Vasomotor Effect of Histamine on Pig and Cattle Coronary Artery In Vitro

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ABSTRACT—Vasomotor effects of histamine were examined in isolated coronary arteries from pigs and cattle. Histamine produced a concentration-dependent contraction in these arteries. These contractile responses were dose-dependently inhibited by diphenhydramine. The slopes of the Schild plots, however, were significantly lower than unity in both species. Cimetidine potentiated the histamine-induced contractions at relatively high doses of histamine (larger than $10^{-5}$ M) in pig coronary arteries, but did not show a significant effect in cattle arteries. After the removal of endothelium, the Schild plot of diphenhydramine against histamine gave a straight line with a $pA_2$ value of 7.80 and slope of 1.00 in pigs, confirming the competitive nature of the antagonism. In cattle, the slope was significantly lower than unity; however, in the presence of cimetidine, it was not significantly different from unity. Dimaprit did not contract the cattle coronary arteries with endothelium, but contracted them after the removal of endothelium. These results suggest that histamine-induced vasoconstriction in pig and cattle coronary arteries is mainly dependent on the $H_1$-receptors in the smooth muscle cells, and that $H_1$- and $H_2$-receptors in the endothelial cells of pigs and $H_2$-receptors in the smooth muscle cells of cattle modify the histamine-induced vasoconstrictions.

Remarkable species differences have been reported concerning the responsiveness of coronary vessels to histamine in vitro. Coronary arteries from monkeys and dogs respond to histamine with relaxations (1, 2), while human, pig and cattle coronary arteries respond with contractions (3–6).

It has been postulated that the species difference might depend on the ability of histamine to activate $H_1$- and $H_2$-receptors in vascular smooth muscle and $H_1$-receptors in endothelium, which possibly mediate the release of prostaglandin I$_2$ (7, 8) or endothelium-derived relaxing factors (1, 9, 10). Little information, however, is available concerning the distribution of $H_1$- and $H_2$-receptors in pig and cattle coronary arteries.

The purpose of the present study was to characterize the subtypes of histamine receptors and to confirm whether vascular responses to histamine are involved in endothelium-dependent mechanisms or not in isolated pig and cattle coronary arteries.

MATERIALS AND METHODS

Coronary arteries from freshly slaughtered pig and cattle were obtained from a local slaughterhouse and transferred to our laboratory immersed in ice-cold physiological salt
solution (119 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl₂, 1.2 mM MgCl₂, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, and 10.0 mM glucose) aerated with a mixture of 95% O₂ and 5% CO₂. The middle part of the left descending coronary arteries was dissected free and cleaned of adhering tissue, and two rings about 3 mm in length were cut. These rings were suspended horizontally between two L-shaped hooks fixing the upper portion to an isometric force transducer (Nihon Kohden Kogyo Co.) and suspended individually in a 15 ml water-jacketed organ bath filled with oxygenated salt solution at 37°C (pH 7.4). The solution was aerated continuously with a mixture of 95% O₂ and 5% CO₂. Changes in KCl concentration in the physiological salt solution were compensated for by an equimolar adjustment of the NaCl concentration.

Rings mounted in the organ chamber were left to equilibrate for 120 min under the optimal resting tension of 1.0 g for the pig and 2.0 g for the cattle coronary arteries. KCl (60 mM) solution was applied every 30 min until the amplitude of the contraction reached a constant value. The isometric tension development was measured continuously by a force transducer.

Cumulative concentration-response curves for the histamine were obtained by adding histamine solution (0.05 ml) directly to the bathing media. In tests with antagonists, the maximum contraction obtained with histamine alone was set as 100%, and subsequent concentration-response curves in the presence of increasing concentrations of antagonists were expressed as a percentage of this maximum in the control curve. After two reproducible control curves had been obtained, the antagonist was added to the bath 30 min before the agonist. Following washout, the concentration-response curve was verified to be the same before adding a new concentration of antagonist. When a competitive antagonist was tested, the log concentration-ratio of EC₅₀ 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 pA₂ values (11).

Endothelium was removed by gently rubbing the intimal surface with a cotton swab wetted with physiological salt solution. The presence or absence of the endothelium was determined morphologically by scanning electron microscopy (JEOL) and functionally by testing the relaxant response to bradykinin (10⁻⁸ M), which was abolished by the endothelium denudation (Fig. 1). Modification of the response to histamine by the removal of

![Fig. 1. Responses to bradykinin (••, 10⁻⁸ M) of pig and cattle coronary arteries with and without endothelium. Each artery was contracted with 2–3 × 10⁻⁶ M PGF₂α; the level before the addition of PGF₂α is shown as horizontal lines just left of each tracing.](image-url)
endothelium was investigated in the same coronary ring or the coronary rings isolated from the same animal.

Drugs used were as follows: histamine dihydrochloride (Nacalai), diphenhydramine hydrochloride (Sigma), cimetidine (Sigma), 2-pyridylethylamine dihydrochloride and dimaprit dihydrochloride (Smith Kline & French), and prostaglandin F2α (Ono).

The results shown in the text, tables and figures are expressed as mean values ± S.E.M. Statistical analyses were made using Student's t-test for paired and unpaired observations. The significance was established when the probability level was equal to or less than 5%.

RESULTS

Histamine-induced vascular response

Figure 2 shows the concentration-response curves to histamine in coronary arteries isolated from pigs and cattle. Cumulative additions of histamine caused a concentration-dependent contraction in pig and cattle coronary arteries.

Table 1 shows the maximal response and pD2 values of histamine. Histamine caused significantly larger vasoconstriction in pigs than in cattle coronary arteries, amounting to 140 and 87%, respectively, of the high potassium-induced contraction. The pD2 value for pig coronary arteries was significantly larger than that for cattle coronary arteries.

H1- and H2-receptor antagonists

Figure 3 shows the effect of an H1-receptor antagonist, diphenhydramine, on histamine-induced vascular responses in pig and cattle coronary arteries. In pig and cattle coronary arteries, diphenhydramine (10⁻⁵ M to 10⁻⁶ M) blocked the histamine-induced contraction to result in a rightward shift of the concentration-response curves.

Table 2 shows the pA2 values of diphenhydramine against histamine in pig and cattle coronary arteries. The pA2 values obtained from Schild plot analysis were 7.78 and 7.63 in pig and cattle coronary arteries with endothelium, respectively. The slope values of the straight lines in each Schild plot were less than 0.1.
Fig. 3. Effects of diphenhydramine on contractile response to histamine in coronary arteries isolated from pigs and cattle. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses). Constrictions induced by $10^{-4}$ M histamine in pigs and cattle were taken as 100%; mean absolute values in arteries were $2035 \pm 95$ mg in pigs ($n = 6$) and $2825 \pm 141$ mg in cattle ($n = 8$). Control (●), diphenhydramine (○): $10^{-8}$ M, △: $10^{-7}$ M, □: $10^{-6}$ M.

Table 2. pA2 values of diphenhydramine against histamine in coronary arteries with and without endothelium

| Coronary artery | Endothelium | Diphenhydramine pA2 | Slope  | r     | N  |
|-----------------|-------------|----------------------|--------|-------|----|
| Pig             | +           | 7.78 ± 0.20          | 0.78 ± 0.06 | 0.91 | 6  |
|                 | −           | 7.80 ± 0.07          | 1.00 ± 0.03* | 0.96 | 6  |
| Cattle          | +           | 7.63 ± 0.15          | 0.68 ± 0.07 | 0.89 | 8  |
|                 | −           | 7.90 ± 0.13          | 0.73 ± 0.03 | 0.86 | 8  |

Each value represents the mean ± S.E.M. *: Not significantly different from unity ($P < 0.05$).

Removal of endothelium

Figure 4 shows the effect of an $H_2$-receptor antagonist, cimetidine, on histamine-induced vascular responses in pig and cattle coronary arteries with endothelium. Cimetidine ($10^{-7}$ M to $10^{-5}$ M), which are sufficient to block $H_2$-receptors (5), showed no significant effects on the histamine-induced contraction in cattle coronary arteries with endothelium. In pig coronary arteries with endothelium, however, cimetidine potentiated the histamine-induced contractions at relatively high concentrations of histamine ($3 \times 10^{-5}$ M and $10^{-4}$ M).

The role of the endothelium was studied in the action of histamine on pig and cattle coronary arteries. Constrictions induced by 60 mM K+ in pig and cattle coronary arteries without endothelium were not significantly different from those of the corresponding arteries with endothelium.

Figure 5 shows the effects of the removal of the endothelium on the histamine-induced contractions in pig and cattle coronary arteries. Histamine-induced contractions were markedly potentiated in the pig coronary
Fig. 4. Effects of cimetidine on contractile response to histamine in coronary arteries with endothelium isolated from pigs and cattle. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses). Contractions induced by 10⁻⁴ M histamine were taken as 100%; mean absolute values in arteries were 1843 ± 192 mg in pigs (n = 6) and 2010 ± 237 mg in cattle (n = 7). *: Significantly different from each corresponding control (P < 0.05). Control (●), cimetidine (○: 10⁻⁷ M, △: 10⁻⁶ M, □: 10⁻⁵ M).

Fig. 5. Concentration-response curves to histamine in coronary arteries isolated from pigs and cattle with and without endothelium. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses). Contractions induced by 60 mM K⁺ were taken as 100%; mean absolute values in arteries were 1985 ± 121 mg in pigs (n = 14) and 2805 ± 210 mg in cattle (n = 9). *: Significantly different from endothelium (+) (P < 0.05).
arteries. The potentiation, however, was not so clear in the cattle coronary arteries.

Table 3 shows the $pD_2$ values and maximal responses to histamine in the coronary arteries with and without endothelium. The removal of the endothelium increased the $pD_2$ value in pig and cattle coronary arteries, and it potentiated the maximal response to histamine in pigs, but not in cattle coronary arteries.

Figure 6 shows the Schild plots of diphenhydramine in contractile response to histamine in pig and cattle coronary arteries with and without endothelium. The calculated $pA_2$ values, slope values and correlation-coefficients are shown in Table 2. The removal of the endothelium increased the slope values in Schild plots in pig and cattle coronary arteries, and the value in pig coronary arteries was not different from unity. However, in cattle coronary arteries, the slope value was different from unity.

Figure 7 shows the effect of cimetidine on histamine-induced contraction in pig and cattle coronary arteries without endothelium. In both arteries, cimetidine showed no significant effects on the histamine-induced contraction.

Figure 8 shows the effect of cimetidine on the antagonism of diphenhydramine against histamine in cattle coronary arteries with and without endothelium. In the presence of cimetidine, the slope parameter was increased in cattle coronary arteries with and without endothelium. In coronary arteries without endothelium, the slope value was not significantly different from unity in the presence of cimetidine.

### Table 3. $pD_2$ values and maximal responses to histamine in coronary arteries with and without endothelium

| Coronary artery | Endothelium | $pD_2$ (S.E.M.) | Max. response (%) | $N$ |
|----------------|-------------|----------------|------------------|-----|
| Pig            | +           | 5.60 ± 0.04    | 144.8 ± 4.0      | 14  |
|                | −           | 5.89 ± 0.05*   | 169.5 ± 10.5*    | 14  |
| Cattle         | +           | 4.71 ± 0.38    | 96.1 ± 4.8       | 9   |
|                | −           | 4.98 ± 0.43*   | 87.1 ± 7.1       | 9   |

Each value represents the mean ± S.E.M. *: Significantly different from endothelium (+) ($P < 0.05$).

**Fig. 6.** Schild plots of diphenhydramine for the contractile response to histamine in coronary arteries isolated from pigs and cattle with and without endothelium. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses).
Fig. 7. Effects of cimetidine on contractile response to histamine in coronary arteries without endothelium isolated from pigs and cattle. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses). Constrictions induced by 10^{-6} M histamine were taken as 100%; mean absolute values in arteries were 3042 ± 168 mg in pigs (n = 6) and 2766 ± 546 mg in cattle (n = 6). Control (●), cimetidine (○: 10^{-5} M).

Fig. 8. Effects of cimetidine on Schild plots of diphenhydramine in contractile response to histamine in coronary arteries with and without endothelium. •–•: with endothelium (Endo.), ○–○: without Endo., •–○: with Endo. and with cimetidine (Cime. 10^{-5} M), ○–○: without Endo. and with Cime. Each point represents the mean value (± S.E.M.) obtained from vessels of different animals (indicated by numbers in parentheses). *: Not significantly different from unity (P < 0.05).

**H_{1} and H_{2}-receptor agonists**

Figure 9 shows the representative tracings of responses to histamine, 2-pyridylethylamine and dimaprit in pig and cattle coronary arteries with endothelium which were preconstricted with prostaglandin F_{2a} (PGF_{2a}). In pig coronary arteries with endothelium, 2-pyridylethylamine, an H_{1}-receptor agonist, caused a weak relaxation at relatively low dose (10^{-7} M) (n = 4). This relaxation was abolished af-
ter removal of the endothelium (not shown). In cattle coronary arteries, 2-pyridylethylamine did not produce a significant relaxation. Dimaprit, an H₂-receptor agonist, did not show any effects on pig and cattle coronary arteries with endothelium which were preconstricted with PGF₂α.

Figure 10 shows the representative tracings from cattle coronary arteries with and without endothelium in response to 2-pyridylethylamine and dimaprit. 2-Pyridylethylamine induced contractions in cattle coronary arteries with and without endothelium, and there was no significant difference between arteries with and without endothelium in the degree of the contractions. Dimaprit did not show any significant effects on cattle coronary arteries with endothelium, but induced weak contractions in denuded arteries (n = 4). These contractions were completely inhibited by cimetidine (10⁻⁴ M) (not shown).

**Fig. 9.** Responses to histamine, 2-pyridylethylamine and dimaprit of pig and cattle coronary arteries with endothelium. Each artery was contracted with 2–3 × 10⁻⁶ M PGF₂α, the level before the addition of PGF₂α is shown as horizontal lines just left of each tracing. Concentrations shown are −log₁₀ molar.

**Fig. 10.** Responses to 2-pyridylethylamine and dimaprit of cattle coronary arteries with and without endothelium. Concentrations shown are −log₁₀ molar.
DISCUSSION

There are many reports about the pharmacological properties of coronary arteries isolated from the experimental animals; however, there have been only a few reports about the domestic animals. The present results demonstrated the different responsiveness to histamine, $H_1$- and $H_2$-antagonists and agonists in isolated coronary arteries from pigs and cattle.

Histamine has been reported to contract pig and cattle coronary arteries in vitro (3, 4, 12, 13), but there are few data about the subtypes of histamine receptors and whether vascular responses to histamine are endothelium-dependent or not in these species (12, 13). As shown in Fig. 2, the isolated pig and cattle coronary arteries were contracted by histamine. These results were similar to previous reports in pigs (12, 13) and cattle (3, 14). In pig coronary arteries, removal of the endothelium increased the maximal response and pD$_2$ value of the histamine-induced contraction (Fig. 5 and Table 3). These results are in agreement with those of Ikenoue et al. (12). They also have reported that the pretreatment of indomethacin ($10^{-6}$ M) did not affect the histamine-induced contraction in pig coronary arteries with endothelium. From their results, they have suggested that the endothelium-dependent relaxation factors (EDRFs) in pig coronary arteries might not contain PGI$_2$. In this experiment, we have not examined the effect of indomethacin on the histamine-induced contraction in pigs, but our unpublished data using methylene blue have shown that the pretreatment of the maximal blue enhanced the histamine-induced contraction to the same degree as the removal of endothelium (Fig. 5).

Moreover, it has been reported that bradykinin-induced relaxation was attenuated by methylene blue but not indomethacin in pig coronary arteries with endothelium (15). In the present experiment, bradykinin induced the endothelium-dependent relaxation, and this response was completely abolished by denudation (Fig. 1). From these results, it is possible to speculate that endothelial cells in pig coronary arteries release EDRF, and it is suggested that the histamine receptors may exist in the endothelium, and modify the histamine-induced response in pig and cattle coronary arteries.

Although diphenhydramine inhibited competitively the histamine-induced contraction in denuded coronary arteries of pigs (Table 2), the slopes of the Schild plots in the antagonism with diphenhydramine significantly deviated from unity in the coronary arteries with endothelium. These results suggest that the endothelium of the pig coronary artery has $H_1$-receptors that may stimulate the release of EDRF. This is in agreement with the previous suggestion (12).

In the cattle coronary arteries, the slope value of the Schild plot was less than unity even after the removal of endothelium, but the slope value was significantly increased and become unity in the presence of $10^{-5}$ M cimetidine (Fig. 8). Cimetidine, however, showed no significant effects on the histamine-induced contractions in cattle coronary arteries with and without endothelium (Figs. 4 and 7). It is possible to suggest that the $H_2$-receptors exist in the smooth muscle cells of cattle coronary artery, which result in the contraction, but it's effect might be very weak. As shown in Figs. 9 and 10, the $H_2$-agonist dimaprit caused no contraction and relaxation in cattle coronary arteries with endothelium, but did cause a weak contraction in denuded arteries. These results confirm the existence of a few $H_2$-receptors in the smooth muscle cells of cattle coronary arteries, which result in the contraction; and they also suggest the existence of $H_2$-receptors in the endothelial cells, which release EDRF.

In pig coronary arteries with endothelium, cimetidine did not show any significant effects on $10^{-7}$ M to $10^{-5}$ M histamine-induced contractions (Fig. 4). These are in agreement with earlier work (12). However, in this experiment, $3 \times 10^{-5}$ M and $10^{-4}$ M histamine-induced contractions were potentiated by cimetidine (Fig. 4). The potentiation by cimetidine was abolished by the removal of the endo-
thelium (Fig. 7). These results are similar to those of Hagen and Paegelow (13). They have mentioned that histamine in pig coronary arteries exerts opposite actions, an H1-receptor-mediated contraction and an H2-receptor-mediated relaxation. However, they have not discussed the location of the two receptors. From our results (Fig. 4), H2-receptors might exist in the endothelium of pig coronary arteries.

In summary, the present results suggest that the pig coronary artery contains mainly H1-receptors in the smooth muscle cells which result in the contraction, and H1- and H2-receptors exist in the endothelial cells which cause the release of EDRF. The cattle coronary artery contains mainly H3-receptors on the smooth muscle cells which result in the contraction, and it contains H2-receptors in the endothelial cells which cause the release of EDRF and H2-receptors on the smooth muscle cells which produce a small contraction.

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