Intravenous Administration of Diphenhydramine Reduces Histamine-Induced Vasodilator Effects in the Retina and Choroid

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PURPOSE. Intravenous administration of histamine causes an increase in choroidal blood flow (ChBF) and retinal vessel diameters in healthy subjects. The receptor mediating this response has not yet been identified. The present study was undertaken to clarify whether H1 receptor blockade with diphenhydramine affects the hemodynamic response of histamine in the choroid and the retina.

METHODS. A randomized, double-masked, placebo-controlled, two-way crossover study was performed in 18 healthy, male, nonsmoking subjects. Histamine (0.32 μg/kg per minute over 30 minutes) was infused intravenously in the absence (NaCl as placebo) or presence of the H1 blocker diphenhydramine (1.0 mg/min over 50 minutes). Ocular hemodynamic parameters, blood pressure, and intraocular pressure were measured before drug administration, after infusion of diphenhydramine or placebo, and after co-infusion of histamine. Subfoveal ChBF and fundus pulsation amplitude (FPA) were measured with laser Doppler flowmetry and laser interferometry, respectively. Retinal arterial and venous diameters were measured with a retinal vessel analyzer. Retinal blood velocity was assessed with bidirectional laser Doppler velocimetry.

RESULTS. Administration of histamine caused a decrease in mean arterial pressure by −4% ± 9% (ANOVA \( P = 0.01 \)). This effect was blunted by coadministration of diphenhydramine (ANOVA, \( P = 0.04 \)). Histamine significantly increased FPA and subfoveal ChBF. Coadministration of diphenhydramine significantly reduced this effect (ANOVA; FPA \( P = 0.001 \), ChBF \( P = 0.049 \)). Histamine significantly increased retinal arterial diameter by +3.5% ± 4.3% and retinal venous diameter by +3.7% ± 2.8%. Again, coadministration of diphenhydramine significantly reduced the vasodilative effect to +0.3% ± 5.5% in retinal arteries (ANOVA, \( P = 0.0006 \)) and to +0.9% ± 2.5% in retinal veins (ANOVA, \( P = 0.004 \)).

CONCLUSIONS. The present data confirm that histamine increases ChBF and retinal vessel diameters in healthy subjects. Administration of the H1 receptor blocker diphenhydramine significantly reduced histamine-induced changes in ocular perfusion parameters. These results strongly indicate that in the retina and choroid, H1 receptors are involved in the histamine-mediated hemodynamic effects in vivo. (Invest Ophthalmol Vis Sci. 2006;47:1096–1100) DOI:10.1167/iovs.05-1174

Whereas the role of histamine in the acute allergic response is well established, the potential physiological role of histamine in the control of vascular tone remains to be clarified fully. Several lines of evidence indicate that histamine acts as a regulator of blood flow in the eye.1–3 In vitro experiments in isolated retinal arteries first demonstrated an endothelium-dependent relaxation of retinal vessels caused by histamine.4 This is in keeping with experiments in humans indicating a vasodilative role of histamine in the retina, paralleled by an increase in choroidal blood flow (ChBF).1–3

However, the exact mechanism underlying this histamine-induced effect is still a matter of investigation. Although there is evidence that the vascular effects of histamine are dependent on the presence of an intact endothelium,1 the receptors mediating this effect have not yet been identified. Until now, three different histamine receptor subtypes have been identified.5–7 The H1 and H2 receptor types are present in the arteriolar smooth muscle, whereas the H3 type has been hypothesized to be the dominant receptor participating in the vasodilator response to histamine.8 Histamine H3 receptors are presynaptic receptors in the central nervous system and on peripheral neurons of the gastrointestinal and bronchial tracts, modulating the endogenous release of a variety of neurotransmitters.9

We showed recently that cimetidine, a H2 receptor antagonist is not able to inhibit the blood flow response to histamine, strongly suggesting that H2 receptors are not responsible for the blood flow effect of histamine in the eye.5 In the present study we hypothesized that administration of diphenhydramine, a histamine type-1 receptor antagonist, alters the vasodilative effect of histamine in the ocular circulation.

MATERIALS AND METHODS

Subjects

Eighteen healthy, male, nonsmoking volunteers were included (age range: 22–32 years, mean: 26.4 ± 2.8 years [SD]). 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 adhered to the guidelines of Good Clinical Practice and the Declaration of Helsinki. Each subject passed a screening examination, including medical history and physical examination; 12-lead electrocardiogram; complete blood count; aspartate transcarbamoylase, \( \gamma \)-glutamyltransferase, alkaline phosphatase, total bilirubin, total protein), total IgE antibodies; hepatitis A, B, and C and HIV serology; urinalysis; and a urine drug screening. Only subjects with IgE plasma levels of less than 100 kU/L were included. Subjects were excluded if any abnormality was found as part of the pretreatment

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screening, unless the investigators considered the abnormality to be clinically irrelevant. Further exclusion criteria were a 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 less than 3 D, and anisometropia of less than 1 D.

Study Design

Subjects were studied in a randomized, balanced, double-masked, two-way crossover design infusing histamine in combination with the H1 receptor antagonist diphenhydramine or a 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 in a dose of 0.32 µg/kg per minute, with a road-ministration of diphenhydramine in a dose of 1.0 mg/min, or placebo on the other study day. Both substances were administered with an infusion pump (Braun, Melsungen, Germany). The total volume load for each volunteer was 88 mL. A time schedule is shown in Figure 1.

On the trial days, subjects arrived after a light breakfast. Baseline hemodynamic parameters were recorded with the subject in a sitting position after the values had stabilized. All subjects were studied with diluted pupils after instillation with tropicamide (Mydriaticum Agepha; Agepha, Vienna, Austria). One drop was applied to the study eye at the beginning of the resting period. Diphenhydramine (Dibodrin, 30 mg ampule; Montavit GmbH, Absam, Austria) or a placebo was given intravenously over a period of 50 minutes. Five minutes after the start of the infusion, ocular hemodynamic parameters were assessed again in a predetermined order (fundus pulsation amplitude [FPA], laser Doppler velocimetry, laser Doppler flowmetry, retinal vessel analyzer). Twenty minutes after the start of the diphenhydramine or placebo infusion, histamine (Mayrhofer Pharmazeutika, Linz, Austria) was administered for 30 minutes. Fifteen minutes after the start of the infusion of histamine, the measurement procedures were repeated. Pulse rate and blood pressure were measured in 5-minute intervals and a real-time electrocardiogram was monitored continuously throughout the study period.

Methods

Noninvasive Measurement of Systemic Hemodynamics

Systolic, diastolic, and mean arterial (MAP) blood pressure were measured every 5 minutes on the upper arm with an automated oscillometer. Pulse rate was automatically recorded from a finger pulse oximeter. An electrocardiogram was recorded continuously with a standard four-lead device (HP-CMS monitor; Hewlett Packard, Palo Alto, CA).

Retinal Vessel Analyzer

The retinal vessel analyzer (RVA; Imedos, Jena, Germany) is a commercially available system that comprises a fundus camera, 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 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 earlier. The system provides excellent reproducibility and sensitivity. In the present study, major temporal arteries and veins were studied. Measurements of retinal venous diameters were taken between 1 and 2 disc diameters from the margin of the optic disc. 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 (model 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) shifts 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. Measurements were performed in main inferior temporal retinal veins.

Calculation of Retinal Blood Flow

Blood flow in retinal veins was calculated based on maximum erythrocyte velocity (Vmax) measured by laser Doppler velocimetry and retinal vessel diameter measured by the RVA, both assessed in retinal veins. Mean blood flow velocity was calculated as (Vmax/2). Blood flow through a specific retinal vein was then calculated as Q = (Vmax/2) · (π · d²/4), where d is the diameter of the vein.

Laser Doppler Flowmetry

Measurements of subfoveal ChBF were performed by laser Doppler flowmetry (model 4000; Oculix Sari), as described by Riva et al. For this purpose, the vascularized tissue was 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 diffusion 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 ChBF.

Laser Interferometry

Pulse synchronous pulsations of the eye fundus were assessed by laser interferometry. The method is described in detail by Schmetterer et al. Briefly, the eye is illuminated along the optical axis by the beam of a single-mode laser diode (λ = 783 nm). The laser power of not more than 100 µW is 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 re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. The FPA has been shown to estimate the pulsatile blood flow component in the choroid.

Measurement of IOP

The IOP was measured with a Goldmann applanation tonometer (Haag Streit, Vienna, Austria).

Statistical Analysis

For data analysis, hemodynamic parameters are expressed as percentage change from baseline (Δ%). Effects of histamine and diphenhydramine...
Baseline parameters were obtained during the 10 minutes before infusion. Results are presented as the mean ± SD. Parameters included Mean arterial pressure (MAP), pulse rate (PR), intraocular pressure (IOP), subfoveal choroidal blood flow assessed with laser Doppler flowmetry (ChBF), retinal arterial and venous diameters, fundus pulsation amplitude (FPA) as assessed with laser interferometry, red blood cell velocity (RBV) assessed with bidirectional laser Doppler velocimetry and calculated retinal blood flow (RBF) in one single vein.

mine on hemodynamic parameters were assessed by two-way ANOVA for repeated measurements, using the absolute values. The interaction between time and treatment was used to assess statistical differences. Planned comparisons were used to calculate differences between diphenhydramine and placebo (treatment effect) and time effects. Results are given as the mean ± SD. Shapiro-Wilks' W test was used to test for normal data distribution. Calculations were performed on computer (Statistica software package; Statsoft, Tulsa, OK).

RESULTS

Systemic Hemodynamics and IOP

Baseline pulse rate and IOP were comparable on both study days (Table 1). Neither administration of diphenhydramine nor placebo affected MAP, whereas administration of histamine decreased MAP by −4% ± 9% (ANOVA, time effect: P = 0.01). Coadministration of diphenhydramine reduced the MAP-lowering effect of histamine (ANOVA, interaction: P = 0.04; placebo versus diphenhydramine: P = 0.04). Infusion of diphenhydramine tended to decrease pulse rate, but this effect did not reach significance (Fig. 2; ANOVA, interaction: P = 0.1). None of the study drugs altered IOP.

ChBF Parameters

As shown in Figure 2, neither placebo nor diphenhydramine affected subfoveal ChBF, as assessed with laser Doppler flowmetry. Histamine, however, increased subfoveal ChBF by +12% ± 16% (ANOVA time effect: P = 0.01). Coadministration of diphenhydramine reduced this histamine-induced increase to +5% ± 13% (ANOVA, interaction: P = 0.04, placebo versus diphenhydramine: P = 0.049), FPA, as measured by the means of laser interferometry was not affected by administration of placebo or diphenhydramine (Fig. 2). Histamine, however, significantly increased FPA by +15% ± 9% (ANOVA, time effect: P = 0.001). Again, this effect was significantly reduced by coadministration of diphenhydramine to +1% ± 5% (ANOVA, interaction: P = 0.001, placebo versus diphenhydramine: P = 0.001).

Retinal Blood Flow

Diphenhydramine alone had no effect on retinal arterial or venous diameters. Histamine increased retinal arterial diameter by +3.5% ± 4.5% (ANOVA, time effect P = 0.0001). Coadministration of diphenhydramine significantly reduced this increase to +0.3% ± 5.5% (ANOVA, interaction: P = 0.004, placebo versus diphenhydramine: P = 0.00006). Administration of histamine increased retinal venous diameter by +3.7% ± 2.8% (ANOVA, time effect: P = 0.000016). Coadministration of diphenhydramine also reduced this vasodilative effect of histamine to +0.9% ± 2.5% (ANOVA, interaction: P = 0.00006, placebo versus diphenhydramine: P = 0.004).

Histamine did not change RBC velocity (Fig. 2). Neither placebo nor diphenhydramine affected RBC velocity (ANOVA, interaction: P = 0.6). Calculated retinal blood flow in retinal veins also remained unchanged after administration of histamine and was not altered by coadministration of diphenhydramine. (ANOVA, interaction: P = 0.3).

DISCUSSION

The vasomotor effects of histamine have been studied in several vascular beds in human and animal experiments. In general, intravenous administration of histamine causes a decline in peripheral vascular resistance, indicated by a decrease in systemic blood pressure. This indicates net vasodilation, although both contractile and relaxing effects, as mediated by the activation of both H1 and H2 receptors, may occur. In contrast to this systemic effect, the effects of histamine on a specific vascular bed are quite variable and hard to predict, because marked species and regional differences have been reported regarding the receptor density and distribution.

In the ocular vasculature, several lines of evidence, mainly based on experiments in isolated vessels, demonstrate the vasodilative role of histamine. These results have been confirmed by in vivo animal experiments, where an increase in retinal blood flow in the rat eye has been observed after intravitreal administration of histamine. The few experimental data available from in vivo human experiments, also confirm this vasodilative role of histamine in the eye. Intravenously administered histamine caused an increase in mean flow velocity in the ophthalmic artery as well as an increase in pulsatile ChBF. In addition, it has recently been shown that histamine increases ChBF and retinal vessel diameter in young, healthy volunteers.

However, interpretation of these results is hampered by the fact that the receptor mediating this vasodilative potential has not yet been identified. It has been recognized that both H1 and H2 receptors are localized uniformly within the inner layers of cerebral arteriolar smooth muscle. Experiments in isolated human subcutaneous resistance arteries indicated that both H1 and H2 receptors are responsible for the histamine effects, but the H2 type seemed to be the dominant receptor participating in the vasodilator responses to histamine in the skin.

In the retina and the choroid, direct evidence of the receptors mediating the vascular effects of histamine is sparse. Studies in isolated bovine retinal arteries showed a concentration-dependent vasodilator effect of histamine. In contrast to the skin, the vasodilative effect in ocular vessels was clearly inhibited by coadministration of the H1 receptor antagonist mepyramine. This indicates that in the eye, the hemodynamic effect was mainly caused by the activation of H1 receptors, although activation of H2 receptors seemed to contribute to the histamine response. As stated earlier this contradictory result may be explained by the fact that the action of histamine on vascular smooth muscle depends on the tissue and species. Furthermore, histamine responses are known to be location and concentration dependent. Even a heterogeneity in the response of segmental vessel to histamine is described.
We recently showed that intravenous administration of cimetidine, an H₂ receptor antagonist, has no inhibitory influence on histamine-induced changes in retinal and choroidal circulation. From this experiment, it was concluded that H₂ receptors do not play a major role in the regulation of ocular blood flow. The results of the present study demonstrate that the histamine-induced increase in retinal vessel diameters and ChBF can be reduced by coadministration of an H₁ receptor antagonist.
antagonist. These results strongly indicate that in the ocular circulation, histamine’s effects are at least partially mediated by H1 receptors. Although diphenhydramine is a potent and highly selective H1 receptor antagonist, we cannot entirely exclude that diphenhydramine also exerts, to a small extent, antagonizing effects on H2 receptors. To exclude this confounding possibility with certainty, a specific H2 receptor agonist would be necessary, but such a receptor agonist is not available for human experiments to date.

In addition, we cannot fully exclude that the retinal vasodilatation observed after administration of histamine is due to a counterregulatory effect caused by the decrease in systemic blood pressure. However, considering that the effect of histamine on blood pressure is very small, it is unlikely that this systemic effect is fully responsible for the vasodilatation at the level of larger arterioles and venules observed in our study. Furthermore, no change in RBC velocity was observed, whereas in our recent experiments, a tendency toward decreased RBC speed was noted. However, in all experiments the effect on RBC velocity and retinal blood flow did not reach significance. Based on the variability of laser Doppler velocimetry measurements in our laboratory, none of these studies had the power to detect subtle differences in RBC velocity with histamine. To clarify whether exogenous histamine may have an effect on retinal RBC velocity, either a study with an increased sample size or a higher dose of histamine is necessary.

The potential sources of endogenous histamine in the human retina are still a matter for investigation. It has been shown that, in the mammalian retina, centrifugal axons contain immunoreactive histamine.22 Whereas the situation in the human retina remains to be clarified, there is evidence that in the macaque retina, these histamine-containing axons originate from the hypothalamus23–24 and terminate in the inner plexiform layer, sometimes adjacent to retinal blood vessels.22 Whether these neurons play a role in the regulation of vascular tone under physiological conditions has yet to be clarified.

Given that some of the effects of histamine are also mediated by H2 receptors,25 one could hypothesize that H2 receptors are also involved in the histamine-induced vascular effects. Although H2 receptors are mainly found in the neurologic tissue, this hypothesis is supported by the fact that H2 receptors have been identified in vascular tissue.26,27 Further investigation is needed to clarify whether H2 receptors play a role in the regulation of ocular vascular tone.

In conclusion, we found that diphenhydramine, in the dose selected, significantly reduced the vasodilative effect of histamine. This result strongly suggests that H1 receptors at least partially contribute to the vasodilative effect of histamine.

**References**


