Real-Time Evaluation of Regional Arterial Stiffening, Resistance, and Ocular Circulation During Systemic Administration of Adrenaline in White Rabbits

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Purpose: To evaluate continuous variations of ocular microcirculation by laser speckle flowgraphy and those of regional stiffening by pulse wave velocity (PWV) and vascular resistance under systemic adrenaline administration in rabbits.

Methods: Six 16-week-old male rabbits were evaluated. The mean blur rates in the retinal vessel (MBR-RV) and choroid (MBR-CH) were measured. We assessed blood pressure (BP), femoral and carotid vascular resistance, and the heart–ankle (ha)-PWV, heart–femoral (hf)-PWV, and femoral–ankle (fa)-PWV. Adrenaline (100, 300, and 1000 ng/kg) was intravenously administered over a 10-minute period during which the parameters were measured simultaneously every 2 minutes.

Results: The MBR-RV and MBR-CH values were dose-dependently increased by the adrenaline in parallel with increased BP. At the load of 100 ng/kg adrenaline, the ΔMBR-RV and ΔMBR-CH showed positive correlations with the variation rate in mean arterial blood pressure. Also, the variation rate in carotid vascular resistance and the Δfa-PWV and Δhf-PWV were significantly positively correlated with both the ΔMBR-RV and ΔMBR-CH. At the 300-ng/kg phase, the correlations between the Δha-PWV and both ΔMBR-RV and ΔMBR-CH were canceled; instead, the Δhf-PWV showed a significant negative correlation with the ΔMBR-RV and ΔMBR-CH. At the 1000-ng/kg phase, Δha-PWV again showed significant positive correlations with the ΔMBR-RV and ΔMBR-CH.

Conclusions: These results indicate the possibility that under a systemic administration of adrenaline in rabbits, not only the BP value but also the vascular resistance and arterial function are related to the variation in ocular microcirculation.

Translational Relevance: A real-time evaluation system of systemic regional arterial function and ocular microcirculation in rabbits was developed.

Introduction

The sympathetic nervous system is known to play a central role in cardiovascular homeostasis,¹ and this system is the effector of the neurogenic control of vascular tone, inducing mainly vasoconstriction of small-resistance arteries.² For the eyes, the sympathetic nervous system has important roles in the control of choroidal blood flow and for maintaining the condition of the retina. Among the major ocular diseases correlated with dysregulations of sympathetic nervous system activity, central serous chorioretinopathy is of paramount importance. Cases of central serous chorioretinopathy show detachment of the macula due to an accumulation of serous fluid and are related to an increase in sympathetic activity.³

Experimental studies in rabbits and monkeys confirmed that a model of central serous chorioretinopathy was created by a repetitive systemic administration of adrenaline.⁴,⁵ The choroidal blood flow is controlled mainly by sympathetic innervation;
the retinal circulation lacks autonomic innervation, shows efficient autoregulation, and is influenced mainly by local factors. In rabbits, it has been confirmed that the $\alpha$- and $\beta$-adrenergic receptors are located in the choroid. An experimental study in rabbits demonstrated that an intravenous administration of adrenaline increased the retinal and choroidal blood flows in parallel with an increase in systemic blood pressure (BP). We thus speculated that the control of ocular microcirculation under systemically altered sympathetic activity is affected mainly by not only the self-regulation of topical circulation but also aspects of systemic arterial vascular function such as BP, arterial stiffness, and vascular resistance.

An experimental study of $\alpha$ adrenaline receptor and $\beta$ adrenaline receptor blockade in rabbits revealed contradictory responses of arterial stiffness between $\alpha$ and $\beta$ stiffness, and vascular resistance. Systemic arterial vascular function such as BP, arterial stiffness, and vascular resistance would change depending on the dose of adrenaline.

Laser speckle flowgraphy (LSFG), a noninvasive quantitative method for determining the ocular blood flow, is based on changes in the speckle pattern of laser light reflected from the fundus of the eye. LSFG is dependent on the movement of erythrocytes in the retina, the choroid, and the optic nerve head, and it can be used to determine the mean blur rate (MBR), which is an indicator of ocular blood flow. Theoretically, the MBR is an index of blood flow velocity and blood flow volume. LSFG has also been used to investigate ocular microcirculation in a rabbit model.

We thus conducted the present study to evaluate continuous variations of (1) the microcirculation in the retinal vessels and choroid by LSFG, and (2) the regional stiffening by pulse wave velocity (PWV) and the vascular resistance of arteries under various doses of intravenous adrenaline in anesthetized rabbits.

### Materials and Methods

#### Animals

A total of six male New Zealand White rabbits (16 weeks old; weight, 2.84–3.44 kg; median, 3.16 kg) housed in the same environment were evaluated. All animal experiments were approved by the Toho University Laboratory Animal Research committee (19-53-358) and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the tenets of the Association for Research in Vision and Ophthalmology.

The experiments were performed with the rabbits under general anesthesia, with induction of anesthesia by ketamine (Ketalar, 35 mg/kg, intramuscular [i.m.]; Daiichi Sankyo Propharma, Tokyo, Japan) and xylazine (Selactar 2%, 5 mg/kg, i.m.; Bayer Japan, Osaka, Japan) and maintenance with isoflurane (end-tidal concentration of 1.5%; Pfizer Japan, Tokyo, Japan). After intubation of the tracheal cannula, the rabbit was mechanically ventilated (FiO$_2$ = 1.0; tidal volume = 6 mL/kg, 40 strokes/min; SN-480-5, Shinano, Tokyo, Japan), and its body temperature was maintained at 37°C using a heating pad. The right ear vein was used for the administration of adrenaline, and the left ear vein was used for continuous maintenance infusions of saline (15 mL/h) and rocuronium bromide (0.6 mg/kg/h; Fuji Pharma, Toyama, Japan).

#### Experimental Protocol

After confirmation that the rabbit’s systemic hemodynamics and ocular circulation state were stable for ≥30 minutes, the experiment was started. Adrenaline (Bosmin injection; Daiichi Sankyo Propharma) was diluted with saline at 100, 300, and 1000 ng/kg and consecutively, intravenously administered over a 10-minute period (0.2 mL/kg/min). Before each adrenaline dosing phase, a 20-minute control period was used. An intravenous administration of adrenaline at 1000 ng/kg is a hypertensive dose for rabbits. We assessed the effects of adrenaline (100, 300, and 1000 ng/kg) as described previously. The scheme of the experimental protocol is shown in Figure 1. All of the parameters were measured simultaneously every 2 minutes during the adrenaline administrations and every 5 minutes during the control periods.

#### Systemic Hemodynamics Evaluation

Figure 2 illustrates the apparatus used to measure the rabbit’s systemic hemodynamics, regional arterial function, and ocular microcirculation. A heparinized catheter was inserted at the right brachial artery, femoral artery (bifurcation), and tibial artery for continuous measurement of the mean arterial blood pressure (MABP, mmHg) using transducers. The carotid and femoral arterial blood flows (mL/min) were measured using an ultrasonic blood flowmeter (TS420; Transonic Systems, Ithaca, NY). The carotid and femoral vascular resistance values were calculated using this basic equation: MABP/carotid or femoral arterial...
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Figure 1. The experimental protocol.

Blood flow.\textsuperscript{20,21} The MABP and heart rate (beats per minute [bpm]) were recorded using the software program LabChart via a PowerLab system (ADInstruments, Bella Vista, New South Wales, Australia).

Measurement of the PWV

For measurements of the PWV, the electrocardiogram, phonocardiogram, and blood pressure values were fed to the LabChart program via the PowerLab system. We measured the length of the artery between the two pressure sensors, the length of the artery from the heart to the proximal femoral artery (hf), and the length from the proximal femoral artery to the end of the tibial artery (fa). We assigned the peak of the second derivative of the pressure waves within a cardiac cycle to the original waves, based on a previous study.\textsuperscript{22} We calculated the heart to the end of the tibial artery (ha)-PWV, hf-PWV, and fa-PWV. The ha-PWV, hf-PWV, and fa-PWV were measured using the distance of the two pressure sensors between the ha, hf, and fa, and the differences in the peak times of two pressure waves were measured between the ha, hf, and fa, respectively.\textsuperscript{5} We calculated the ha-PWV by using the blood pressure of the brachial and tibial arteries, because the time between the closing sound of the aortic valve and the notch of the brachial pulse wave is estimated to be equal to the time between the opening sound of the aortic valve and the rise of the brachial pulse wave.\textsuperscript{21}

LSFG Valuation

The MBR images were obtained using the LSFG-MRC device (Softcare, Iizuka, Japan), and the MBR in the retinal vessels and choroid area were calculated by LSFG Analyzer software (Softcare). The LSFG-MRC consists of a fundus camera equipped with a diode laser (830-nm wavelength) and a charge-coupled device image sensor ($750 \times 360$ pixels). The principles and application of this method have been described elsewhere.\textsuperscript{10–14} Figure 3A provides a fundus photograph of the rabbit. Rectangular bands were placed at the retinal vessel and at the choroid area, avoiding retinal vessels (Fig. 3B). Within a 5-second period tuned to the cardiac cycle, MBR images were
Figure 3. The method for analyzing the mean blur rate in retinal vessel (MBR-RV) and the choroid area (MBR-CH) using the LSFG-MRC device. (A) Fundus photograph of rabbit. (B) Color-scale map of the total measurement area; the rectangular measurement areas were placed at the retinal vessel (1) and choroid area (2). (C) The pulse waves show changes in the MBR (tuned to the cardiac cycle for 5 seconds). The average MBR-RV and MBR-CH values were determined from this panel.

recorded from the rectangular area, and the average MBR was displayed on a computer screen (Fig. 3C). The MBR of the choroid (MBR-CH) was also displayed on the screen.

The MBR of the retinal vessel (MBR-RV) and the MBR of the choroid area (MBR-CH) were recorded three times at each time point, and the mean value was then calculated. In each experiment, the rectangular bands were stored in the software program, and the same rectangular bands were used for all analyses. Mydriasis was induced by topical tropicamide (Mydrin-M ophthalmic solution 0.4%; Santen Pharmaceutical Co., Osaka, Japan). Only the left eye was used in all of the experiments.

Intraocular Pressure Control

To keep the intraocular pressure (IOP) stable during each experiment, a 25-gauge infusion cannula was inserted into the vitreous cavity of the rabbit through the pars plana, as described previously.18 This infusion cannula was connected to a bottle of intraocular irrigating solution (BSS Plus; Alcon Japan, Tokyo, Japan) for the stabilization of IOP during the experiment. The IOP was set at 10 mmHg by changing the height of the bottle. The IOP was confirmed using an Icare TONOLAB tonometer (Revenio Group, Helsinki, Finland) at the start and end of the experiment.

Statistical Analyses

The data of the continuous variables are presented as mean ± standard error. The MBR-RV and MBR-CH were evaluated based on the rate of change from the baseline value before the administration of each of the three doses of adrenaline. The time courses of the changes in the MABP of the brachial artery, heart rate, carotid and femoral vascular resistance, ha-PWV, hf-PWV, fa-PWV, %MBR-RV, and %MBR-CH were analyzed by a repeated measures analysis of variance with the Dunnett test as a post hoc test. The Kruskal–Wallis test was used for comparisons of the baseline MBR-RV and MBR-CH values with those at each adrenaline dose, in arbitrary units. We used a univariate regression to determine the correlation coefficients among the change rate (%) of systemic hemodynamics, the percentage of each of the PWVs, and the ocular microcirculation parameters (%MBR-RV and %MBR-CH) during the experiment for each administered dose of adrenaline. $P < 0.05$ was accepted as significant. The JMP-10.0 program (SAS Institute, Cary, NC) was used for the statistical analyses.
Results

For the 100-ng/kg adrenaline dose, the baseline arbitrary units for MBR-RV and MBR-CH were 20.3 ± 2.8 and 12.8 ± 0.8, respectively; for the 300-ng/kg dose, they were 22.3 ± 3.6 and 17.8 ± 2.5, respectively; and, for the 1000-ng/kg dose, they were 28.7 ± 2.4 and 13.4 ± 1.6, respectively. There was no significant difference in the MBR-RV or the MBR-CH between these baseline units (P = 0.21 and P = 0.23, respectively).

The time courses of the changes in the heart rate, MABP, %MBR-RV, %MBR-CH, carotid and femoral vascular resistance, ha-PWV, hf-PWV, and fa-PWV during the experiment are shown in Figure 4. The heart rate tended to decrease during administration of the adrenaline but did not reach significance at any of the adrenaline doses. At the 100-ng/kg dose of adrenaline, the MABP tended to increase, but not significantly (from 40.2 mmHg to 43.4 ± 0.7 mmHg). At 300 and 1000 ng/kg of adrenaline, the MABP was significantly increased (from 40.2 mmHg to 49.2 ± 1.4 mmHg and from 42.0 mmHg to 55.8 ± 1.3 mmHg, respectively). Similarly, the administration of 100 ng/kg adrenaline tended to increase the MBR-RV and MBR-CH values (18.0% ± 3.4% and 21.0% ± 3.5%, respectively).

With the administration of 300 and 1000 ng/kg of adrenaline, both the MBR-RV and MBR-CH were significantly increased (31.8 ± 4.3% and 42.7 ± 6.7%, respectively). The percent femoral vascular resistance was significantly increased from the 300-ng/kg dose (12.1% ± 1.4% at the 300-ng/kg dose and 35.0% ± 4.1% at the 1000-ng/kg dose). The percent carotid vascular resistance was significantly increased only at the dose of 1000 ng/kg of adrenaline (28.0% ± 5.2%). The ha-PWV did not show a characteristic variation at any of the adrenaline doses. The hf-PWV did not show significant variations but tended to decrease at all of the adrenaline doses. The fa-PWV tended to be increased depending on the dose of adrenaline, but the differences did not reach significance. The time course of mean value of the MBR-RV and MBR-CH is shown in Supplementary file.

The Table summarizes the results of the univariate regression analysis among the variation rate (Δ) of MBR-RV and MBR-CH and the systemic circulation parameters by each dose of adrenaline (100, 300, and 1000 ng/kg). The ΔMABP showed a strong positive correlation with the ΔMBR-RV (correlation coefficient r = 0.87), but the correlation decreased in a dose-dependent manner and was r = 0.43 at the 1000-ng/kg dose. The ΔMABP showed a significant positive correlation with the ΔMBR-CH (r = 0.66) at 100 ng/kg and was r = 0.40 at 300 ng/kg but returned to r = 0.66 at the 1000-ng/kg dose. The correlation between the variation rate of femoral vascular resistance and the ΔMBR-RV increased in a dose-dependent manner, and it became a significant positive correlation at the 300-ng/kg dose (r = 0.33) and at the 1000-ng/kg dose (r = 0.54).

The correlation between the variation rate of femoral vascular resistance and the ΔMBR-RV showed a significant positive correlation at each dose (r = 0.42, r = 0.30, and r = 0.34, respectively). The variation rate of carotid vascular resistance and the ΔMBR-RV were positively and significantly correlated at all adrenaline doses: 100 ng/kg (r = 0.39), 300 ng/kg (r = 0.31), and 1000 ng/kg (r = 0.31). The variation rate of carotid vascular resistance showed a significant positive correlation with the ΔMBR-CH at

| Adrenaline Dose | ΔMBR-RV r | ΔMBR-CH r |
|-----------------|-----------|-----------|
| 100 ng/kg/min   |           |           |
| ΔMABP           | 0.87**    | 0.66**    |
| ΔFemoral vascular resistance | 0.15 | 0.42** |
| ΔCarotid vascular resistance | 0.39** | 0.54* |
| Δha-PWV         | 0.50**    | 0.43**    |
| Δhf-PWV         | 0.27*     | 0.61**    |
| Δfa-PWV         | 0.63**    | -0.26     |
| 300 ng/kg/min   |           |           |
| ΔMABP           | 0.63**    | 0.40*     |
| ΔFemoral vascular resistance | 0.33* | 0.30* |
| ΔCarotid vascular resistance | 0.31* | 0.04 |
| Δha-PWV         | -0.11     | -0.28     |
| Δhf-PWV         | -0.31*    | -0.44**   |
| Δfa-PWV         | 0.13      | 0.12      |
| 1000 ng/kg/min  |           |           |
| ΔMABP           | 0.43**    | 0.66**    |
| ΔFemoral vascular resistance | 0.54* | 0.34** |
| ΔCarotid vascular resistance | 0.34* | 0.27 |
| Δha-PWV         | 0.52**    | 0.33*     |
| Δhf-PWV         | 0.22      | 0.60**    |
| Δfa-PWV         | 0.16      | -0.40**   |

*P < 0.05.
**P < 0.001.
Figure 4. Time courses of the effects of the systemic adrenaline administration (100, 300, and 1000 ng/kg/min) on the heart rate, MABP of the brachial artery, the MBR-RV and MBR-CH, femoral and carotid vascular resistance, and the ha-PWV, hf-PWV, and fa-PWV in the anesthetized rabbits ($n = 6$). Closed symbols: significantly different from the corresponding control value of each parameter at $P < 0.05$. 
the 100-ng/kg dose ($r = 0.54$), but the significance was canceled at the 300-ng/kg and 1000-ng/kg doses.

The $\Delta ha$-PWV was significantly positively correlated with both the $\Delta MBR$-RV and $\Delta MBR$-CH at 100 ng/kg ($r = 0.50$ and $r = 0.43$, respectively), but, conversely, during the 300-ng/kg phase the $\Delta ha$-PWV tended to be negatively correlated with the $\Delta MBR$-RV and $\Delta MBR$-CH. At 1000 ng/kg of adrenaline, the $\Delta ha$-PWV was again significantly and positively correlated with the $\Delta MBR$-RV and $\Delta MBR$-CH ($r = 0.52$ and $r = 0.33$, respectively). The $\Delta hf$-PWV was significantly positively correlated with both the $\Delta MBR$-RV and $\Delta MBR$-CH values at the 100-ng/kg dose ($r = 0.27$ and $r = 0.61$, respectively). On the other hand, during the 300-ng/kg phase, the $\Delta hf$-PWV was significantly negatively correlated with the $\Delta MBR$-RV and $\Delta MBR$-CH ($−0.31$ and $−0.44$, respectively); at the 1000-ng/kg dose, the $\Delta hf$-PWV tended to be positively correlated with the $\Delta MBR$-RV ($r = 0.22$) and was significantly positively correlated with the $\Delta MBR$-CH ($r = 0.60$). Finally, the $\Delta fa$-PWV at the 100-ng/kg dose was significantly positively correlated with the $\Delta MBR$-RV and did not show a significant correlation with either the $\Delta MBR$-RV or the $\Delta MBR$-CH at the 300-ng/kg dose. During the 1000-ng/kg phase, the $\Delta fa$-PWV was significantly negatively correlated with the $\Delta MBR$-CH.

### Discussion

Arterial stiffness is a marker of both arteriosclerosis and vascular function, the latter of which is required to maintain efficient systemic and ocular microcirculation.23–26 The function of blood vessels is to nourish the living body and maintain homeostasis. The autonomic nervous system, comprised of the two primary branches (i.e., sympathetic and parasympathetic nervous systems), plays an essential role in the regulation of vascular wall contractility and tension. The sympathetic and parasympathetic nerves work together to balance the functions of autonomic effector organs.27 The activation of the sympathetic nervous system takes place via $\alpha$ and $\beta$ adrenaline receptors, which are located on the blood vessel wall.28 Adrenaline is used as a primary adrenaline receptor agonist due to (1) its high potency to stimulate all of the subtypes of $\alpha$ and $\beta$ adrenaline receptors, and (2) its relative lack of reuptake into adrenergic nerve endings.7

A disruption of the ocular blood flow by impairments in sympathetic neural control occurs in various ocular diseases such as glaucoma29,30 age-related macular degeneration,31,32 and central serous chorioretinopathy.3–5 A determination of the precise relationship between the ocular blood flow and systemic hemodynamics by adrenaline loading could thus significantly contribute to clarification of the pathology of sympathetic nervous system–related ocular diseases.

Research using rabbits has confirmed differing effects of adrenaline on the retinal and choroidal blood flows between topical and systemic administrations of adrenaline. The systemic administration of adrenaline was observed to increase the retinal and choroidal blood flows in parallel with the increase in the systemic BP of rabbits, whereas a topical adrenaline administration decreased the retinal and choroidal blood flows without a systemic change in BP.8 However, it has not been known whether the regional PWV and/or vascular resistance strongly influence retinal and choroidal circulation under adrenaline loading, or whether the sites of arterial stiffness and resistance would differ depending on the dose of adrenaline. We thus conducted simultaneous measurements of systemic and regional arterial function and ocular circulation by LSFG, as doing so could clarify some unclear points and provide more useful information about these parameters under adrenoreceptor stimulation.

Our present results demonstrated that the systemic administration of adrenaline to rabbits increased the MABP, MBR-RV, MBR-CH, and femoral and carotid vascular resistance in a dose-dependent manner, whereas the rabbits’ heart rate, ha-PWV, hf-PWV, and fa-PWV did not show significant variation at any of the three doses of adrenaline used herein (Fig. 4). Our previous experiment revealed that the MABP and femoral vascular resistance were increased with the adrenaline doses of 300 and 1000 ng/kg without significant variations in heart rate or ha-PWV.19 Stimulation with an $\alpha$ adrenaline receptor led to vasoconstrictive action, and $\beta$ adrenaline receptor stimulation led to vasodilatory action.33 We speculate that, in the present experiment, the vasoconstrictive action and the vasodilatory action were activated by systemic adrenaline administration and both actions were then offset; as a result, the PWV parameters did not show significant variations. However, the authors of a study using the same type of rabbit experiment reported that a 10,000-ng/kg dose of adrenaline increased the ha-PWV.21

In another laser Doppler flowmetry study of rabbits, the systemic administration of 1000 ng/kg adrenaline increased the retinal and choroidal blood flows by 22%.8 In light of the above reports, our present experimental results concerning the variations in systemic hemodynamics and function and ocular circulation caused by the systemic administration of adrenaline appear to be valid data.
It has been confirmed that the choroidal vessels in rabbit have strong autoregulation. The choroidal vessels have \(\alpha\) and \(\beta\) adrenaline receptors, and direct electrical stimulation of the ocular sympathetic nerves or local administration of adrenaline caused choroidal vasoconstriction. A direct administration of adrenaline into the eye thus resulted in decreased choroidal and retinal blood flows. We speculate that, in the setting of systemic administration of adrenaline, the systemic vascular reaction due to the stimulation of the \(\alpha\) and \(\beta\) adrenaline receptors outweighed the local effects in the eye and resulted in the increase in choroidal and retinal vessel blood flows.

Contradictory responses of arterial stiffness between the elastic artery and the muscular artery were confirmed by an experimental study of \(\alpha\)-receptor and \(\beta\)-receptor blockade in rabbits. In a clinical investigation of the regional PWV in patients with diabetes and ischemic heart disease, arterial stiffness was found to play different roles in the muscular and elastic arteries. This finding may be misleading in attempts to determine the relationship between systemic and ocular blood flows in adrenaline loading. It has also been unclear whether regional arterial stiffness and resistance would strongly influence ocular microcirculation under systemically administered adrenaline loading and whether the sites of arterial stiffness and resistance would change depending on the dose of adrenaline. We therefore next evaluated the correlations of the continuous variations of the MBR-RV, MBR-CH, MABP, vascular resistance, and regional PWV with each dose of adrenaline, and we obtained some interesting knowledge. At the low load of 100 ng/kg of adrenaline, variations of the MBR-RV and MBR-CH showed good positive correlations with variations of MABP, and the variations of carotid vascular resistance, ha-PWV, and hf-PWV were significantly positively correlated with the variations of both the MBR-RV and MBR-CH. We suspect that these positive correlations are the result of increasing cardiac output due to \(\beta_1\) adrenaline receptor stimulation and vasoconstriction caused by \(\alpha\) adrenaline receptor stimulation due to the systemic administration of low-dose adrenaline.

On the other hand, during the 300-ng/kg dose phase, the correlations between the \(\Delta\)ha-PWV and both the \(\Delta\)MBR-RV and \(\Delta\)MBR-CH were canceled; instead, the \(\Delta\)hf-PWV showed significant negative correlations with the \(\Delta\)MBR-RV and \(\Delta\)MBR-CH. We speculate that this phenomenon may represent the Windkessel effect. Elastic fibers provide reversible elasticity to the elastic arteries. This allows the aorta to deform elastically under an applied hemodynamic load, with no permanent deformation and no energy dissipation when the load is removed (i.e., the Windkessel effect). In the cardiovascular system, the Windkessel effect dampens the pulsatile flow from the left ventricle so that the distal vasculature receives almost constant perfusion. If the Windkessel effect is compromised, the microvasculature of downstream organs, especially the brain and kidney, may be damaged.

At the high load of 1000 ng/kg of adrenaline in the present experiment, the negative correlations between the \(\Delta\)hf-PWV and both \(\Delta\)MBR-RV and \(\Delta\)MBR-CH were canceled. The 1000-ng/kg load of adrenaline might cause elastic artery dysfunction and then compromise the Windkessel effect. Our experimental results indicate that the elastic and muscular arteries may have different functions in the background of increasing retinal and choroidal blood flows due to the systemic administration of adrenaline. Differences in the correlations with variations in the systemic parameters between the \(\Delta\)MBR-RV and \(\Delta\)MBR-CH may be specific to rabbits due to the autoregulation of rabbit choroidal vessels, but we speculate that these phenomena are similar to the mechanism in humans that functions to protect peripheral organs against systemic sympathetic stimulation. Notably, the IOP remained stable during each experiment; thus, the effect of variations of IOP on our present findings can be ignored. We believe that our findings provide clues that elucidate the crosstalk of systemic hemodynamics, elastic and muscular arterial function, and ocular microcirculation under stimulation of \(\alpha\) and \(\beta\) adrenaline receptors.

There are some study limitations to address. First, the sample size was only six rabbits. There is a possibility that this small sample size slightly affected the correlation coefficients. More detailed experiments with larger samples are needed. Second, the MBR-RV and MBR-CH were recorded three times at each time point, and the mean value was then calculated. The rabbits’ heart rates were \(\sim 200\) bpm. Because the MBR used herein was the mean value of MBR during one cardiac cycle, one time point was calculated by 50 heart-rate analyses. Nevertheless, the number of MBR readings per time point was low. Finally, it was reported that the length ratio of elastic and muscle arteries differs between rabbits and humans, so further investigation in humans is necessary. Additional studies are also warranted to investigate the relationships among systemic hemodynamics, function, and ocular circulation under the systemic stimulation of adrenaline receptors. Studies examining the stimulation or blocking of \(\alpha\) and \(\beta\) adrenaline receptors together and separately could also be informative. Additional validation experiments are needed to clarify the pathology of sympathetic nervous system-related ocular diseases.
In conclusion, our LSFG-MRC experiment confirmed that the MBR-RV and MBR-CH are dose-dependently increased by systemic administration of adrenaline in anesthetized New Zealand White rabbits. However, there is a possibility that not only systemic BP values but also regional vascular resistance and elastic and muscular arterial functions are related to the variations in ocular microcirculation.

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