Effect of histamine and cimetidine on retinal and choroidal blood flow in humans

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It was recently demonstrated that intravenous administration of histamine causes an increased mean flow velocity in the ophthalmic artery (18). This increase is paralleled by a vasodilatation of retinal vessels and an increase in choroidal blood flow (24). However, the mechanism underlying this effect is unclear, because different subtypes of histamine receptors exist and the receptor responsible for the ocular vasodilator effects of histamine in vivo has yet to be identified (3, 4, 8). Recent experiments in other vascular beds have identified both H1 and H2 receptors as possible candidates for vascular effects, but the H2 type seems to be the dominant receptor participating in the vasodilator responses of histamine in many vascular beds (21). Thus the current study aimed to test the hypothesis that administration of cimetidine, a histamine type 2 receptor antagonist, is able to modify the blood flow response of intravenous histamine in the choroid and retina.

MATERIAL AND METHODS

Subjects. Eighteen healthy male nonsmoking volunteers were included (age range: 21–35 yr, mean: 25.5 yr, SD: 3.0 yr). The nature of the study was explained, and all subjects signed a written informed consent form 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 and the Declaration of Helsinki. Each subject passed a screening examination that included medical history and physical examination, 12-lead electrocardiogram, complete blood count, activated partial thromboplastin time, thrombin time, fibrinogen, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate transcarbamylase, y-glutamyltransferase, alkaline phosphatase, total bilirubin, and total protein), total IgE antibodies, hepatitis A, B, and C and human immunodeficiency virus serology, urine analysis, and a urine drug screening. Only subjects with IgE plasma levels of <100 kU/l were included. Subjects were excluded if any abnormality was found as part of the pretreatment screening unless the investigators considered the abnormality to be clinically irrelevant. Further exclusion criterion was history of migraine or other types of headaches. Moreover, an ophthalmic examination, including slit-lamp biomicroscopy and indirect funduscopy, was performed. Inclusion criteria were normal ophthalmic findings, ametropia of ≤3 diopters, and anisometropia of <3 diopters.

Study design. The dose of cimetidine was selected on the basis of results of a pilot study (n = 4; mean age: 26.1 yr, age range: 22–31 yr). In this open-dose escalation study, increasing doses of cimetidine of 0.3, 1, 1.6, and 2.3 mg/min were administered for 15 min at each step. Systemic hemodynamic measurements and blood flow measurements were performed using the same techniques as outlined for the main study at the end of each infusion step.

THE IMPORTANCE OF HISTAMINE as a regulator of vascular tone has been demonstrated for several vascular beds (6, 12). On the basis of these experiments, it has been hypothesized that histamine may also play a role in the regulation of ocular blood flow. This hypothesis is supported by several in vitro experiments indicating a vasodilatory role of histamine in the ocular circulation (7). Data of human experiments on the effect of histamine on ocular blood flow is, however, sparse.

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In the main experiment, subjects were studied in a randomized, double-masked, two-way crossover design with histamine in combination with the H$_3$ receptor antagonist cimetidine or placebo. Two study days were scheduled for each subject with washout periods of at least 5 days between study days. On both study days, histamine was administered intravenously at a dose of 0.32 μg·kg$^{-1}$·min$^{-1}$ with coadministration of either cimetidine at a dose of 2.3 mg/min or placebo.

On the trial days, subjects arrived after having ingested a light breakfast. Baseline hemodynamic parameters were recorded with subjects in a sitting position after the values had stabilized. Cimetidine (Cimetag, 200 mg; SmithKline Beecham Pharma, Brunn/Gebirge, Austria) or placebo was given intravenously for 30 min. Fifteen minutes after the start of the histamine infusion, the measurement procedures were repeated again. Pulse rate and blood pressure were measured in 5-min intervals, and real-time electrocardiogram was monitored continuously throughout the study period. The dose of histamine was chosen on the basis of our previous investigations on the effect of systemic nitric oxide synthase inhibition on histamine-induced headache and on ocular vascular effects after intravenous histamine administration (17, 24).

**Noninvasive measurement of systemic hemodynamics.** Systolic, diastolic, and mean arterial blood pressures (SBP, DBP, and MAP) were measured every 5 min on the upper arm with the use of an automated oscillometric device. Pulse pressure amplitude (PPA) was calculated as PPA = SBP − DBP. Pulse rate was automatically recorded from a finger pulse oxymetric device. An electrocardiogram was monitored continuously with the use of a standard four-lead device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

**Zeiss retinal vessel analysis.** The retinal vessel analyzer (RVA; Heidelberg, Jena, Germany) is a commercially available system that comprises a fundus camera (Zeiss FF 450; Jena, Germany), a video camera, a high-resolution video recorder, a real-time monitor, and a personal computer with vessel diameter-analyzing software. The RVA allows for a precise determination of retinal vessel diameter with a 50 times lower than the maximum level allowed for constant illumination of the retina at the wavelengths mentioned. The system provides excellent reproducibility and sensitivity (13). In the present study, major temporal arteries and veins were studied. Measurements of retinal venous diameters were taken between 1 and 2 disk diameters from the margin of the optic disk. Red blood cell (RBC) velocity was measured at the same locations as retinal vessel diameters by using bidirectional laser-Doppler velocimetry.

**Laser-Doppler velocimetry.** In the present study, we used a fundus camera-based system with a single-mode laser diode at a centerline wavelength of 670 nm (Oculix 4000; 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 vessel. The maximum Doppler shift corresponds to the centerline erythrocyte frequency. Because of the smaller vessel diameter of retinal arteries compared with retinal veins, aiming the laser beam on the vessel surface is more difficult in retinal arteries. Accordingly, measurements were done in a major inferior temporal retinal vein only (15).

**Calculation of retinal blood flow.** Retinal blood flow was calculated on the basis of the measurements of maximum erythrocyte velocity ($V_{\text{max}}$) obtained using laser-Doppler velocimetry and on measurements of retinal vessel diameters obtained using the RVA. Mean blood flow velocity in retinal veins was calculated as ($V_{\text{max}}/2$). Blood flow through a specific retinal vein was then calculated as $Q = (V_{\text{max}}/2) \times (\pi \times d^2/4)$, where $d$ is the diameter of the vein.

**Laser-Doppler flowmetry.** Measurement of subfoveal choroidal blood flow was performed using laser-Doppler flowmetry (Oculix 4000; Oculix Sarl) introduced by Riva et al. (14). For this purpose, the vascularized tissue is illuminated by coherent laser light. Scattering on moving 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 frequency spectrum of scattered light from which mean RBC velocity, blood volume, and blood 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 using laser interferometry. The method was described in detail by Schmetterer and et al. (18). Briefly, the eye is illuminated by the beam of a single-mode laser diode ($\lambda = 783$ nm) along the optical axis. The laser power of not more than 100 μW is

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**Table 1. Baseline parameters of ocular and systemic hemodynamic measurements on the two study days**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Histamine Day</th>
<th>Cimetidine/ Histamine Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>82±7.5</td>
<td>80±7.1</td>
</tr>
<tr>
<td>Pulse rate, beats/min</td>
<td>67±9.2</td>
<td>68±11.3</td>
</tr>
<tr>
<td>Intracocular pressure, mmHg</td>
<td>13.1±1.5</td>
<td>12.5±1.7</td>
</tr>
<tr>
<td>Choroidal blood flow, arbitrary units</td>
<td>10.2±2.3</td>
<td>10.5±2.2</td>
</tr>
<tr>
<td>Arterial vessel diameter, μm</td>
<td>120.8±14.4</td>
<td>122.4±15.3</td>
</tr>
<tr>
<td>Venous vessel diameter, μm</td>
<td>144.9±19.1</td>
<td>144.7±17.1</td>
</tr>
<tr>
<td>Fundus pulsation amplitude, μm</td>
<td>4.2±1.4</td>
<td>4.3±1.4</td>
</tr>
<tr>
<td>Retinal blood velocity, cm/s</td>
<td>2.0±0.6</td>
<td>2.0±0.7</td>
</tr>
<tr>
<td>Retinal blood flow, μl/min</td>
<td>16.4±4.3</td>
<td>16.2±4.2</td>
</tr>
</tbody>
</table>

Baseline parameters were obtained during the 10 min before infusion. The averaging period for hemodynamic measurements with laser-Doppler flowmetry and laser-Doppler velocimetry was 120 s. Results are presented as means ± SD ($n = 18$). Retinal blood flow denotes blood flow through one specific vein and not total blood flow.

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Fig. 1. Effects of increasing doses of cimetidine on pulse rate (bpm, beats/min) (pilot study, $n = 4$; means ± SD).
much lower than the limit set by the American National Standards Institute. The light is reflected at both the front side of the cornea and the retina. The two reemitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. The maximum distance change between cornea and retina is called fundus pulsation amplitude (FPA) and estimates the pulsatile blood flow component in the choroid (16, 19).

Measurement of intraocular pressure. The intraocular pressure (IOP) was measured with a Goldmann applanation tonometer.

Statistical analysis. For data description and statistical analysis, hemodynamic parameters were expressed as percent changes from baseline (Δ%). Effects of histamine and cimetidine on hemodynamic parameters were assessed using a two-way ANOVA model for repeated measurements. Results are given as means ± SD. Post hoc analysis was performed using planned comparison. Shapiro-Wilks’

Table 2. Systemic hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>Histamine Day</th>
<th>Cimetidine/Histamine Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Placebo</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>120±7</td>
<td>117±9</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>62±9</td>
<td>61±7</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>82±7</td>
<td>80±7</td>
</tr>
<tr>
<td>PPA, mmHg</td>
<td>58±9</td>
<td>57±8</td>
</tr>
<tr>
<td>PR, beats/min</td>
<td>66±11</td>
<td>66±7</td>
</tr>
</tbody>
</table>

Values are means ± SD of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure amplitude (PPA), and pulse rate (PR) flow as recorded at the 3 time points.
W-test was used to test for normal data distribution. Calculations were performed using the Statistica software package (StatSoft).

RESULTS

Dosage study. As depicted in Fig. 1, administration of cimetidine induced a dose-dependent decrease in pulse rate (ANOVA, $P = 0.015$). Other systemic and ocular hemodynamic parameters remained unaffected by administration of cimetidine (data not shown).

Main study. Baseline values of hemodynamic and ocular blood flow parameters were comparable between study days as summarized in Table 1.

Systemic hemodynamics and IOP. As shown in Fig. 2, administration of cimetidine induced a significant decrease in pulse rate (ANOVA, $P = 0.021$), which was not altered by coadministration of histamine. Administration of histamine tended to decrease MAP when placebo was coadministered (−4 ± 9%), but this effect was not different on the cimetidine study day (ANOVA, $P = 0.17$). PPA was not altered by either histamine or cimetidine administration (Table 2). IOP remained unchanged during the whole study period on both study days (data not shown).

Choroidal blood flow parameters. Administration of cimetidine alone did not affect choroidal blood flow or FPA (Fig. 2). Histamine increased FPA as assessed with laser interferometry and choroidal blood flow as assessed with laser-Doppler flowmetry by $+11 \pm 5$ and $+14 \pm 15\%$, respectively (ANOVA, $P < 0.001$ each). Cimetidine did not modify the effects of histamine on FPA or choroidal blood flow (FPA: $P = 0.51$; choroidal blood flow: $P = 0.63$).

Retinal blood flow parameters. Cimetidine had no effect on retinal arterial or venous diameters, retinal RBC velocity, or retinal blood flow. Administration of histamine increased retinal arterial diameters and retinal venous diameters by $+2.8 \pm 4.2\%$ (ANOVA, $P = 0.009$) and $+3.0 \pm 3.6\%$ (ANOVA, $P = 0.002$), respectively. RBC velocity in retinal veins tended to decrease after administration of histamine (−8.3 ± 21.2%, $P < 0.13$), but this effect was not significant. Calculated retinal blood flow remained unchanged after administration of histamine (ANOVA, $P = 0.6$). None of these effects were modified by coadministration of cimetidine.

DISCUSSION

Whereas in several vascular beds the importance of histamine as a local regulator of blood flow has generally been accepted, the effect of histamine on ocular blood flow is less clear. Previous in vivo experiments in animal models did not indicate for a role of histamine in ocular blood flow regulation (1, 5), but other investigators have provided evidence that histamine may induce vasodilation in the posterior part of the eye (7, 24).

Direct evidence of a vasodilatory effect of histamine has been recognized in isolated bovine retinal arteries, where a concentration-dependent vasodilator effect of histamine was observed. These results observed in isolated vessels have been confirmed in animal experiments, in which a pronounced increase in retinal blood flow in the rat has been observed following intravitreal administration of histamine (10). However, little is known about the mechanism underlying the vasodilator effect of histamine in the eye. The effect of histamine on isolated vessels was mainly attributed to the activation of H$_1$ receptors, although activation of H$_2$ receptors appears to contribute to the histamine response as well (7). Neither H$_1$ nor H$_2$ antagonists completely abolished the vasodilator response to histamine, but combined treatment with both antagonists totally suppressed the relaxing effect of histamine (7). In addition, the vasodilatory effect of histamine was dependent on the endothelium, indicating a role of nitric oxide in the signaling cascade (7). This finding is in keeping with a previous report from our group (17) showing that the effects of histamine in the ophthalmic artery and the choroid were diminished by systemic nitric oxide synthase inhibition, supporting the concept that the vasodilator actions of histamine in the eye at least partially depend on nitric oxide.

Our data indicate that in vivo H$_2$ receptors do play only a minor role in mitigating the hemodynamic effects of histamine, because neither the histamine-induced vasodilator effects in the larger retinal vessels nor the effects on choroidal blood flow parameters were altered by cimetidine. Could these results be false negative? We cannot entirely exclude that higher doses of cimetidine may affect blood flow in the eye. On the other hand, cimetidine was administered in a hemodynamically active dose as shown by the well-known effect of the drug to induce a decrease in pulse rate (11).

This result is somewhat unexpected, because in other vascular beds, H$_2$ receptors do contribute to histamine-induced vascular effects. It has been known for a long time that the action of histamine on vascular smooth muscle may vary depending on the tissue and species. Furthermore, histamine responses are known to be location and concentration dependent. Even a segmental vessel heterogeneity of the response to histamine has been described, explaining differences between in vitro and in vivo studies (22, 23).

The potential sources of histamine in the retina are still a matter of discussion. On one hand, histamine does not cross the blood-retinal barrier. On the other hand, mast cells, another potential source of histamine, are not present in the retina (20). Hence, histamine is likely to originate from neurons. In particular, horizontal cells have been proposed to be a source of histamine in the retina. (2) This indicates that histamine acts as a local neurotransmitter or neuromodulator in the retina and contributes to the blood flow regulation in the posterior pole of the eye.

In conclusion, we found that cimetidine in the selected dose was not able to modify the blood flow response to histamine. It remains to be elucidated whether the histamine-induced changes in ocular blood flow are mediated via H$_1$ receptors or by other hitherto unidentified mechanisms.

GRANTS

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