Retinal Artery Response to Acute Systemic Blood Pressure Increase during Cold Pressor Test in Humans

Taiji Nagaoka, Fumibiko Mori, and Akitoshi Yoshida

PURPOSE. The purpose of this study was to investigate the response of the retinal arteriole to an acute increase in systemic blood pressure (BP).

METHODS. Sixteen healthy volunteers underwent a 5-minute cold pressor test (CPT) on the left hand. Retinal blood flow (RBF) was determined using a laser Doppler velocimetry system that enables simultaneous measurements of blood velocity and vessel diameter in a retinal artery every 30 seconds for the duration of the CPT. The mean arterial BP (MABP) was measured every 30 seconds during the test.

RESULTS. The MABP immediately increased and reached the maximum level (from 85.1 ± 2.7 to 103.7 ± 5.0 mm Hg; P < 0.01) 1.5 ± 0.2 minutes after the CPT began. The peak increase in velocity (from 30.6 ± 1.3 to 46.1 ± 2.1 mm/sec; P < 0.01) occurred at 1.6 ± 0.2 minutes, resulting in an increase in RBF (from 10.1 ± 1.0 to 14.3 ± 1.4 μL/min; P < 0.01). The peak decrease in diameter (115.8 ± 5.0 to 105.1 ± 5.0 μm; P < 0.01) occurred at 2.9 ± 0.3 minutes and the RBF returned to the baseline value. The increases in MABP significantly correlated with the decreases in diameter (r = −0.674, P = 0.0059).

CONCLUSIONS. The CPT caused an acute increase in MABP that was associated with a transient increase in RBF and blood velocity, followed by constriction of the retinal arterioles and return of RBF to baseline. The results suggest that constriction of the retinal arterioles plays an important role in the maintenance of RBF in response to an acute increase in systemic BP.

A autoregulation is the ability of the peripheral vasculature to maintain blood flow despite changes in perfusion pressure. In the eye, the retinal blood flow (RBF) has been reported to be autoregulated in response to an acute increase in systemic blood pressure (BP). However, the exact mechanism of this autoregulation is unclear.

The physiologic importance of autoregulation is thought to be the protection of the capillary network against the disruptive effects of elevated capillary pressure when systemic BP increases. Systemic hypertension has been reported to be a major risk factor in the progression of diabetic retinopathy.2–4 and retinal vascular autoregulation in response to raised systemic BP is impaired in patients with diabetes.5 Therefore, it is important to investigate the mechanism of autoregulation of RBF for the clinical evaluation of retinal disorders.

There is a long latent period before constriction occurs in response to an increase in intravascular pressure during the autoregulatory process.6 In addition, many vasoactive reactions are thought to occur at different times and sites in the microvasculature to maintain proper perfusion to the tissue.7 Therefore, it is necessary to detect dynamic changes in the retinal arterioles in response to increases in systemic BP to evaluate autoregulation of RBF. A stabilized laser Doppler velocimetry (LDV) system allows measurement of the vessel diameter and blood velocity in retinal arterioles simultaneously within a short time8–10 and allows evaluation of the dynamic changes in the RBF.

The purpose of this study was to investigate the dynamic response of the retinal arterioles to an acute increase in systemic BP in humans. For this purpose, we used the stabilized LDV system and the cold-pressor test (CPT), which causes an increase in systemic BP by sympathetic activation of the autonomic nervous system.

METHODS

Subjects

Measurements were obtained from 16 eyes of 16 healthy volunteers (14 men, 2 women; mean age, 27.7 ± 6.6 years [SD]; range, 20–44 years). The procedure followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects after the study was fully explained. All subjects had normal visual acuity better than 1.0, clear media, and no history of ocular and systemic disease or therapy. The temperature in the examination room was maintained in a constant range from 22°C to 24°C. The subjects were asked to abstain from drinking coffee and smoking for at least 2 hours before the test. To prepare for the test, each subject rested for 10 to 15 minutes in a quiet room before the test began.

The mean arterial BP (MABP) and heart rate (HR) were estimated by electronic sphygmomanometer (EP888i; Colin, Tokyo, Japan). Intracocular pressure (IOP) was monitored by applanation tonometry (Haag Streit, Bern, Switzerland). The axial length of each eye was measured by A-mode ultrasound (Ocucan; Alcon Surgical, Irvine, CA) to compute the intraocular light-scattering geometry for the laser Doppler measurement. Pupil dilation was achieved using a combination of 0.5% tropicamide and 1% phenylephrine eye drops.

RBF Measurement

In the present study, a stabilized LDV system (Canon Laser Blood Flowmeter [CLBF], model 100; Canon, Tokyo, Japan) was used for in vivo estimation of the blood flow in the major temporal retinal arterioles. Feke et al.5,9 and Yoshida et al.10,11 recently described this device. In those studies, the investigators used a prototype device. The device is now commercially available. The CLBF allows noninvasive measurement of the absolute values of the red blood cells (RBCs) flowing in the centerline of the vessel, based on the bidirectional LDV.12 The probing red laser (wavelength, 675 nm) is emitted from a fundus-camera–like measuring head. The red Doppler-shifted light scattered from the flowing RBCs in the retinal artery is detected simultaneously in two directions separated by a fixed angle. The signals from the two photomultiplier tube detectors undergo computer-controlled spectrum analysis, and sequential measurements of the centerline velocity of the retinal blood cells over a 2-second time interval are performed automatically.

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performed every 30 seconds for 5 minutes of the CPT and 5 minutes after the end of the CPT.

**Statistical Analysis**

All data are expressed as the mean ± SE. For statistical analysis, we used analysis of variance for repeated measurements followed by post hoc comparison with the Dunnett procedure. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Time Course of Response**

Table 1 shows the baseline systemic and retinal circulations. Individual differences in the time course of the responses to the CPT were observed in our study. To avoid underestimation of the actual effectiveness of the test and to minimize the individual variability in the latency of the response to the CPT, we defined the time point at which the peak responses of velocity and diameter were observed as T1 and T2, respectively. The average T1 and T2 were 1.6 ± 0.2 and 2.9 ± 0.3 minutes of the CPT, respectively (Table 2). There were significant time differences between MABP and diameter (T2; $P = 0.002$) and between velocity (T1) and diameter (T2; $P = 0.004$).

**Systemic Circulatory Response**

After the beginning of the CPT, all subjects had an acute steep increase in MABP from 85.1 ± 2.7 to 103.7 ± 5.0 mm Hg (average peak increase; 21.3% ± 2.7%, $P < 0.01$; Table 2) at 1.5 ± 0.2 minute of CPT. After that, at T1 and T2, the MABP was still significantly ($P < 0.01$) increased, by 15.6% ± 3.2% and 15.4% ± 3.1%, respectively (Fig. 1). The increase in MABP at T2 is almost the same as that at T1. The subjects then showed a concomitant progressive return of MABP toward baseline, but the significant increase in MABP persisted to the end of the CPT. HR significantly increased by 14.9% ± 2.7% and 11.4% ± 2.0% at T1 and T2, respectively (Fig. 1).

**Reproducibility of RBF Measurements**

The averaged coefficients of variation of velocity, diameter, and RBF at five baseline measurements obtained from all 16 subjects in this study were 13.9% ± 1.2%, 4.1% ± 0.6%, and 16.9% ± 1.4%, respectively.

**Velocity Response**

The velocity increased with a roughly parallel time course of MABP in all cases. At T1, the velocity significantly ($P < 0.01$) increased from 30.6 ± 1.3 mm/sec to 46.1 ± 2.1 mm/sec (average peak increase; 51.0% ± 3.6%, $P < 0.01$; Fig. 1). After that, the increased velocity declined slightly, but the velocity was still significantly ($P < 0.01$) increased, by 16.2% ± 6.1%, at T2 compared with baseline. At the end of the CPT (5 minutes), the velocity showed a sustained and significant ($P < 0.05$) increase of 14.1% ± 2.8%.

**Cold Pressor Test**

The subject’s left hand was immersed up to the wrist in water at 4°C for 5 minutes and then removed from the water while the subject remained still for 5 minutes.

**Study Protocol**

The experiment consisted of a 5-minute baseline period, followed by a 5-minute CPT and a 5-minute recovery period. At baseline, five measurements of each parameter were obtained every minute at a single site along a major temporal arteriole, and the average of the measurements was defined as the baseline. Measurements of parameters were performed every 30 seconds for 5 minutes of the CPT and 5 minutes after the end of the CPT.

**Table 2. Time of Peak Response after Start of CPT**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP</td>
<td>1.5 ± 0.2*</td>
</tr>
<tr>
<td>Velocity</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>Diameter</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE. * $P < 0.01$ in comparison with the diameter.
Diameter Response
In contrast to the velocity, the diameter did not change significantly at T1 (Fig. 1). After that, the diameter decreased significantly at T2 ($P < 0.01$) from 115.8 ± 5.0 μm to 105.1 ± 5.0 μm (average peak decrease, -9.3% ± 1.1%, $P < 0.01$; Fig. 1). In most cases, the peak change in diameter had a longer latency than that of MABP and velocity. At the end of the CPT, the decreased diameter approached baseline but still showed a significant ($P < 0.05$) decrease of -3.9% ± 1.3%.

RBF Response
At T1, the RBF significantly ($P < 0.01$) increased by 44.3% ± 4.1% accompanied by a rapid increase in velocity (Fig. 1). At T2, the increased RBF rapidly returned to baseline. The RBF observed at T2 was not significantly ($P > 0.05$) different from baseline. At the end of the CPT, there was still no significant change in RBF compared with baseline.

RVR Response
After the initiation of the CPT, the RVR immediately decreased by -15.5% ± 4.1% ($P < 0.01$) at T1. After that, the RVR inversely started to increase by 31.7% ± 8.2% ($P < 0.01$) of the averaged values at T2.

Response of Each Parameter after CPT
Five minutes after the end of the CPT, each parameter returned to a level that was not statistically different from baseline (Fig. 1).

Relationship between MABP and Vessel Diameter
The peak increases in MABP significantly and inversely correlated with the peak decreases in diameter ($r = -0.674$, $P = 0.0059$; Fig. 2).

DISCUSSION
In the present study, the CPT caused an acute increase in MABP and a transient increase in RBF that corresponded with the increase in blood velocity (Fig. 1). After that, vessel diameter began to decrease and the transiently increased RBF returned to baseline. These findings suggest that constriction of retinal arterioles plays an important role in the regulation of RBF in response to an acute increase in systemic BP.

In two previous human studies in which conventional LDV was used, the RBF was maintained until the MABP increased to an average of 115 mm Hg (41% of baseline)\textsuperscript{5} or 112 mm Hg (30% of baseline).\textsuperscript{5} In the present study, the average peak MABP was 103.7 mm Hg (21.3% of baseline), which is smaller than in the previous studies. Our finding that RBF was finally maintained in response to the increase in MABP is consistent with previously published results. However, the transient increase in RBF observed in our study was not mentioned in the two previous studies. Because it was not technically possible to measure velocity and take photographs simultaneously when using a conventional LDV technique, the dynamic RBF response to the physiologic stimuli could not be precisely evaluated in the previous studies. Our results also indicate that the stabilized LDV system has superior temporal resolution and...
allows the evaluation of the dynamic change in the retinal circulation.

In the present study, we first showed that there were differences in the latency of the response to the CPT (Table 2). Our findings that the peak changes in MABP were observed at 1.5 minutes are consistent with previous findings that the peak responses in MABP occurred in the second minute of the CPT. These findings indicate that that the sympathetic neural system is completed at 1 to 2 minutes after the start of the CPT. In addition, the velocity changed along a time course that was roughly parallel to that of the MABP in the present study, which indicates that the change in velocity is dependent on the increase in intravascular pressure when MABP increases during the CPT.

In the present study, the increase in MABP and the peak decrease in diameter were 15.4% (13.2 mm Hg) and 9.3% at T2 (Fig. 1). The response of retinal arterioles to the increase in MABP was studied previously by Blum et al., who used the retinal vessel analyzer. They reported that isometric exercise causes a significant increase of 22.8 mm Hg in MABP and a decrease of 5.5% in the diameter of the retinal arterioles. Their results are roughly in agreement with ours in the magnitude of the response to the increase in MABP. These results indicate that constriction of retinal arterioles plays an important role in RBF autoregulation when systemic BP increases.

The details of the mechanisms by which RBF is autoregulated remain to be clarified. Because the CPT is the stimulus for the increase in systemic BP and HR by the activation of the sympathetic nervous system, it is necessary to take into account the neural influence on our data. Although we did not measure the activity of the sympathetic nervous system, the significant increases in the MABP and HR that were observed in the present study (Fig. 1) indicate that the sympathetic nervous system in response to the CPT in our subjects. Some investigators suggest that the sympathetic nervous system, which affects the peripheral vascular bed to a different extent in many organs, plays some role in regulation of RBF. Conversely, sympathetic nerves are thought to be nonfunctional in the retinal vascular bed. If the constriction of the retinal arterioles originates in the sympathetic neural system, it should have occurred within several seconds and corresponded with the elevation in arterial pressure. Our findings that the peak decrease in diameter was proceeded by the peak changes in MABP and velocity (Table 2) are consistent with the latter findings. Taken together, it is reasonable to consider that the sympathetic neural system may not play a major role in the constriction of retinal arterioles that was observed in the present study, in response to the CPT.

The details of the sites at which RBF is autoregulated remain to be clarified. Figure 2 shows that the peak change in vessel diameter significantly correlated with the peak increase in MABP. Harder found that the pressure elevation in isolated cerebral arteries caused membrane depolarization in vascular smooth muscle, resulting in vessel constriction. His findings indicate that intravascular pressure directly alters the vascular smooth muscle transmembrane potential. Although the exact mechanism responsible for the constriction of the retinal arterioles was not clarified in the present study, our results indicate that the magnitude of the retinal arteriole’s constriction is mainly dependent on the magnitude of the local increase in intravascular pressure. It is therefore reasonable to conclude that the CPT-induced constriction of the retinal arterioles observed in our study is mainly dependent on the increase in intravascular pressure. Moreover, these findings indicate that the RBF may be mainly autoregulated in the retinal arterioles when systemic BP increases.

Myogenic contraction is defined as the contraction of vessels and increase in vascular resistance in response to an increase in pressure. In the present study, it is surprising that the RVR significantly decreased in contrast to the transient increase in RBF at T1 (early phase) and then increased at T2 (late phase; Fig. 1). This opposite change in the RVR is similar to a phenomenon that was observed in cat mesenteric arterioles by Johnson and Intaglietta. They suggest that the auto-regulatory myogenic contraction in response to increased arterial BP is necessarily preceded by a brief passive vascular expansion that is expected to produce a transient reduction in resistance. Although we could not observe the dilation of the measured retinal arterioles, the early reduction in RVR observed in the present study may be caused by the passive expansion at any site in the retinal microvasculature in response to an acute increase in MABP and RBF.

Because there were individual differences in the time course of the response to CPT in the present study, it is important to evaluate the dynamic change in each parameter when autoregulation of RBF is examined. Our data showed that the stabilized LVD system enables analysis of the dynamic change in diameter and velocity of the retinal arterioles in response to the acute increase in MABP in the human retinal circulation. It is likely that the timing of the onset, as well as the time course of the hemodynamic change, may identify the nature of vasoregulation.

The present results may be clinically significant. Systemic hypertension may exacerbate some retinal disorders, such as diabetic retinopathy. In addition, it has been reported that the autoregulation of RBF is impaired in diabetes mellitus. Our finding that the transiently increased RBF returned to baseline when the retinal arterioles constricted (Fig. 1) suggests that the impaired function of the retinal arterioles causes the failure of the autoregulatory mechanism and increases the pressure in the capillaries, which leads to edema or hemorrhage.

In conclusion, the CPT caused an acute increase in MABP, which was associated with a transient increase in the RBF and blood velocity, followed by constriction of the retinal arterioles and return of the RBF to baseline. Our results suggest that constriction of the retinal arterioles plays an important role in the maintenance of RBF in response to an acute increase in systemic BP.

References


