Mechanism of Histamine Actions in Human Coronary Arteries

Noboru Toda

Helical strips of human coronary arteries contracted in response to histamine concentration dependently, they relaxed with low concentrations and contracted with high concentrations. Treatment with cimetidine potentiated contraction in the strips with intact and damaged endothelium to a similar extent and attenuated relaxation. Removal of endothelium abolished relaxation and potentiated contraction in the cimetidine-treated strips. Methylene blue increased the contractile response to histamine in the strips with endothelium but did not alter the response in the damaged-endothelium strips. Histamine-induced relaxations in the intact strips were suppressed or abolished by treatment with ETYA, AA861, a lipooxygenase inhibitor, and by chlorpheniramine but were unaffected by indomethacin. Chlorpheniramine also abolished amine-induced contraction. It may be concluded that histamine-induced contraction in human coronary arteries is mediated by H₁ receptors in smooth muscle, and relaxation is mediated by H₂ receptors in smooth muscle and H₁ receptors in endothelium. Also, stimulation of the endothelial H₁ receptor liberates vasodilator substance and possibly activates smooth muscle guanylate cyclase to accumulate cellular cyclic guanosine monophosphate. (Circulation Research 1987;61:280–286)

Responsiveness to histamine differs considerably in a variety of blood vessels from different animal species. In response to histamine, dog mesenteric, gastroepiploic, and renal arteries relax, but dog cerebral arteries of the proximal portion, pulmonary and mesenteric arteries, and pulmonary and portal veins constrict. Coronary arteries from monkeys and dogs respond to the amine with relaxations, while human coronary arteries respond with contractions. Histamine constricts proximal middle cerebral arteries from dogs but dilates the distal arteries. These differing actions may be derived from the ability of histamine to activate histaminergic H₁ and H₂ receptors in vascular smooth muscle and to also activate H₁ receptors in endothelium, which possibly mediate the release of prostaglandin (PG) I₂ or endothelium-derived relaxing factor.

Intravenous injections of histamine provokes coronary vasospasm in patients with variant angina, and endogenous histamine is regarded as one candidate for the genesis of the vasospasm. Large amount of histamine may be released by antigen–antibody reactions as well as by many clinical drugs commonly used, such as morphine and d-tubocurarine. Similar coronary vasospasm is also produced by the amine in miniature swine in which the coronary artery is denuded and the high cholesterol diet is fed. However, the mechanism of histamine action in human coronary arteries has not yet been determined.

Therefore, the present study was undertaken to further clarify the response to histamine of coronary arteries isolated from human cadavers and to pharmacologically analyze the mechanism of its action in relation to vascular endothelium and H₁ and H₂ receptors.

Materials and Methods

Ventral, interventricular, and circumflex branches of the left coronary artery were isolated from human hearts (17 males, aged 54 to 84, and 9 females, aged 26 to 68) during autopsy up to 6 hours after death. Causes of death were stomach, rectum, lung, pharyngeal, tongue, cystic, and gall bladder cancers, liver cirrhosis, and stroke. Within 6 hours after death, both the human coronary artery contraction caused by high K⁺, acetylcholine, and norepinephrine and the function of human and monkey coronary artery endothelium are well retained. Patients treated with drugs, such as H₁ and H₂ receptor–blocking agents and aspirin, that might interfere with the response to histamine of coronary arteries were excluded. Medium-sized arteries of 1–2-mm o.d. were removed from the heart. The arteries were cleaned and helically cut into strips approximately 20 mm long. The specimen was vertically fixed between hooks in a muscle bath containing modified Locke-Ringer solution, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O₂–5% CO₂. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 2.0 g, which is optimal for inducing the maximum contraction. Constituents of the solution were as follows (in mM): NaCl 120, KCl 5.4, NaHCO₃ 25.0, CaCl₂ 2.2, MgCl₂ 1.0, and dextrose 5.6. The pH was 7.35–7.41. Before the experiments began, the prepa-
Contractions induced by 30 mM K⁺ were obtained first. Responses to agonists were obtained under resting conditions or in arterial strips partially contracted with prostaglandin F₂α, the contraction being in a range between 20–35% of the contraction induced by 30 mM K⁺. Contractions induced by agonists were presented as values relative to those induced by 30 mM K⁺, and relaxations were presented as values relative to those induced by 10⁻⁴ M papaverine. Cumulative concentration-response curves for agonists were obtained by adding the drugs directly to the bathing media. Preparations had been treated for 20–30 minutes with blocking agents before the concentration-response curve for agonists was obtained. Endothelium was removed by gently rubbing the intimal surface with cotton pellets. The endothelial function was determined by testing the relaxant response to substance P. The endothelium was also determined by the silver staining procedure. Modification of the response to histamine by removal of endothelium was investigated in coronary arteries isolated from the same human cadavers.

The results shown in the text, table, and figures are expressed as mean values ± SEM. Statistical analyses were made using the Student’s t test for paired and unpaired observations. Drugs used were histamine dihydrochloride (Kanto Chemical Co., Inc., Tokyo), d-chlorpheniramine maleate (Shionogi & Coop., Ltd., Osaka, Japan), cimetidine (Fujisawa Pharmaceutical Co., Osaka), indomethacin (Sigma Chemical Co., St. Louis, Mo.), methylene blue (Nakarai Chemicals, Ltd., Kyoto, Japan), AA861 (2,3,5-trimethyl-6-[12-hydroxy-5,10-dodecadiynyl]-1,4-benzo-quinone; Takesa Chemicals Industries, Ltd., Osaka), ETYA F₂α (Ono Pharmaceutical Co., Osaka), substance P (Protein Research Foundation, Osaka), and papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka).

Results

The addition of histamine (10⁻⁷–5 × 10⁻⁵ M) produced a concentration-dependent contraction in 33 (from 18 cadavers) of 53 human coronary arterial strips. In the remaining 20 strips (from 8 cadavers), histamine in low concentrations (2 × 10⁻⁶–5 × 10⁻⁷ M) elicited relaxations from the resting level when some active tone spontaneously developed (n = 6) or from the level partially contracted with PGF₂α (n = 14). Removal of endothelium from strips obtained from the same heart abolished or markedly suppressed the amine-induced relaxation. Relaxations induced by 10⁻⁷ M substance P (62.1 ± 4.9%, n = 18, relative to those induced by 10⁻⁴ M papaverine) in arteries partially contracted with PGF₂α were abolished by removal of endothelium. Contractions induced by 30 mM K⁺ in arterial strips with and without endothelium were 1,809 ± 152 and 1,491 ± 159 mg, respectively, which was not significantly different (n = 33, paired comparison). In arteries from which the endothelium was removed, histamine-induced contractions were significantly potentiated when compared as relative values to 30 mM K⁺-induced contractions. Quantitative data are summarized in Figure 1. Mean values of the apparent median effective concentration (EC₅₀) of histamine in the arteries with and without endothelium were [3.01 ± 0.79] (n = 18) and [0.87 ± 0.26] × 10⁻⁶ M (n = 18), respectively, the difference being statistically significant (p < 0.02). Amin-induced contractions were markedly suppressed or reversed to relaxations by treatment with 10⁻⁶ M chlorpheniramine (n = 7), as observed in a previous study.⁴

Contractile responses to histamine in concentrations up to 10⁻⁵ M were reproducible in control media after the third series of experiments. Therefore, the third concentration-response curve was taken as a control, and antagonists were added before the fourth curve was obtained, when responses to the amine before and after treatment with pharmacologic antagonists were compared in the same preparations. Treatment with 10⁻⁷ M cimetidine significantly potentiated the contractile
response to histamine in the arteries with and without endothelium (Figure 2). In 11 arterial strips with intact endothelium that showed amine-induced relaxations, cimetidine partially attenuated relaxation (Figure 3). Mean values of relaxations at $10^{-7}$ M histamine before and after the H$_2$ antagonist were 58.2 and 34.2%, respectively, of papaverine-induced relaxations, and those at $5 \times 10^{-5}$ M histamine were 79.7 and 56.6%, respectively (Table 1). Histamine-induced relaxation in cimetidine-containing media was reproducible following repeated rinsing with drug-free media.

In cimetidine-treated preparations with both intact and damaged endothelium obtained from the same hearts, the effect of methylene blue was compared in a concentration of $10^{-5}$ M in which the 30 mM K$^+$-induced contraction was not influenced, and the relaxant response to acetylcholine, but not histamine$^5$ and papaverine, was suppressed in dog coronary arteries (unpublished data). This concentration is also reported to inhibit the cyclic guanosine monophosphate (cGMP) accumulation caused by acetylcholine in bovine intrapulmonary arteries.$^{20}$ The addition of methylene blue contracted control and rubbed strips by 166±37 and 94±22 mg ($n=7$), respectively. Histamine-induced contraction in control arteries was significantly potentiated by treatment with methylene blue, while the response was not influenced in arteries with damaged endothelium (Figures 3 and 4). In 3 strips treated with cimetidine in which relaxation was elicited by low concentrations of histamine in the presence of endothelium, relaxation was abolished or reversed to a contraction by treatment with methylene blue (Table 1), and contraction caused by high concentrations of the amine was markedly potentiated (Figure 3). Treatment with AA861, a lipoxygenase inhibitor,$^{21}$ in a concentration of $10^{-3}$ M, which was sufficient to
markedly suppress relaxation of dog coronary arteries caused by acetylcholine \((n=7)\) but not relaxation caused by nitroglycerin \((n=6)\) (N. Toda, unpublished data) and histamine,\(^5\) abolished histamine-induced relaxation or reversed it to contraction in 3 out of 3 strips with endothelium that was exposed to cimetidine-containing media (Table 1). The contraction induced by high concentrations of histamine in the arterial strips with and without endothelium was not potentiated by \(10^{-5}\) M AA861 but rather was attenuated \((n=4)\). Contractions due to \(K^+\) in human (present study) and porcine coronary arteries (N. Toda, unpublished data) were moderately inhibited by this concentration of AA861, which suggests a nonspecific impairment in contractility. In all 4 arterial strips with endothelium in which relaxation was induced by low concentrations of histamine in the presence of cimetidine, relaxation was abolished almost completely by \(10^{-3}\) M ETYA (Figure 5 and Table 1), which suppressed acetylcholine-induced relaxation but did not alter relaxation caused by nitroglycerin in dog coronary arteries (N. Toda, unpublished data). Treatment with \(10^{-6}\) M chlorpheniramine abolished relaxation induced by histamine up to \(5 \times 10^{-7}\) M in 5 out of 5 strips with endothelium treated with cimetidine (Table 1) and suppressed contraction by the amine or reversed it to relaxation (Figure 6). The concentrations of chlorpheniramine and cimetidine used in the present study did not significantly attenuate the contractile response to \(30\) mM \(K^+\) in 3 human coronary arteries or the relaxant response to papaverine \((10^{-6} \text{ to } 10^{-4}, n=3)\). Treatment with \(10^{-6}\) M indomethacin did not significantly alter the contractile response to histamine in the arteries with endothelium \((n=10)\) or affect relaxation in response to low concentrations of histamine (Figure 5 and Table 1).

**Discussion**

In human coronary arterial strips, histamine produced a concentration-dependent contraction, or a relaxation in low concentrations (up to \(5 \times 10^{-7}\) M) and a contraction in the higher concentrations, while isolated monkey and dog coronary arteries respond to the amine only with a relaxation.\(^1,4,5\) The contractile response to histamine was markedly suppressed or abolished by chlorpheniramine, an \(H_1\) antagonist. The

### Table 1. Modification by Blocking Agents of Relaxation Induced by Histamine in Coronary Arterial Strips With Intact Endothelium

<table>
<thead>
<tr>
<th>Blockers</th>
<th>(10^{-7}) M histamine</th>
<th>(5 \times 10^{-7}) M histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Control</td>
</tr>
<tr>
<td>Cimetidine (10^{-5}) M</td>
<td>11</td>
<td>-34.2±5.7†</td>
</tr>
<tr>
<td>Methylene Blue (10^{-5}) M</td>
<td>3</td>
<td>+8.0±3.3§</td>
</tr>
<tr>
<td>AA861 (10^{-5}) M</td>
<td>3</td>
<td>-5.6±5.2§</td>
</tr>
<tr>
<td>ETYA (10^{-5}) M</td>
<td>4</td>
<td>-2.0±1.1§</td>
</tr>
<tr>
<td>Indomethacin (10^{-6}) M</td>
<td>4</td>
<td>-48.0±5.5</td>
</tr>
<tr>
<td>Chlorpheniramine (10^{-6}) M</td>
<td>5</td>
<td>-1.2±1.2‡</td>
</tr>
</tbody>
</table>

Data, except for preparations with cimetidine, were obtained from preparations exposed to \(10^{-5}\) M cimetidine-containing media.

*Relaxations induced by \(10^{-4}\) M papaverine and contractions induced by \(30\) mM \(K^+\) were taken as 100% relaxation and contraction, respectively; —, relaxation; +, contraction; \(n\), number of preparations used; †, significantly different from control, \(p<0.01\); ‡, \(p<0.02\); §, \(p<0.05\).
relaxation was attenuated and the contraction was potentiated by treatment with cimetidine in the strips with and without endothelium to a similar extent. These findings suggest that the contraction is mediated by histaminergic H₂ receptors, while H₁ receptors located in smooth muscle are involved in the amine-induced relaxation. Similar conclusions have been drawn regarding the pulmonary vasculature of guinea pigs and dogs and isolated dog arteries.

Removal of endothelium abolished the relaxant response of human coronary arteries to substance P or low concentrations of histamine and, by comparison as relative values to 30 mM K⁺-induced contractions, potentiated the contraction induced by histamine. Treatment with indomethacin did not potentiate the contraction or attenuate the relaxation. The concentration of this inhibitor is approximately 10 times as high as that sufficient to abolish a possible release of PGI₂ from dog renal arteries stimulated by angiotensin II. Release of PGI₂ from endothelium in human coronary arteries is, if any, minimal, but plays an important role in causing a relaxation in dog mesenteric and gastroepiploic arteries in response to histamine. Treatment with methylene blue significantly potentiated the amine-induced contraction in the coronary arterial strips treated with cimetidine only when the endothelium was not damaged, and abolished the relaxation induced by low concentrations of histamine. Nonspecific increments in the arterial contraction could be excluded since the responses to histamine were compared in paired strips with intact and damaged endothelium. Methylene blue, a guanylate cyclase inhibitor, is postulated to interfere with the action of substances that liberate relaxing factor from endothelium by inhibiting the synthesis of cGMP. ETYA, an inhibitor of lipoxygenase and cyclooxygenase, suppressed the histamine-induced relaxation in the presence of cimetidine, while a cyclooxygenase inhibitor, indomethacin, was ineffective. Treatment with AA861, a selective 5-lipoxygenase inhibitor, reversed the histamine-induced relaxation to a contraction in the cimetidine-treated strips. This inhibitor, in a concentration of 10⁻⁵ M, decreases the lipoxygenase activity from guinea pig peritoneal leukocytes by about 90% and suppresses the endothelium-dependent relaxation by acetylcholine in dog coronary arteries, while the relaxation induced accumulation of cellular cGMP. ETYA, an inhibitor of lipoxygenase and cyclooxygenase, suppressed the histamine-induced relaxation in the presence of cimetidine, while a cyclooxygenase inhibitor, indomethacin, was ineffective. Treatment with AA861, a selective 5-lipoxygenase inhibitor, reversed the histamine-induced relaxation to a contraction in the cimetidine-treated strips.

**Figure 5.** Responses to histamine of human coronary arterial strip with endothelium in control media and those containing 10⁻¹ M cimetidine (Cim.), cimetidine plus 10⁻⁴ M indomethacin (Indometh.), and cimetidine plus 10⁻³ M ETYA. Preparation was partially contracted with 10⁻³ M PGF₂α; level prior to addition of PGF₂α is shown as horizontal line just left of each tracing. In upper left insert is relaxation by 10⁻² M substance P (Sub. P) in this preparation contracted with 30 mM K⁺. Concentrations of histamine from 1 to 4 = 2 x 10⁻⁴, 10⁻⁵, 5 x 10⁻⁶, and 2 x 10⁻⁷ M, respectively; PA = 10⁻⁴ M papaverine.

**Figure 6.** Responses to histamine of human coronary arterial strip with endothelium exposed to 10⁻¹ M cimetidine-containing media, before and after treatment with 10⁻⁴ M chlorpheniramine. Preparation was partially contracted with 2 x 10⁻³ M PGF₂α. Concentrations of histamine from 1 to 5 = 2 x 10⁻⁴, 10⁻⁵, 5 x 10⁻⁶, 2 x 10⁻⁷, and 10⁻⁸ M, respectively; PA = 10⁻⁴ M papaverine.
Histamine on Human Coronary Artery

by nitroglycerin and histamine, independent of endothelium, in the dog arteries is not attenuated (Toda\textsuperscript{3} and unpublished data). The scavenging action is not seen with the concentration used, and the antioxidant action is not evident as compared with its action on 5-lipoxygenase activity (Y. Maki, personal communication; Toda\textsuperscript{3}). Furthermore, relaxations induced by low concentrations of histamine in cimetidine-treated preparations with endothelium were suppressed by treatment with chlorpheniramine, as seen in Figure 6 and Table 1. These findings may support the hypothesis that histamine stimulates endothelial H\textsubscript{1} receptors and activates phospholipase A\textsubscript{2}, which produces vasodilator substance from arachidonic acid by a catalysis of lipoxygenase. The substance liberated from endothelium appears to activate smooth muscle guanylate cyclase and accumulate cellular cGMP, which results in the relaxation of human coronary arteries. Further study on biochemical determination of enzyme activation and reaction products is involved to improve the validity of this hypothesis.

Evidence supporting the idea that vasoconstrictor substance is liberated from endothelium of human coronary arteries stimulated by histamine was not obtained in the present study. In the human artery, histamine appears to act on H\textsubscript{1} receptors in endothelium and produce relaxing factor(s), and on H\textsubscript{2} and H\textsubscript{3} receptors in smooth muscle, which elicit contractions and relaxations, respectively (Figure 7). The amine that relaxes monkey coronary arteries is also expected to act on these sites\textsuperscript{2}; however, the involvement of these actions quantitatively differs from that in the human arteries in which the contractile response mediated by smooth muscle H\textsubscript{2} receptors is appreciably predominant. Although dog coronary arteries respond to histamine with relaxations as do monkey arteries, the response is mediated exclusively by smooth muscle H\textsubscript{2} receptors.\textsuperscript{3}

Histamine-induced contractions in human conduit coronary arteries were potentiated by impairment of endothelium and blockade of H\textsubscript{2} receptors. This may be the case in the human arteries in vivo, as observed in in situ swine coronary arteries.\textsuperscript{15} In contrast, contractions of human coronary arteries induced by acetylcholine and norepinephrine did not significantly differ in intact and the endothelium-damaged strips.\textsuperscript{4,12} Histamine in plasma is increased by antigen–antibody reactions and by some therapeutic agents and endogenous substances, including basic polypeptides, alcaloids, and others,\textsuperscript{14} and mast cells, a major source of endogenous histamine, are increased around zones of recent thrombosis and atheromatous arteries and in edematous areas of the vascular wall.\textsuperscript{24,25} Endogenous histamine, like exogenously applied histamine,\textsuperscript{15} may be one of the key substances responsible for the localized coronary vasospasm.

**Acknowledgments**

The authors thank Drs. T. Okamura, I. Shimizu, and B. Ka for their excellent technical assistance.

**References**

3. Toshimitsu Y, Uchida K, Kojima S, Shimo Y: Histamine responses mediated via H\textsubscript{1} and H\textsubscript{2} receptors in the isolated portal vein of the dog. J Pharm Pharmacol 1983;36:404–405

![Figure 7. Schematic presentation of possible mechanisms of histamine action in human coronary arteries. Squares in endothelium and smooth muscle represent histaminergic H\textsubscript{1} and H\textsubscript{2} and relaxing factor receptors. Possible responses mediated by different sites of action, 1 to 3 in right side, are demonstrated in the left side. Heavy line represents observed response to histamine. RF, relaxing factor.](http://circres.ahajournals.org/content/58/2/285.full)

KEY WORDS • human coronary artery • histamine • endothelium-dependent relaxation • histamine H1 and H2 receptors
Mechanism of histamine actions in human coronary arteries.

N Toda

Circ Res. 1987;61:280-286
doi: 10.1161/01.RES.61.2.280

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/61/2/280