Mechanisms of Histamine-Induced Relaxation in External and Internal Ophthalmic Arteries

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Purpose. Mechanisms that underlie the relaxant response to histamine were examined in dog external (a branch of external carotid artery) and internal (a branch of internal carotid artery) ophthalmic arteries (EOA and IOA).

Methods. Changes in isometric tensions were recorded in helical strips of the arteries with and without the endothelium.

Results. Histamine predominantly produced relaxations in EOA and IOA, partially contracted with prostaglandin (PG) F\textsubscript{2a}. The relaxation of IOA almost was abolished by treatment with cimetidine (10\textsuperscript{-5} mol/l), whereas the response of EOA was partially attenuated by treatment with cimetidine or chlorpheniramine (10\textsuperscript{-6} mol/l) and was abolished with their combined treatment. Endothelium denudation depressed the relaxation in EOA but did not affect the response of IOA. The response to histamine of EOA was inhibited by treatment with indomethacin (10\textsuperscript{-6} mol/l) or tranylcypromine (10\textsuperscript{-4} mol/l), a PGI\textsubscript{2} synthesis inhibitor, only when the endothelium was present, but additional treatment with chlorpheniramine did not further inhibit relaxation. On the other hand, IOA’s response to histamine was not inhibited by indomethacin, despite the presence of endothelium.

Conclusions. The histamine-induced relaxation in EOA may be associated with the release of vasodilator PGI\textsubscript{2} through the activation of H\textsubscript{1} receptors in the endothelium and with the direct action on H\textsubscript{2} receptors in smooth muscle, whereas the relaxation in IOA is mediated exclusively by H\textsubscript{2} receptors in smooth muscle. Invest Ophthalmol Vis Sci. 1993;34:41-48.

Histamine is detected biochemically\textsuperscript{1} and histologically\textsuperscript{2} in blood and in the vascular wall. The amine released from mast cells and other tissues or applied exogenously in vivo lowers systemic blood pressure by dilating resistance vessels via an activation of H\textsubscript{1} or H\textsubscript{2} receptors.\textsuperscript{3} However, histamine may be a vasospastic substance, because the amine caused contraction in human conduit coronary arteries\textsuperscript{4} and in the main trunk of human cerebral arteries.\textsuperscript{5} Remarkable species differences have been reported regarding the vascular actions of histamine. Coronary arteries from monkeys and dogs respond with relaxations,\textsuperscript{6,7} whereas human, pig, and cattle coronary arteries respond with contractions.\textsuperscript{8-10} Furthermore, there are marked differences in the amine-induced responses of a variety of blood vessels from the same species. Dog mesenteric, gastrointestinal, and renal arteries relax,\textsuperscript{6} but dog cerebral (proximal portion), pulmonary, and portal veins constrict.\textsuperscript{6,11,12} These different responses seem to be derived from differential actions on H\textsubscript{1} and H\textsubscript{2} receptors localized in vascular smooth muscle and on H\textsubscript{1} receptors in the endothelium that may be responsible for the release of prostaglandin (PG) I\textsubscript{2}\textsuperscript{13,14} or endothelium-derived relaxing factor (EDRF).\textsuperscript{4,15}

Large amounts of histamine reportedly are present in several ocular structures, including the retina, choroid, and optic nerve,\textsuperscript{16-18} and light stimulation
changes the content in the retina and the optic nerve. Circadian rhythm of histamine metabolism also has been found in the ocular structures. These findings suggest that histamine in the mammalian visual system may be physiologically important. However, no information is available concerning the response of opthalmic arteries to histamine. In the dog, two opthalmic arteries supply blood to the ocular tissues; the external (EOA) and internal opthalmic arteries (IOA) anatomically originate from external and internal carotid arteries, respectively. Because responses to vasoconstrictor substances such as 5-hydroxytryptamine, noradrenaline, and bradykinin in dog internal carotid arteries reportedly are different from those in the external carotid arteries, it would be intriguing to determine whether these opthalmic arteries respond similarly to histamine. Therefore, the present study was undertaken to compare the responses of EOA and IOA isolated from the same dogs to histamine and to characterize the mechanism of histamine's action regarding the histaminergic receptor subtype and vascular endothelium.

**MATERIALS AND METHODS**

All experimental procedures that used animals conformed to the ARVO Resolution on the Use of Animals in Research. Mongrel dogs of either sex, weighing 8 to 14 kg, were anesthetized with intravenous injections of sodium pentobarbital (30 mg/kg) and killed by bleeding from the carotid arteries. The eyeballs, attached with optic nerves and extraocular tissues, were rapidly removed from the orbital cavities. EOA (0.4–0.7 mm outer diameter) and IOA (0.3–0.6 mm outer diameter) were isolated. Both arteries were cut into helical strips approximately 10 mm long. Cross-sectional areas of the strips were calculated from the length and weight of the strips (density weight = 1). The specimens were histologically verified by AgNO3 staining. The endothelium of arterial strips was removed by gently rubbing the intimal surface with a cotton pellet. Removal of the endothelium was histologically verified by AgNO3 staining.

The results shown in the text and in the figures are expressed as mean values ± SEM. Statistical analyses were made using Student's paired and unpaired t-tests for two groups and Tukey's method after one-way analysis of variance for more than three groups. Drugs used were histamine hydrochloride (Kanto Chemical, Tokyo); d-chlorpheniramine maleate (Scherings, Kenilworth, NJ); cimetidine (Fujisawa Co., Osaka, Japan); indomethacin (Sigma Chemical Co., St. Louis, MO); N5-nitro-L-arginine (L-NA); substance P (Peptide Institute Inc., Minoh, Japan); PGF2α, EP2, D2, A2, and sTXA2 (9,11-epithio-11,12-methano thromboxane A2; Ono Co., Osaka); beraprost sodium (Toray-Kaken Co., Tokyo); tranylcypromine hydrochloride (Nakarai Chemicals Ltd., Kyoto, Japan); dl-norepinephrine hydrochloride (Sankyo Co., Tokyo); and papaverine hydrochloride (Dainippon Co., Osaka).
RESULTS

Endothelial Integrity of the Strips
The intimal surface in the intact and rubbed artery strips was histologically examined by silver staining. The typical example in an EOA is shown in Figure 1. The histologic endothelial integrity was clearly differentiated in the intact and rubbed strips. Similar results were obtained in the IOA strips. Figure 2 illustrates endothelial function determined by substance P (10^{-7} mol/l) in the EOA and IOA treated with indomethacin (10^{-5} mol/l). Substance P-induced relaxations in the EOA with and without the endothelium were 71.4 ± 4.0% and 0.4 ± 0.4% relative to those induced by 10^{-4} mol/l papaverine (P < 0.001; n = 8), respectively; those in the IOA were 62.4 ± 8.5% and 1.8 ± 1.8% (P < 0.001; n = 8), respectively. In the endothelium-intact EOA and IOA, the responses were markedly suppressed by treatment with indomethacin plus 10^{-5} mol/l L-NA, a nitric oxide (NO) synthase inhibitor (45.7 ± 8.1% inhibition of control in EOA, P < 0.001, n = 8; and 45.0 ± 13.5% inhibition in IOA, P < 0.005, n = 7, respectively).

Modification by Endothelial Denudation of the Response to Histamine
In helical strips of EOA and IOA, the addition of histamine in concentrations ranging from 2 × 10^{-8} to 10^{-5} mol/l produced a concentration-related relaxation. The response of EOA was significantly suppressed, but not abolished, by removing the endothelium (Fig. 2, left), whereas the response of IOA was not influenced (Fig. 2, right). Quantitative data are summarized in Figure 3.

![FIGURE 1. Silver staining of intact (left) and rubbed (right) external ophthalmic artery strips. The strips were stained with a method by Abrol et al.\textsuperscript{16} (Bar = 10 µm.)](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933395/)

![FIGURE 2. Typical recordings of response to substance P (SP 10^{-7} mol/l; under treatment with 10^{-5} mol/l indomethacin) and histamine (H) of dog external (left) and internal (right) ophthalmic artery strips with (upper) and without (lower) the endothelium partially contracted with prostaglandin F\textsubscript{2α}. The strips were obtained from the same dog. Concentrations of histamine (1 to 5), 2 × 10^{-8}, 2 × 10^{-7}, 5 × 10^{-7}, 2 × 10^{-6}, and 2 × 10^{-5} mol/l, respectively; PA indicates 10^{-4} mol/l papaverine.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933395/)
Responses of EOA to Histamine

In EOA strips with the endothelium, histamine-induced relaxations in 24 of 28 EOA and contractions in the remaining four. The contractile response was abolished by treatment with \(10^{-6}\) mol/l chlorpheniramine. The relaxant response was significantly attenuated by treatment with \(10^{-6}\) mol/l chlorpheniramine (Fig. 4, left) or \(10^{-5}\) mol/l cimetidine (Fig. 4, right) and abolished by their combined treatment. Histamine-induced relaxations were not inhibited by treatment with \(10^{-5}\) mol/l L-NA. The mean relaxations at \(5 \times 10^{-7}\) mol/l histamine in control and L-NA-treated strips were 28.1 ± 9.7% and 25.2 ± 13.2%, respectively, and the mean relaxations at \(2 \times 10^{-6}\) mol/l were 55.6 ± 13.3% and 53.4 ± 14.1% (n = 5), respectively. Treatment with \(10^{-4}\) mol/l indomethacin significantly reduced the relaxation in the strips with the intact endothelium (Fig. 5, left). The remaining relaxation was not influenced by additional treatment with chlorpheniramine, but was abolished by cimetidine. Treatment with \(10^{-4}\) mol/l tranylcypromine markedly inhibited the relaxant response to histamine of the endothelium-intact strips (Fig. 6). On the other hand, the response of the strips without the endothelium was not...
Histamine-Induced Relaxation in Ophthalmic Artery

Responses of IOA to Histamine

In IOA strips, histamine did not produce contractions. Treatment with chlorpheniramine did not significantly alter the relaxant response of IOA to histamine, but additional treatment with cimetidine abolished the response (Fig. 8, left). The histamine-induced relaxation almost was abolished by cimetidine alone (Fig. 8, middle), but were not significantly altered by treatment with indomethacin and indomethacin plus chlorpheniramine (Fig. 8, right).

DISCUSSION

The present study demonstrated that mechanisms of the histamine-induced relaxation differed in the dog
DOG INTERNAL OPHTHALMIC ARTERY Endothelium (+)

FIGURE 8. Modification by chlorpheniramine (Chlor.; 10⁻⁶ mol/l) and chlorpheniramine plus cimetidine (Cim.; 10⁻⁵ mol/l, left), by cimetidine and cimetidine plus chlorpheniramine (middle), and by 10⁻⁶ mol/l indomethacin (IM) and indomethacin plus chlorpheniramine (right) of the response to histamine in dog internal ophthalmic artery strips. Relaxations induced by 10⁻⁴ mol/l papaverine were taken as 100%; mean absolute values in control strips and those treated with chlorpheniramine and chlorpheniramine plus cimetidine in the left panel were 237 ± 19 mg, 233 ± 17 mg (n = 8), and 226 ± 14 mg (n = 6), respectively. Those in control and those treated with cimetidine and cimetidine plus chlorpheniramine in the middle panel were 179 ± 25 mg, 186 ± 37 mg, and 211 ± 25 mg (n = 5), respectively. Those in control and those treated with indomethacin and indomethacin plus chlorpheniramine in the right panel were 239 ± 10 mg, 239 ± 22 mg (n = 14), and 254 ± 21 mg (n = 7), respectively. Significantly different from control, *P < 0.01; **P < 0.05. Significantly different from the value in the arteries treated with chlorpheniramine, °P < 0.01.

EOA and IOA. In the EOA, the amine-induced relaxation was partially attenuated by treatment with chlorpheniramine or cimetidine and was abolished by the combined treatment, as seen in monkey basilar, middle cerebral, and coronary arteries, and in dog mesenteric and gastroepiploic arteries. In contrast, the amine-induced relaxation in the IOA was not significantly affected by chlorpheniramine, but was markedly suppressed by cimetidine alone. Similar results were observed in monkey temporal arteries, dog distal middle cerebral arteries, and in dog coronary and renal arteries. These findings suggest that the histamine-induced relaxation is mediated by both H₁ and H₂ receptor subtypes in the EOA, but by only the H₂ receptor subtype in the IOA.

Removal of the endothelium significantly suppressed the relaxant response of EOA to histamine, as reported in dog mesenteric arteries, rat thoracic aorta, and guinea pig pulmonary arteries but not the response of the IOA. Therefore, the EOA response partially depends on the endothelium, whereas the response of the IOA is endothelium independent. Two different mechanisms that underlie the endothelium-dependent relaxation induced by histamine have been reported: the mediation by EDRF seen in the human coronary artery and umbilical blood vessel and the involvement of PGI₂ in the dog mesenteric and gastroepiploic arteries. In the present study, treatment with indomethacin significantly reduced the response of the EOA to histamine. However, this effect was observed only when the endothelium was not damaged. The remaining relaxation in the indomethacin-treated strips was not influenced by additional chlorpheniramine, but was abolished by cimetidine. In addition, the relaxant response of the EOA with the damaged endothelium was eliminated by treatment with cimetidine alone. Magnitudes of the inhibition of the amine-induced relaxation by indomethacin or chlorpheniramine in the intact EOA strips were similar to that caused by endothelium denudation (Figs. 3, 4, and 5 left). However, treatment with L-NA in a concentration (10⁻⁵ mol/l) sufficient to inhibit the production and release of EDRF did not alter the response, whereas the NO synthase inhibitor suppressed the response to substance P.

In EOA strips, a PGI₂ analog and PGE₂ produced a relaxation, whereas the other cyclooxygenase products, including PGA₂, PGD₂, PGF₂α, and TXA₂ analog,
elicited no response or a contraction, as seen in dog cerebral artery. In addition, the relaxant response to histamine was attenuated by tranilcmypromine, a PGI\textsubscript{2} synthetase inhibitor. These findings strongly suggest that the histamine-induced relaxation in the EOA is associated with the release of PGI\textsubscript{2}, but not EDRF, through activation of H\textsubscript{1} receptor subtype in the endothelium and with direct action on H\textsubscript{2} receptor in the smooth muscle. Similar results have been reported in dog mesenteric and human pulmonary arteries. On the other hand, the relaxant response to histamine in the IOA was not inhibited by treatment with indomethacin, despite the presence of endothelium, suggesting that the response is not mediated by the release of vasodilator PGs. The number of mast cells, a major source of endogenous histamine, increased around areas of recent thrombosis and atheromatous arteries. Endothelial-dependent mechanisms would be impaired under pathologic conditions, such as hypertension, atherosclerosis, etc. Human coronary conduit arteries respond to histamine with relaxations when the endothelial function is reserved, but respond with contractions when the endothelium is damaged, suggesting that amine acts as a coronary vasospastic substance. This is not the case for the ophthalmic arteries, because histamine-induced contractions in dog EOA and IOA—if they occurred at all—were only slight, and only relaxations were observed, even when the endothelium was denuded. PGI\textsubscript{2} released intraluminally from large arteries would contribute to dilatation of downstream arteries and arterioles and to inhibition in platelet aggregation and adhesion.

Differences in the mechanism of histamine-induced relaxation in the two ophthalmic arteries used may be associated with differential origins of the arteries—that is, the internal carotid artery for IOA and the external carotid artery for EOA. In the dog distal middle cerebral artery that shares the same origin with the IOA, the relaxant response to histamine is abolished by treatment with cimetidine, as seen in the IOA. However, the response of superficial temporal arteries, a branch of the external carotid artery, is endothelium-independent and is abolished by cimetidine. Vascular responsiveness to the agents is not necessarily identical, even if the vessels originated from the same trunk.

The present studies revealed different mechanisms of histamine action in the EOA and IOA. Ophthalmic blood flow may be controlled by the arteries that have the ability to respond differently to endogenous chemical substances, such as histamine (this study) and nicotine (unpublished data). Furthermore, our data clearly indicate that endothelial cells play an important role in the relaxation of vascular smooth muscle by liberating PGI\textsubscript{2} (with histamine in EOA) or EDRF (with substance P in EOA and IOA), even in the very small arteries.

**Key Words**

dog, endothelium, external and internal ophthalmic arteries, histamine, prostaglandins.

**References**


