Participation of H_1-Receptors in Histamine-Induced Contraction and Relaxation of Horse Coronary Artery *In Vitro*

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ABSTRACT. The mechanisms of histamine-induced contraction and relaxation were investigated in rings isolated from a middle part of the left descending coronary arteries of horses. Intact and endothelium-denuded preparations were compared. Rings of horse coronary arteries contracted in response to histamine in a concentration dependent manner, but some of them relaxed with lower concentrations and contracted with higher concentrations. Removal of the endothelium abolished the relaxation and potentiated the contraction. The pD_2 values were 4.70±0.08 in the rings with intact endothelium and 4.95±0.08 in endothelium-denuded rings. Histamine-induced contractions in intact and denuded preparations were not affected by an H_2-antagonist, cimetidine, but were inhibited by an H_1-antagonist, diphenhydramine in non-competitive manner in the rings with endothelium and in competitive manner in denuded rings. After precontraction with $PGF_{2\alpha}$ or norepinephrine, histamine relaxed preparations with intact endothelium (pD_2 value, 7.80±0.11), although histamine-induced relaxations were not observed in denuded preparations. The relaxation was competitively inhibited by diphenhydramine. Relaxing response was significantly attenuated by methylene blue, quinacrine, L-nitro-arginine, gossypol and AA861 but not by indomethacin. These results suggest that the histamine-induced contraction and relaxation in horse coronary arteries are mediated mainly by H_1-receptors in the smooth muscle and endothelium, respectively, and H_1-receptor activation of endothelial cells may liberate vasodilator substance(s).—KEY WORDS: coronary artery, endothelium-dependent relaxation, H_1-receptor.

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Although histamine has been recognized to be one of powerful stimulants of coronary vessel in a variety of animals, responsiveness of coronary arteries to histamine differs considerably in different animal species. Coronary arteries isolated from dogs and monkeys respond to this amine with relaxation [7, 15], while those from pig, bovine and human do so with contraction [5, 11, 16, 17]. These species differences may be derived from the ability of histamine to activate histaminergic H_1- and H_2-receptors in vascular smooth muscle cells and also to activate H_1-receptors in endothelial cells, which may in turn activate the release of endothelium-derived relaxing factor (EDRF) [15, 18] or the release of prostaglandin I_2 [13, 14].

It has been well known that sudden cardiac death occurs in young, apparently healthy horses [6]. However, the cause of sudden cardiac death has not been fully clarified. Coronary artery spasm is suggested to be one of the factors in sudden cardiac death. Little is known about the pharmacological properties of the horse coronary artery. To our knowledge, histamine action on the horse coronary artery has not yet been investigated. The present study was undertaken to clarify the response to histamine of coronary arteries isolated from horse and to analyze pharmacologically the mechanisms of its action in relation to the vascular endothelium and the distribution of H_1- and H_2-receptors.

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MATERIALS AND METHODS

Measurement of mechanical activity: Coronary arteries were isolated from freshly slaughtered horse at two local slaughterhouses and transferred to our laboratory in oxygenated ice-cold physiological salt solution. The physiological salt solution was composed of (mM): NaCl 119, $CaCl_2$ 1.6, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 10.0. The middle part of the left descending coronary artery was dissected free and cleaned of adhering tissue, and two rings about 3 mm in length were cut. Each coronary ring was placed in a 15 m*l* water-jacketed organ bath filled with oxygenated physiological salt solution at 37°C (pH 7.4), mounted between two L-shaped hooks fixing the upper portion to an isometric force transducer

(Nihon Kohden Kogyo Co., Tokyo), and each bath was aerated with a mixture of 95% CO_2 and 5% O_2. The isometric tension developed was recorded on a pen-recorder (Nihon Kohden Kogyo Co., Tokyo). The resting tension was adjusted to 2 g sufficient to induce the maximum contraction.

Endothelium was removed by gently rubbing the intimal surface with a cotton swab wetted with physiological salt solution. The integrity of the endothelial cells was determined morphologically by a silver staining procedure [2] or a scanning electron microscopy (JEOL) and functionally by testing the relaxant response to 10^{-7} M bradykinin, which was abolished by the endothelium denudation. Modification of the response to histamine by the removal of endothelium was investigated in the same coronary ring or the coronary rings isolated from the same animal.

Experimental procedure: Before the start of experiments, the coronary ring was allowed to equilibrate for at least 120 min in the bathing media, during which time the bathing fluids were replaced every 15 min. The contractile response to 60 mM K^+ was first obtained. The rings were washed three times with fresh media and equilibrated for 60 min. Cumulative concentration-contractile response curves for histamine were obtained by adding histamine directly to the bathing media. To examine the cumulative concentration-relaxation curves for histamine, the rings were partially contracted with norepinephrine (10^{-6}M). After the contraction was stabilized, histamine was added cumulatively to the bathing media. In tests with antagonists, an antagonist was added to the bathing media 30 min before adding histamine. The maximum contraction or relaxation obtained with histamine alone was set as 100%, and subsequent concentration-response curves in the presence of antagonist were expressed as a percentage of this maximum in the control curve.

When a competitive antagonist was tested, the log concentration-ratio of EC_{50} values (i.e., concentration producing half-maximum response) in the absence or presence of antagonist was calculated and plotted against the logarithm of antagonist concentration to obtain pA_2 values [1]. In experiments using some metabolic inhibitors, relaxations in coronary arteries were represented as a percentage of the contraction generated by 10^{-6}M norepinephrine.

Drugs: Histamine dihydrochloride, methylene blue, indomethacin and quinacrine dihydrochloride were obtained from Nacalai tesque. Diphenhydramine hydrochloride, cimetidine, bradykinin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and gossypol acetic acid were obtained from Sigma. *dl*-Norepinephrine (Sankyo), L-nitro-arginine (Aldrich Chemical Company, Inc.) and AA861 (2, 3, 5-trimethyl-6-[12-hydroxy-5, 10-dodecadiynyl]-1, 4-benzo-quinone) (Takeda) were used. All drugs but indomethacin and AA861 were dissolved in distilled water. AA861 was dissolved in ethanol, and indomethacin was dissolved in physiological salt solution. All drugs were made fresh on the day of use. Further dilutions to the desired concentrations were made with physiological salt solution. The final concentration of ethanol (0.1%) had no effects on the resting tension and relaxing responses to agonists.

Statistical analysis: The results are expressed as mean values ±S.E.M. Statistical analysis was made using Student's *t*-test for paired and unpaired observations. P values of less than 0.05 were considered significant.

RESULTS

Contractile response to histamine: Figure 1 shows the effect of histamine on horse coronary arteries and the effect of endothelium removal. Cumulative addition of histamine produced concentration-

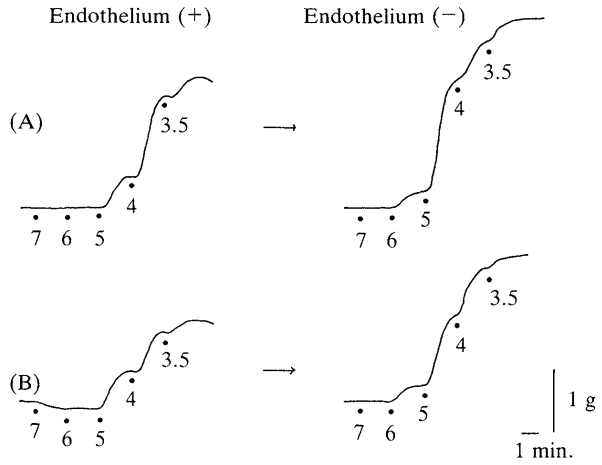

Fig. 1. Representative tracing to illustrate the effects of histamine on horse coronary arteries with and without endothelium. Some horse coronary arteries (7 out of 12 horses) with endothelium show only a contractile response to histamine (A), and the others show a weak relaxing response at relatively low concentrations (10^{-7}M and 10^{-6}M) of histamine (B). Histamine was added to the bath at points indicated; figures indicate cumulative concentration (-logM).

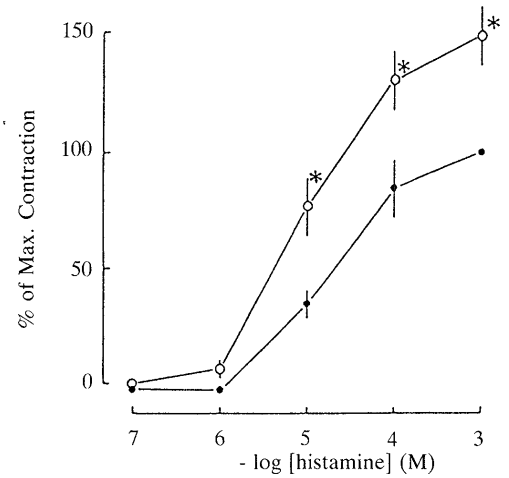

Fig. 2. Concentration-effect curves to histamine on horse coronary artery with (●) and without (○) endothelium. The histamine-induced response in artery with endothelium was estimated, then the endothelium was removed by rubbing the intimal surface, and modification of the response to histamine by the removal of endothelium was investigated. Contraction induced by 10^{-3}M histamine in arteries with endothelium was taken as 100%; mean absolute value was 1473±145 mg. The removal of the endothelium did not affect significantly the 60 mM KCl-induced contractile response. Each point represents mean values from 9 animals. Vertical bars represent S.E.M. * Significantly different from endothelium (+) ($p<0.05$, paired *t*-test).

dependent contractions ((A) in Fig. 1). In 5 out of 12 coronary arteries ((B) in Fig. 1), histamine produced a weak relaxation at 10^{-7}M and 10^{-6}M. Removal of the endothelium enhanced the contractile response and abolished the relaxation.

Figure 2 shows the concentration-response curves to histamine in horse coronary arteries with and without endothelium isolated from the same horse. The arteries without endothelium responded to histamine more strongly than those with endothelium. The pD_2 values were significantly ($P<0.05$) larger in coronary arteries without endothelium (4.95±0.08) than those with endothelium (4.70±0.08) (Table 1).

H_1- and H_2-antagonist effect on contractile response to histamine: Contractile responses to histamine in concentrations up to 10^{-4}M were reproducible in control media only after the third series of experiments. Therefore, the third concentration-response curve was taken as a control. Antagonists were added before the fourth curve was obtained, and the responses to histamine before and after

Table 1. The pD_2 values of histamine-induced contraction and relaxation in horse coronary arteries with and without endothelium

Endothelium	Histamine	pD_2
+	contraction	4.70±0.08
−	contraction	4.95±0.08[a)]
+	relaxation	7.80±0.11[b)]

Each value represents the mean value ± S.E.M. of 9 animals.
a) Significantly different from endothelium (+) ($P<0.05$).
b) Significantly different from contraction (endothelium (+) and (−)) ($P<0.001$).

treatment with antagonists were compared in the same preparation. An H_1-receptor antagonist, diphenhydramine, abolished the relaxation and shifted the concentration-response curve for histamine to the right in a non-parallel fashion in coronary arteries with endothelium, but in a parallel fashion in arteries without endothelium ((a) in Fig. 3). The calculated pA_2 value of diphenhydramine against histamine was 7.82±0.05 (n=8) without endothelium. The slope of Schild plot was 0.95±0.04, which was not significantly different from unity ((b) in Fig. 3). In coronary arteries with endothelium, however, the slope of Schild plot was 0.67±0.05, which was significantly different from unity. An H_2-receptor antagonist, cimetidine (10^{-6}M and 10^{-5}M), showed no significant influence on the histamine-induced concentration-response curve in horse coronary arteries with and without endothelium (data not shown).

Relaxation-response to histamine: The results shown in Fig. 1 suggest that histaminergic receptors are present on the endothelium. This possibility was examined in the following experiments. In the presence of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (2.8×10^{-5}M)- or norepinephrine (10^{-6}M)-induced tone, histamine (10^{-8}M- 10^{-6}M) relaxed the horse coronary arteries concentration- dependently (Fig. 4). Histamine-induced relaxation was abolished or converted to the contractile response by removing the endothelium (Fig. 4). The effect of the removal was confirmed by the absence of the bradykinin-induced relaxation and the evidence of cellular loss with no $AgNO_3$ staining and/or with the scanning electron microscopy.

H_1-and H_2-antagonist effect on histamine-induced relaxation: The pD_2 value of histamine for relaxation was 7.80±0.11, which was significantly

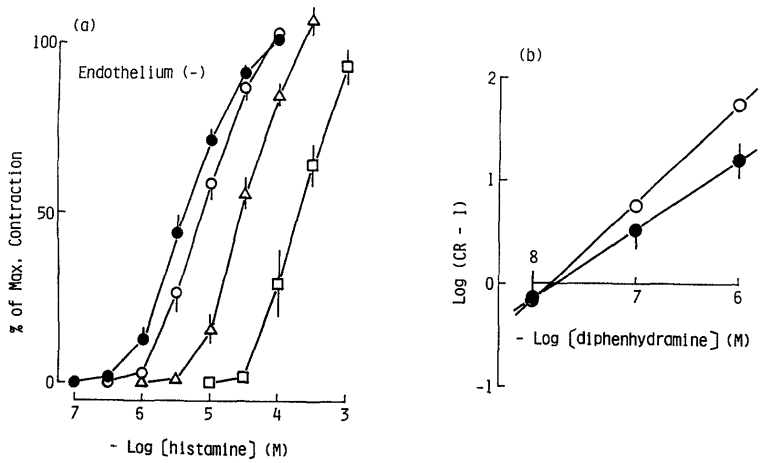

Fig. 3. Effect of diphenhydramine on the concentration-effect curves to histamine in horse coronary arteries without endothelium (●; control, diphenhydramine 10^{-8}M (○), 10^{-7}M (△), 10^{-6}M (□)) (a), and the Schild plots for antagonistic effect of diphenhydramine (●; with endothelium, ○; without endothelium) (b). Each point represents the mean values from 8 animals. Vertical bars represent S.E.M. CR; An equieffective concentration-ratio of histamine, i.e., concentration of agonist producing 50% maximal response (EC_{50}) in the presence of diphenhydramine/EC_{50} in the absence of antagonist.

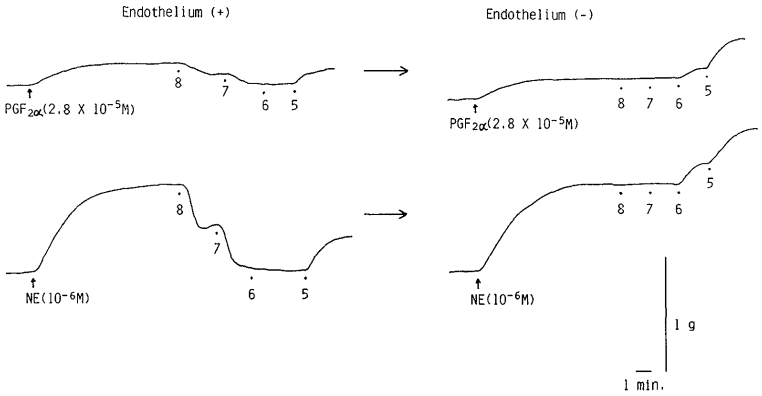

Fig. 4. Representative tracing to illustrate the histamine-induced relaxations in horse coronary artery with endothelium, and its abolition by denudation of the endothelium. Histamine was added to the bath at points indicated; figures indicate cumulative concentration (-log M). NE; norepinephrine. $PGF_{2\alpha}$; prostaglandin $F_{2\alpha}$.

($P<0.001$) greater than that for the contraction in coronary arteries with or without endothelium (Table 1). Cimetidine (10^{-7}M - 10^{-5}M) did not significantly influence histamine-induced relaxation ((a) in Fig. 5). Diphenhydramine (10^{-7}M - 10^{-5}M) blocked the histamine-induced relaxation in a concentration dependent manner ((b) in Fig. 5). The pA_2 value of diphenhydramine against histamine-induced relaxation was 8.14±0.06 (n=7), and the slope value of Schild plot was 1.05± 0.04, which was not significantly different from unity.

Endothelium-dependent mechanisms in histamine-induced relaxation: Preincubation with methylene blue (10^{-5}M), quinacrine (10^{-6}M, 10^{-5}M), AA861 (10^{-5}M), L-nitro-arginine (10^{-5}M, 10^{-4}M) and gossypol (10^{-5}M) resulted in significant attenuation of the relaxing response of histamine (Fig. 6a, b). Treatment of rings with indomethacin (10^{-5}M) showed no significant effect on the relaxing response to histamine (Fig. 6a). The treatment with

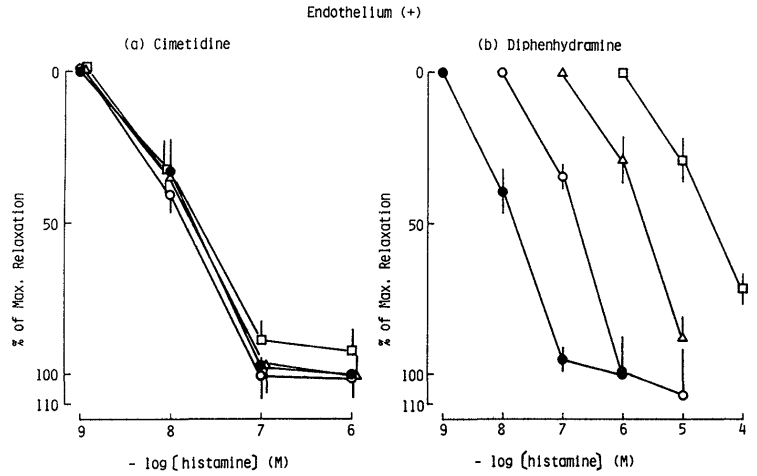

Fig. 5. Effects of cimetidine and diphenhydramine (●; control, 10^{-7}M (○), 10^{-6}M (△), 10^{-5}M (□); cimetidine (a) and diphenhydramine (b)) on histamine-induced relaxations of horse coronary arteries with endothelium precontracted by norepinephrine (10^{-6}M). The relaxation obtained with 10^{-6}M histamine alone was set as 100%. Each point represents the mean values from 9 animals. Vertical bars represent S.E.M.

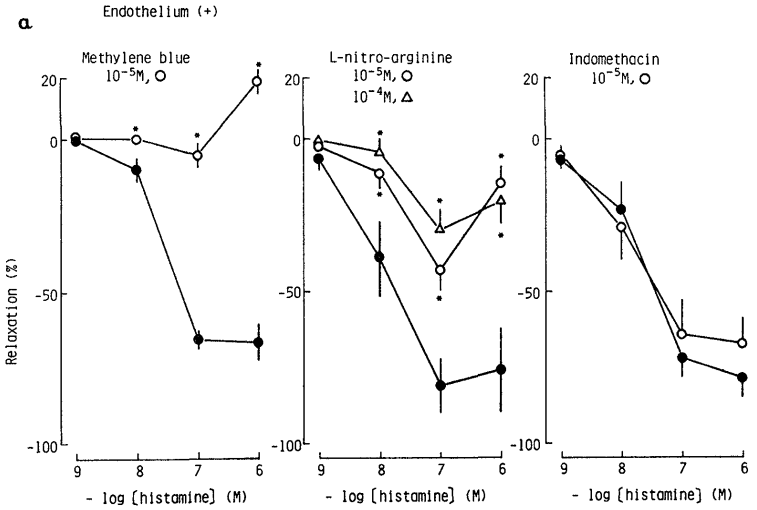

Fig. 6a. Effects of methylene blue, L-nitro-arginine and indomethacin on histamine-induced relaxations of horse coronary arteries with endothelium. Norepinephrine (10^{-6}M)-induced contraction was set as 100%. Each point represents the mean values from 4 to 8 animals. Vertical bars represent S.E.M. *Significantly different from control (●) ($p<0.05$).

methylene blue, quinacrine, AA861, L-nitro-arginine, indomethacin and gossypol did not affect the basal tone or the contractile response to 10^{-6}M norepinephrine.

DISCUSSION

The present results show that histamine has a dual effect on the horse coronary artery. Histamine at higher concentrations was capable of contracting all coronary arteries, but in some coronary arteries

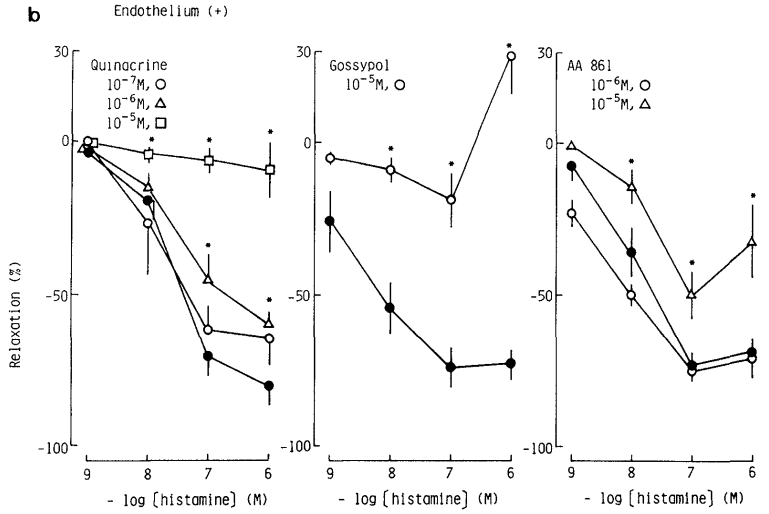

Fig. 6b. Effects of quinacrine, gossypol and AA861 on histamine-induced relaxations of horse coronary arteries with endothelium. Norepinephrine (10^{-6}M)-induced contraction was set as 100%. Each point represents the mean values from 4 to 8 animals. Vertical bars represent S.E.M. *Significantly different from control (●) ($p<0.05$).

histamine at relatively lower concentrations induced the relaxation. The removal of the endothelium from the coronary arteries completely abolished the histamine-induced relaxation, and significantly potentiated the histamine-induced contraction. These results suggest that histaminergic receptors are present in the endothelial cells of the horse coronary artery; the stimulation of these receptors releases the EDRF. At present, it has been considered that one of the EDRFs is nitric oxide [4], which is sensitive to methylene blue treatment indicating that its action was on the soluble form of guanylate cyclase [3]. One of the EDRFs in this experiment may be nitric oxide, because L-nitro-arginine [9, 10] significantly attenuated histamine-induced relaxation (Fig. 6a). EDRFs released by histamine may not contain a prostanoid compound, because pretreatment with indomethacin did not alter the histamine-induced relaxation (Fig. 6a). Quinacrine, AA861 and gossypol also significantly reduced the relaxation induced by histamine (Fig. 6b). This suggests that 5-lipoxygenase may be involved in the generation of EDRF, as suggested by Minami and Toda [8] in dog femoral artery.

In the coronary arteries without endothelium, the histamine-induced contraction was inhibited competitively by an H_1-receptor antagonist, diphenhydramine, with a pA_2 value of 7.82, which is consistent with the value from other experiment [1]. In the coronary arteries with endothelium, however, the competitive inhibition was not observed. These results suggest the presence of the vasodilator histaminergic receptors in the endothelium.

The contractile response in coronary arteries with and without endothelium was not affected by an H_2-receptor antagonist, cimetidine, indicating that H_1-receptors on the smooth muscle cells mediate the contraction in the horse coronary artery.

The histamine-induced relaxation was observed in all horse coronary arteries pre-contracted with $PGF_{2\alpha}$ or norepinephrine. This relaxation was competitively antagonized by diphenhydramine with a pA_2 value of 8.14, which was not significantly different from those in the contraction, but cimetidine showed no significant effect on it (Fig. 5). These results indicate that in the horse coronary artery histamine stimulates H_1-receptors located on the endothelial cells which consequently cause relaxation, and also stimulates H_1-receptors on the smooth muscle cells which result in the additional contraction. Thus, the response to histamine may be observed as the sum of two opposite effects via both H_1-receptors located on the endothelial cells and on the smooth muscle cells.

As the pD_2 value of histamine for relaxation was significantly greater than those for contraction (Table 1), some horse coronary arteries might show the relaxation in response to lower concentrations of histamine (Fig. 1).

The present results indicate that the response of

the horse coronary arteries to histamine is quite similar to that of the human coronary arteries [16] which show the relaxations in lower concentrations of histamine (2×10^{-8}M, 10^{-7}M) and the contractions in response to higher concentrations, while the relaxations are suppressed by the removal of the endothelium and by the H_1-receptor antagonist. Satoh and Inui [12] and Van de Voorde and Leusen [18] have shown that the histamine-induced relaxations were mediated exclusively by H_1-receptors on the endothelial cells in guinea pig pulmonary arteries and rat thoracic aortas. These results are also consistent with the present ones.

In summary, the present findings show that the horse coronary arteries contain H_1-receptors both on the endothelial cells which cause relaxation and on the smooth muscle cells which cause contraction. Activation of H_1-receptors on the endothelial cells appear to release EDRF(s).

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REFERENCES

1. Arunlakshana, O. and Schild, H. O. 1959. Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* 14: 48–58.
2. Caplan, B. A., Gerrity, R. G., and Schwartz, C. J. 1974. Endothelial cell morphology in focal areas of *in vivo* Evans Blue uptake in the young pig aorta. I. Quantitative light microscopic findings. *Exp. Mol. Pathol.* 21: 102–117.
3. Ignarro, L. J. 1989. Endothelium-derived nitric oxide: actions and properties. *Fed. Am. Soc. Exp. Biol. J.* 3: 31–36.
4. Ignarro, L. J. 1990. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu. Rev. Pharmacol. Toxicol.* 30: 535–560.
5. Ikenoue, K., Kawakita, S., and Toda, N. 1986. Vasoconstrictor responses of isolated pig coronary arteries. *Folia Pharmacol. Japon* 87: 281–290 (in Japanese with English abstract).
6. Kiryu, K., Nakamura, T., Kaneko, M., Oikawa, M., and Yoshihara, T. 1987. Cardiopathology of sudden cardiac death in the race horse. *Heart Vessels Suppl.* 2: 40–46.
7. Konishi, M., Toda, N., and Yamamoto, M. 1981. Different mechanisms of action of histamine in isolated arteries of dog. *Br. J. Pharmacol.* 74: 111–118.
8. Minami, Y. and Toda, N. 1989. Possible involvement of 5-lipoxygenase products in the generation of endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.* 250: 1055–1060.
9. Moore, P. K., al-Swayeh, O. A., Chong, N. W. S., Evancs, R. A., and Gibson, A. 1990. L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.* 99: 408–412.
10. Palmer, R. M. J., Rees, D. D., Ashton, D. S., and Moncada, S. 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.* 153: 1251–1256.
11. Ratz, P. H. and Flaim, S. F. 1984. Mechanism of 5-HT contraction in isolated bovine ventricular coronary arteries. Evidence for transient receptor-operated calcium influx channels. *Circ. Res.* 54: 135–143.
12. Satoh, H. and Inui, J. 1984. Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. *Eur. J. Pharmacol.* 97: 321–324.
13. Toda, N., Konishi, M., and Miyazaki, M. 1982. Involvement of endogenous prostaglandin I_2 in the vascular action of histamine in dogs. *J. Pharmacol. Exp. Ther.* 223: 257–262.
14. Toda, N. 1984. Endothelium-dependent relaxation induced by angiotensin II and histamine in isolated arteries of dog. *Br. J. Pharmacol.* 81: 301–307.
15. Toda, N. 1986. Mechanisms of histamine-induced relaxation in isolated monkey and dog coronary arteries. *J. Pharmacol. Exp. Ther.* 239: 529–535.
16. Toda, N. 1987. Mechanism of histamine actions in human coronary arteries. *Circ. Res.* 61: 280–286.
17. Toda, N. and Okamura, T. 1989. Endothelium-dependent and -independent responses to vasoactive substances of isolated human coronary arteries. *Am. J. Physiol.* 257: H988–H995.
18. Van de Voorde, J. and Leusen, I. 1983. Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur. J. Pharmacol.* 87: 113–120.