Intravenously Administered Histamine Increases Choroidal but not Retinal Blood Flow

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PURPOSE. To determine the effect of intravenously administered histamine on both retinal and choroidal blood flow in humans.

METHODS. A randomized, double-masked, two-way crossover study was performed in 14 healthy volunteers. Placebo or histamine was administered intravenously in stepwise increasing doses (0.08 µg/kg/min, 0.16 µg/kg/min, and 0.32 µg/kg/min). Retinal vessel diameters were measured with a retinal vessel analyzer, and retinal venous blood speed was assessed by bi-directional laser Doppler velocimetry. Using these parameters retinal blood flow was calculated. Subfoveal and pulsatil choroidal blood flow were measured with laser Doppler flowmetry and laser interferometry, respectively.

RESULTS. After infusion of histamine pulsatil choroidal blood flow increased by 5 ± 3%, 9 ± 8%, and 14 ± 7% (P = 0.001, ANOVA) and subfoveal choroidal blood flow by 8 ± 11%, 15 ± 11%, and 15 ± 12% (P = 0.003, ANOVA). Retinal arterial and venous vessel diameter significantly increased by 3 ± 4%, 2 ± 4%, and 3 ± 5% (P = 0.047, ANOVA) and 1 ± 2%, 3 ± 2%, and 3 ± 2% (P = 0.015, ANOVA), respectively. Red blood cell velocity in major retinal veins tended to decrease by −9 ± 12%, −9 ± 20%, and −15 ± 12%, but this effect did not reach levels of significance. Calculated retinal blood flow was not changed by administration of histamine (−7 ± 14%, −8 ± 20%, and −8 ± 12%, P = 0.28, ANOVA).

CONCLUSIONS. Intravenous histamine in the selected doses increased choroidal blood flow. Retinal vessels showed a small diameter increase, whereas red blood cell speed decreased, resulting in an unchanged total retinal blood flow. This may result from local differences in the receptor distribution in the posterior part of the eye. (Invest Ophthalmol Vis Sci. 2004;45: 2337–2341) DOI:10.1167/iovs.03-1235

The widely recognized importance of local mediators controlling blood flow has markedly extended our understanding of the ocular circulation. As one of these putative mediators, histamine is of experimental interest because of its potent effects on vascular function, especially in pathologic conditions such as inflammation and hypersensitivity reactions.¹,² The vascular responses to histamine in any organ, however, show a wide variability between species and vessels, emphasizing the importance of gaining knowledge in human ocular tissue.

In the human retina, there is evidence that histamine, as in the brain, plays a role as an endogenous modulator of ocular blood flow.³,⁴ Results of a previous study in healthy volunteers have demonstrated that intravenous administration of histamine causes an increase in pulsatile choroidal blood flow.³ However, no information is yet available concerning the effects of histamine on retinal blood flow in humans.

The present study was performed to gain more insight into the role of histamine on ocular blood flow regulation in humans.

MATERIALS AND METHODS

Subjects

Fourteen healthy male nonsmoking volunteers were included (age range, 22–33 years; mean, 27.6 ± 3.95 years). The nature of the study was explained and all subjects signed a written informed consent to participate. The study protocol was approved by the Ethics Committee of Vienna University School of Medicine and followed the guidelines of Good Clinical Practice (GCP) and the Declaration of Helsinki. Each subject passed a screening examination including medical history and physical examination, 12-lead electrocardiogram, complete blood count, coagulation parameters, clinical chemistry, total IgE-antibodies, blood serology, urine analysis, and a urine drug-screening. To minimize the risk of allergic reactions which are commonly associated with elevated circulating plasma levels of IgE antibodies, only subjects with IgE plasma levels of <100 kU/L were included.

Exclusion criteria were history of migraine or other types of headaches. Since sex hormones have been found to be strong vasoactive substances, which could possibly bias the results, women were not included in the study. Moreover, an ophthalmic examination, including slit lamp biomicroscopy and indirect funduscopy, was performed. Inclusion criteria were normal ophthalmic findings, ametropia of <5 diopters and anisometropia of <1 diopter.

Study Design

Subjects were studied in a randomized, double-masked, placebo-controlled, two-way crossover design. As flush symptoms occurred in every subject during histamine infusion, mostly during the highest histamine dosage, the double-blind conditions could not be maintained throughout the whole study period. Two study days were performed. On one study day, histamine was administered intravenously in stepwise increasing doses (0.08 µg/kg/min, 0.16 µg/kg/min, and 0.32 µg/kg/min). Each dose was infused for 30 minutes using a volume-controlled pump. To maintain double-blind conditions, three syringes containing physiologic saline solution were prepared and infused on the other study day.

Baseline ocular hemodynamic parameters were recorded in a sitting position after the values had stabilized. Afterward histamine-diphosphate (0.125 mg/ml; Mayrhofer Pharmazeutika, Linz, Austria) or placebo (physiologic saline) was administered intravenously over a period of 30 minutes for each of the three dosages. Ten minutes after the start of each infusion step, ocular hemodynamic parameters were assessed again in a predetermined order. Pulse rate and real time...
electrocardiogram were monitored continuously throughout the study period. The dose of histamine was chosen based on previous findings of the effect of systemic nitric oxide synthase inhibition on histamine-induced headache and on ocular vascular effects after intravenous histamine administration.8

Methods
Systolic, diastolic, and mean blood pressures (SBP, DBP, MAP) were measured on the upper arm using an automated oscillometric device. Pulse rate was automatically recorded from a finger pulse-oxymetric device. An electrocardiogram was monitored continuously using a standard four-lead device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Retinal Vessel Analyzer (RVA)
The Zeiss RVA (Carl Zeiss, Jena, Germany) is a commercially available system which comprises a fundus camera (Zeiss FF 450; Carl Zeiss), a video camera, a high resolution video recorder, a real-time monitor, and a personal computer with a vessel diameter analyzing software (Imedos, Jena, Germany). The RVA allows the precise determination of retinal vessel diameter with a time resolution of 25 readings/s. The fundus was illuminated with light in the range of wavelengths between 567 and 587 nm. In this spectral range, the contrast between retinal vessels and the surrounding tissue is optimal. Retinal irradiance was approximately 220 μW/cm², which is approximately 50 times lower than the maximum level allowed for constant illumination of the retina at the wavelengths mentioned above. The system provides excellent reproducibility and sensitivity.6 In the present study, major temporal arteries and veins were studied. Measurements of retinal venous diameters were taken between one and two disc diameters from the margin of the optic disc. Red blood cell velocity was measured at the same locations as retinal vessel diameters by using bi-directional laser Doppler velocimetry.

Laser Doppler Velocimetry

In the present study, a fundus camera-based system with a single mode laser diode at a center wavelength of 670 nm was used (Oculix Sarl, Arbaz, Switzerland). The principle of blood flow velocity measurement by laser Doppler velocimetry is based on the optical Doppler effect. Laser light, which is scattered by moving particles (e.g., erythrocytes), is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessels.10 The maximum Doppler shift corresponds to the centerline erythrocyte frequency. Measurements were done in main inferior temporal retinal veins.

Calculation of Retinal Blood Flow
Retinal blood flow was calculated based on these measurements of maximum erythrocyte velocity (Vmax) using laser Doppler velocimetry and retinal vessel diameters using the RVA. Mean blood velocity in retinal veins was calculated as (Vmax/1.6).6,11 Blood flow through a specific retinal vein was then calculated as Q = (Vmax/1.6) * (π * d²/4) where d is the diameter of the vein.

Laser Doppler Flowmetry
Measurement of subfoveal choroidal blood flow was performed by laser Doppler flowmetry (Oculix 4000; Oculix Sarl) introduced by Riva et al.12 For this purpose the vascularized tissue is illuminated by coherent laser light. Scattering on moving red blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast, static scatterers in tissue do not change light frequency, but lead to randomization of light direction impinging on RBCs. This light diffusing in vascularized tissue leads to a broadening of the spectrum of scattered light, from which mean RBC velocity (vel), the blood volume (vol), and the blood flow (flow) can be calculated in relative units. In the present study laser Doppler flowmetry was performed in the fovea to assess choroidal blood flow.

Laser Interferometry
Pulse synchronous pulsations of the eye fundus were assessed by laser interferometry. The method is described in detail by Schmetterer et al.13 Briefly, the eye is illuminated by the beam of a single mode laser diode (λ = 783 nm) along the optical axis. The light is reflected at both the front side of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. The method has been shown to estimate the pulsatile blood flow component in the choroid.14,15

Measurement of Intraocular Pressure
The IOP was measured with a Goldmann applanation tonometer (Haag-Streit Applanation Tonometer AT900; Haag-Streit, Koeniz, Switzerland).

Statistical Analysis
For data description and statistical analysis, hemodynamic parameters were expressed as percentage change from baseline (Δ%). Effects of histamine on hemodynamic parameters were assessed by ANOVA for repeated measurements. Results are given as mean ± SD. A P-value of < 0.05 was considered as the level of significance. Calculations were performed using the Statistica software package (Statsoft, Melbourne, Australia).

RESULTS
Intravenous administration of histamine in the selected doses was well tolerated by all subjects. Flush symptoms were obvious in all subjects, but none experienced headache or discomfort. No adverse event occurred during the study.

Systemic Hemodynamics and Intraocular Pressure
Baseline values of hemodynamic variables and ocular blood flow parameters are summarized in Table 1. No statistical difference in blood pressure and pulse rate was found between the two study days at baseline conditions. As shown in Table 2,
administration of histamine significantly decreased MAP from 79 ± 7 mm Hg to 75 ± 8 mm Hg, 74 ± 7 mm Hg, and 75 ± 6 mm Hg at the three administered doses, respectively (P = 0.046, ANOVA, n = 14). Placebo did not alter mean arterial pressure. Neither histamine nor placebo affected intraocular pressure.

### Choroidal Blood Flow

As shown in Figure 1, histamine significantly increased pulsatile choroidal blood flow assessed with laser interferometry by 4.7 ± 3.3%, 9.0 ± 8.0%, and 13.6 ± 7.0% (P = 0.001 vs. placebo, ANOVA, n = 14) at the three administered doses. Subfoveolar choroidal blood flow assessed with laser Doppler flowmetry increased by 8 ± 11%, 13 ± 11%, and 13 ± 12% after infusion of histamine. This effect was statistically significant both versus placebo and versus baseline (P = 0.003 vs. placebo, ANOVA, n = 14, P = 0.03 vs. baseline). Placebo had no consistent effects on choroidal blood flow parameters.

### Retinal Blood Flow

As shown in Figure 2, retinal arterial diameter assessed with the Zeiss retinal vessel analyzer significantly increased by 2.8 ± 4.1%, 2.2 ± 3.6%, and 3.4 ± 4.8% after administration of histamine (P = 0.047 vs. placebo, ANOVA, n = 14). Effects of stepwise increasing doses of histamine on retinal venous diameter were also significant (1.1 ± 1.8%, 2.9 ± 2.3%, and 2.9 ±

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**Table 2.** Hemodynamic Parameters and Intraocular Pressure at Baseline and after Administration of Placebo and Histamine, Respectively

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Baseline</th>
<th>Placebo Step 1</th>
<th>Placebo Step 2</th>
<th>Placebo Step 3</th>
<th>Histamine Baseline</th>
<th>Histamine Step 1</th>
<th>Histamine Step 2</th>
<th>Histamine Step 3</th>
</tr>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
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<td>79 ± 8</td>
<td>78 ± 7</td>
<td>78 ± 8</td>
<td>79 ± 7</td>
<td>75 ± 8</td>
<td>74 ± 7</td>
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<td>PR (bpm)</td>
<td>71 ± 11</td>
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<td>65 ± 10</td>
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<td>70 ± 8</td>
<td>69 ± 11</td>
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<tr>
<td>PA (mm Hg)</td>
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<td>53 ± 10</td>
<td>54 ± 13</td>
<td>58 ± 9</td>
<td>57 ± 9</td>
<td>57 ± 8</td>
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<td>IOP (mm Hg)</td>
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Results are presented as means ± SD (n = 12). MAP, mean arterial pressure; PR, pulse rate; PA, arterial pulse amplitude; IOP, intraocular pressure.

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**Figure 1.** Influence of stepwise increasing doses of histamine (open triangles) or placebo (filled squares) on mean arterial pressure (MAP), pulse rate (PR), choroidal blood flow, and fundus pulsation amplitude (FPA). Data are presented as means ± SD. Asterisks indicate significant changes versus placebo (two-way repeated measure ANOVA).
1.9%, \( P = 0.015 \) vs. placebo, ANOVA, \( n = 14 \)). After infusion of histamine red blood cell velocity in major retinal veins tended to decrease by \(-9 \pm 12\%\), \(-9 \pm 20\%\), and \(-13 \pm 12\%\), but this effect did not reach the level of significance. Thus, calculated retinal blood flow was not altered by administration of histamine \((-7 \pm 14\%, -4 \pm 20\%, \text{and} -8 \pm 12\%\) after the three doses of histamine, respectively).

**DISCUSSION**

The results of this study indicate that intravenously administered histamine dose-dependently increased choroidal but not retinal blood flow. Histamine did however increase retinal arterial and venous diameters, combined with a tendency toward decreased red blood cell velocity.

Several lines of evidence, mainly gained from in vitro experiments, suggest that histamine may play a role in ocular blood flow regulation. This hypothesis is supported by the presence of histamine in rat and bovine retinas at concentrations comparable to those measured in the brain, where histamine is known to act as an endogenous modulator of the circulation in different physiological and pathologic conditions.\(^5,16,17\) Furthermore, specific histamine bindings in human retina were observed similar to those found in the brain.\(^18\) This strongly suggests the presence of histamine \(H_1\) and \(H_2\) receptors in retinal blood vessels.

A strong increase in retinal blood flow caused by histamine has been reported in rats after intravitreal administration of histamine.\(^{19}\) In addition a concentration-dependent relaxation of bovine isolated retinal small arteries induced by intravitreal administration of histamine was demonstrated.\(^{20}\) These results are, however, in contrast to the work of Yu and coworkers,\(^{21}\) who measured contractile dose–response curves of cat isolated ophthalmic artery in response to histamine. The authors reported a contraction primarily in the proximal segment of the artery.

Interpretation of these results and comparison to our data may, however, be difficult. First, species differences could at least partially account for these contradicting results.\(^{18,19,20}\) Second, interpretation of these results is hampered by the fact that several subtypes of histamine receptors have been identified,\(^{22,23,24}\) and that responses may considerably depend on the size of the vessels studied. Furthermore, different administration routes and dosages could account for the contradicting results.
In humans, evidence for the influence of histamine on ocular blood flow is sparse. In an in vitro study performed in a human posterior ciliary artery, histamine induced a dilation at low concentrations and a constriction at higher concentrations. Intravenous administration of histamine was shown to cause different effects in cerebral and ocular vascular beds in healthy humans. In that study, a 25% increase in mean flow velocity (MFV) in the ophthalmic artery and a 10% increase in pulsatile choroidal blood flow was shown, but no histamine-induced change in MFV in the middle cerebral artery was observed. These results are in keeping with the data of the present study, where a consistent increase in subfoveal and pulsatile choroidal blood flow in the order of 10%–15% was observed. In a recent study a decrease in blood speed in the middle cerebral artery, together with vasodilatation in the temporal and radial arteries, was found after histamine infusion. This effect is compatible with the decreased retinal blood speed and increased vessel diameters found in retinal branch veins in this study.

An important finding of the present study is that major arteries and veins dilated, whereas red blood cell velocity as observed in retinal veins tended to decrease after administration of histamine, resulting in an unchanged retinal blood flow. Based on this finding, one could hypothesize that histamine has different effects on retinal resistance vessels and major retinal arteries and veins. Direct investigation of the microcirculation would be necessary to elucidate this question. However, diameter measurements of these smaller vessels are currently not possible because of the limited resolution of the instruments available.

In conclusion, the data of the current experiment suggest that chorioidal but not retinal blood flow changes after intravenous administration of histamine in the selected doses. Hence, histamine may act as an endogenous modulator of chorioidal perfusion. In addition, the present study may reflect the complex distribution of histamine receptor subtypes within the eye, which needs to be further elucidated.

References