered in part by selecting specific ranges of BP in addition to varying IOP to extend results of previous studies in which one or the other parameter was altered. Nonetheless, the relationship between relative BF$_{ONH}$ changes and OPP observed in our study follows the general pattern of autoregulation classically defined by altering only the IOP.\textsuperscript{57}

This BP-dependent BF$_{ONH}$ change induced by rapid IOP changes may have important clinical implications. In glaucoma, and particularly in normal-tension glaucoma, the vascular factors associated with the development of glaucomatous optic neuropathy include both IOP\textsuperscript{58-60} and systemic BP.\textsuperscript{31,32,57,61} These patients often have significant diurnal IOP variation\textsuperscript{56,62-64} and greater reductions in nocturnal BP than do healthy people.\textsuperscript{34-36} 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 BF$_{ONH}$. For instance, in a recent study by Choi et al.,\textsuperscript{36} 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.\textsuperscript{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 BF$_{ONH}$ was actually increased by 200%, despite of different techniques used for the BF measurement and different magnitude of the IOP reduction that initiates the responses. However, this response was absent when the IOP changes took a longer time (~20 seconds) in rabbits,\textsuperscript{53} suggesting again that it is the myogenic 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 anesthesia on the BF$_{ONH}$ response during low-BP cannot be excluded, although the dose difference of the anesthetic between the BP groups was negligible and depends largely on the response of individual animals. Second, despite excellent repeatability of LSFG measurement of BF$_{ONH}$, 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,\textsuperscript{41} the depth has never been verified in monkeys.\textsuperscript{73} In addition, the maximum temporal resolution of LSFG for the BF$_{ONH}$ measurement is only 2 seconds, 4 seconds for each measurement. Thus, the measurement during the initial state after the OPP change may not capture the maximum BF$_{ONH}$; 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 autoregulation of the BF$_{ONH}$, 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 BF$_{ONH}$.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{A schematic view shows the effects of high-, medium-, and low-BP on the IOP-induced BF$_{ONH}$ changes during IOP$_{10-30}$. The dotted curve represents a fraction of an autoregulation curve derived from the relative BF$_{ONH}$ changes at corresponding OPP in the study. The marks along the curve show the same 20-mm Hg IOP changes (horizontal lines), but different magnitudes of relative BF response (vertical lines). The latter depends on the OPP levels where the IOP is altered. Corresponding BP and IOP and OPP for each BP group are listed below the graph.}
\end{figure}
References


