Role of the Endothelium and Smooth Muscle Tone in the Dilator Response of the Rabbit Coeliac Artery to Histamine

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Abstract—The role of the endothelium and vascular tone was studied on histamine-induced relaxation and contraction of the rabbit coeliac artery. Histamine contracted the tissue via the release of noradrenaline (NA), an action blocked by prazosin and mepyramine but not influenced by endothelial removal. Tachyphylaxis readily developed to histamine-induced contractions. After a moderate tone (40–55%) was induced with NA, histamine relaxed the tissues concentration-dependently. This relaxation was absent when the endothelium was removed indicating that the receptors involved are located on the endothelium. When the tone was increased to 80–85% with NA, relaxation could only be demonstrated after blocking H_1-receptors. Removal of the endothelium did not influence this response. The relaxant effect of histamine in both preparations, however, was blocked by metiamide indicating that it is H_2-receptor mediated. In the rabbit coeliac artery, endothelial H_2-receptors are readily activated at moderate tone while muscular H_2-receptors are stimulated at high tone after blocking H_1-receptors. The predominance of H_2-receptors in this artery may serve the physiological function of vasodilation in the blood vessels of the stomach.

Histamine has a dual effect on blood vessels. In general, there is a vasoconstriction which is blocked by H_1-receptor antagonists such as mepyramine whilst the vasodilator response, the dominant effect in-vivo, can only be demonstrated in in-vitro preconstricted vessels treated with H_1-receptor blocking drugs (Edvinsson & Owman 1975; Owen 1977; Tayo & Bevan 1986). The importance of the endothelium in vascular relaxation by some agents has been reviewed (Vanhoutte & Rimelle 1983; Furchgott 1984). In general, acetylcholine (ACh), adenosine triphosphate, A23187, and many other substances relax many arterial segments and lose this relaxant property in the absence of the endothelium (see Furchgott 1984). Endothelium-dependent relaxation after histamine is not well documented. Van de Voorde & Leusen (1983) and Toda (1984) found that histamine-dependent relaxation of the rat aorta and dog mesenteric artery was endothelium-dependent. In the rabbit renal artery histamine-induced relaxation is endothelium-independent and unlike the rat aorta which is H_1-mediated, it is mediated via H_2-receptor activation (Tayo & Bevan 1986). In the present work, the actions of histamine on the rabbit coeliac artery have been studied. In addition, the roles of vascular tone and the endothelium in these actions were investigated.

Materials and Methods

Drugs
The following drugs were used: acetylcholine chloride, histamine dihydrochloride, (−)-noradrenaline bitartrate, atropine sulphate, indomethacin, (±)-propranolol hydrochloride (all obtained from Sigma, St. Louis, MO, USA), metiamide (SmithKline Beecham, UK), prazosin hydrochloride (Pfizer, UK) and mepyramine maleate (May &

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Baker, UK). Acetylcholine, histamine and noradrenaline (NA) were prepared daily and stock solutions of the other drugs were prepared and aliquots taken for daily use.

Tissue preparation
Male white New Zealand rabbits, 2·8–3·4 kg, were stunned and bled. The coeliac artery was quickly removed and placed in a petri dish containing a physiological salt solution (PSS) with the following composition (mM): Na^+ 144·2; K^+ 4·9; Ca^{2+} 1·6; Mg^{2+} 1·2; Cl^- 126·7; HCO_3^- 25·0; SO_4^{2-} 1·19; glucose 11·1 and EDTA 0·024 and was bubbled with 95% O_2 −5% CO_2. Care was taken to avoid the collapse of the vessels which could lead to unintentional rubbing of the endothelium. The artery was carefully cleaned of adherent tissues under a dissecting microscope and cut into two 3 mm ring segments. Each segment was set up in a 20 mL tissue bath at 37°C and pH 7·4, for isometric recording of tension changes (Bevan & Osher 1972). The tissues were allowed to equilibrate for 60 min and the PSS was replaced every 20 min. The tissues were then stretched to a predetermined optimum resting tension of 1·8 g. After a further 60 min their responses were determined by adding histamine (< 0·2 mL) directly to the bath.

Contraction studies
Cumulative responses were obtained to histamine and NA. The response was allowed to plateau before the addition of the next higher concentration. Because there was tachyphylaxis to histamine, a sequential concentration-effect test was carried out in which 30 min was allowed after each concentration of histamine. This was also performed when NA was used, to establish that the tachyphylaxis was not a generalized phenomenon in the tissue. The tissue was allowed 60 min rest after a concentration-effect test before the next test was conducted. In some experiments, tissues that had produced tachyphylaxis to histamine were further exposed to

NA to test for cross-tolerance. Prazosin (0·1 μM) and mepyramine (0·01 μM) were added to the bath 60 min before histamine and NA to block α_1-adrenoceptors and H_1 histamine receptors, respectively.

Relaxation studies

Tone was induced with NA (5 μM) which elicited 40–55% of the maximal response. When contractions had reached a steady state, relaxation responses were obtained to increasing concentration of histamine in the presence of propranolol and atropine to block β-adrenoceptors and muscarinic receptors, respectively. In some experiments 80–85% tone was induced with NA (20 μM) in the presence of mepyramine (1 μM) and the above procedure was carried out. One of each of the paired segments served as a time control and received NA alone. This was particularly necessary in this tissue because spontaneous relaxation almost invariably follows contractions to 3 μM NA. Preparations in which this spontaneous relaxation was more than 10% were excluded from the study.

Effect of endothelial removal on contraction and relaxation

In some experiments the endothelium was first removed by rubbing with a stainless steel wire. The effects of histamine (contractions and relaxations) were studied in such preparations in a manner similar to intact vessels. If relaxation to ACh (0·1 μM) was less than 10% of maximal relaxation of tone of the control vessel, the rubbing was considered successful. In addition, rubbed segments were routinely subjected to histological examination with the en-face silver staining technique of Poole et al (1958) at the end of each experiment.

Characterization of histamine receptors mediating relaxation in the intact and rubbed segments

Responses were obtained to increasing concentrations of histamine before and after 60 min pretreatment with metiamide. In a few experiments, the tissues were treated with mepyramine in addition to metiamide. From the plots the concentration of histamine at which the response was halved was obtained and the ratio of this concentration to the concentration producing the same effect in the absence of antagonist was calculated. The K_B values of metiamide were calculated from:

$$K_B = \frac{[\text{Antagonist}]}{(\text{Concentration ratio} - 1)}$$

Control tissues received histamine but no antagonist.

Statistical analysis

Results are expressed as mean $\pm$ s.e.m. Statistical analyses were by Student's t-test and the differences were accepted as significant when $P < 0.05$.

Results

Contraction studies

Histamine (0·3–600 μM) caused a concentration-related contraction of the coeliac artery (Fig. 1). After the first cumulative or sequential concentration-response test there was immediate tachyphylaxis to histamine. The second and
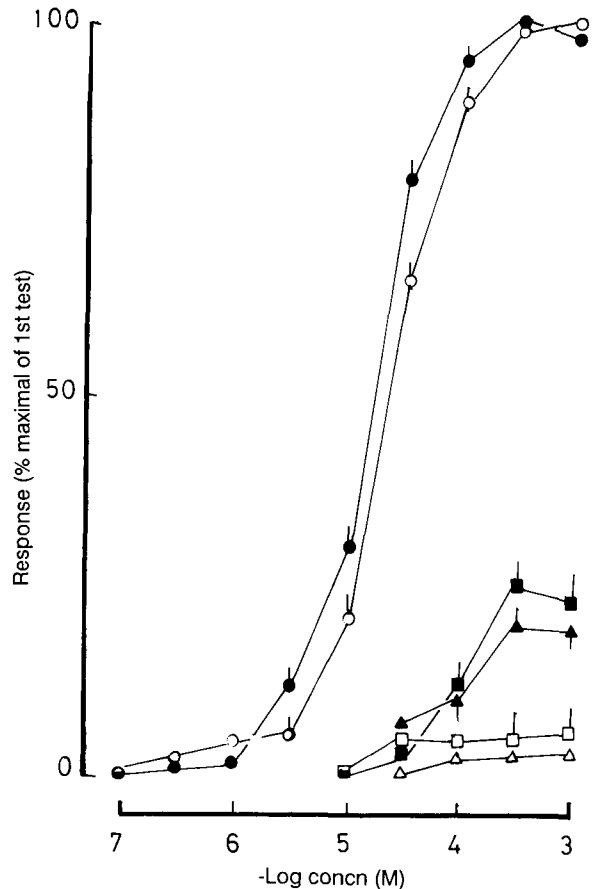

Fig. 1. Concentration-response curves of histamine-induced contractions of the coeliac artery showing the effect of endothelium and tachyphylaxis on the responses. Open symbols—endothelium intact, filled symbols—endothelium removed. Circles denote the first concentration—effect curve; squares, the 2nd and triangles the 3rd. Each point is the mean $\pm$ s.e.m. of 8 observations. * $P < 0.05$ compared with control.

third tests gave only about 5% of the initial maximal response (Fig. 1). Repeated washing over 2 h did not prevent this tachyphylaxis. The EC50 of histamine in the first test was $1.47 \pm 0.22 \times 10^{-5}$ M (n = 8). Even though there was immediate tachyphylaxis to histamine, responses to NA (0·1 μM) were reproduced over a period of time (Fig. 2). Prazosin (0·1 μM) reduced NA-induced contractions significantly ($P < 0.05$). In addition, in the presence of prazosin (0·1 μM) histamine could not elicit a contraction.

Relaxation studies

Moderate tone. Tone was induced to about 40–55% of maximal with 5 μM NA. ACh (0·1 μM) was added to the bath to test the ability of the tissues to relax. The bathing fluid was replaced and moderate tone was induced before a concentration-effect was carried out for histamine (0·1–100 μM) in the presence of atropine and propranolol. Histamine (0·1–100 μM), in the absence of H_1-receptor blocking drugs, relaxed the coeliac artery in a concentration-related manner (Fig. 3a). The maximal relaxation of tone after histamine was $48 \pm 3\%$ (n = 8). The IC50 was $1.47 \pm 0.15 \times 10^{-6}$ M (n = 8). Some tissues were exposed to mepyramine (0·1 μM) before
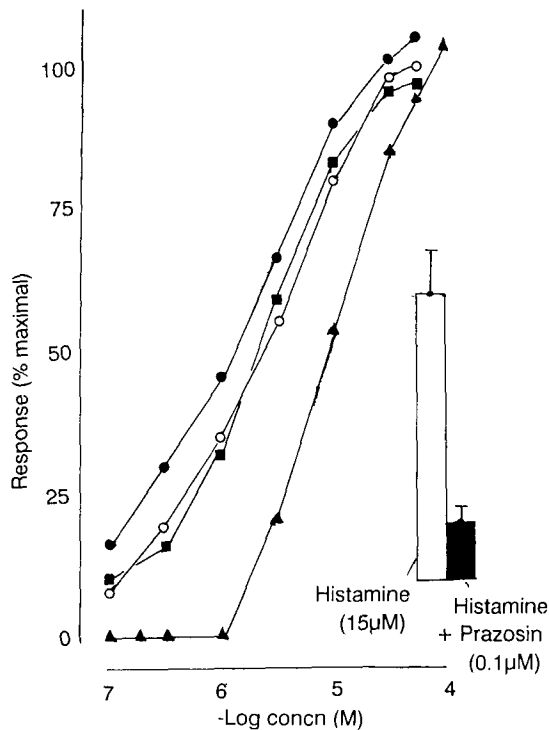
FIG. 2. Concentration-response curves of noradrenaline in the coeliac artery in the presence of endothelium (O), in the absence of endothelium (●); after tachyphylaxis to histamine but with intact endothelium (■); and after prazosin (▲) (0·1 μM). Each point is the mean ± s.e.m. of 5 observations.

histamine. Mepyramine enhanced the effect of histamine significantly (not shown).

Effect of "high" tone on relaxation. When tone was increased to 80–85% of maximal with 20 μM NA, histamine (0·1 μM) did not relax the tissue. However, if the tissues were pretreated with mepyramine (1·0 μM) 60 min before histamine there was a graded relaxation (Fig. 3b). The maximal
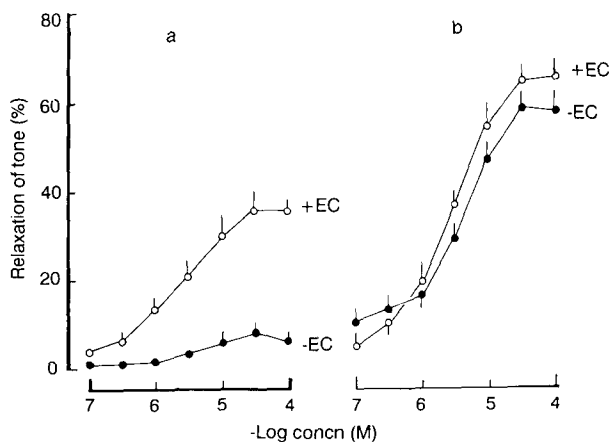

FIG. 3. Concentration-relaxation curves of histamine in the coeliac artery in the presence (O) and in the absence of the endothelium (●). In (a) about 40–55% tone was applied to the vessels. In (b) 80–85% tone was applied in the presence of mepyramine (1 μM). Each point is the mean ± s.e.m. of 8 and 6 observations respectively in (a) and (b). + EC and − EC denote intact and rubbed segments, respectively.

relaxant effect of histamine was 75 ± 5%. The IC50 value of histamine was 2·14 ± 0·25 × 10⁻⁶ M (n = 6). The IC50 value of histamine did not differ in moderate and high-tone vessels $(P > 0.05)$.

Effect of rubbing the endothelium

Contraction. Rubbed segments also contracted on exposure to histamine. Sub-maximal concentrations of histamine produced significantly greater effects $(P < 0.05)$ in the rubbed segments (Fig. 1). There was, however, no difference $(P > 0.05)$ between the EC50 values after rubbing (1·47 ± 0·22 × 10⁻⁵ M) and in control (1·20 ± 0·12 × 10⁻⁵ M); denuded vessels also showed tachyphylaxis to histamine (Fig. 1). Unlike control vessels, denuded segments retained 25 ± 3% and 20 ± 2% of the initial maximal during the second and third tests, respectively (Fig. 1). The responses to histamine were significantly higher $(P < 0.05)$ in denuded segments in the second and third determinations (Fig. 1). In addition, the EC50 values of histamine in the denuded vessels that showed tachyphylaxis were not significantly different from those of denuded vessels before tachyphylaxis 1·20 ± 0·12 × 10⁻⁵ M (first test, denuded) and 1·06 ± 0·11 × 10⁻⁵ M (second and third tests, denuded) $(P > 0.05)$. Histological examinations suggested successful rubbing of the endothelium.

Relaxation. Rubbed segments did not relax in response to histamine when the tone on the vessel was 40–55% (Fig. 3a); however, when tone was raised to about 80% in the presence of mepyramine (0·01 μM), there was a relaxation (Fig. 3b). There was no significant difference in the effect of histamine in the intact and rubbed segments treated with mepyramine at high tone (Fig. 3b). Rubbed segments did not relax to ACh (0·1 μM) but they relaxed to papaverine.

Effect of tachyphylaxis of the contractions on subsequent relaxations

In some experiments, vessels that had contracted to histamine and exhibited tachyphylaxis were used to determine if there was a cross-tachyphylaxis between the contraction and the relaxation. They were contracted with NA (5 μM) before relaxations were obtained to histamine (1·0–100 μM). Such vessels relaxed to histamine to a similar extent to those that had not previously been exposed to histamine (Fig. 4). In addition, such vessels dilated to ACh (0·1 μM).
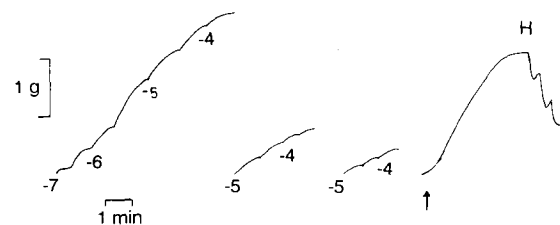

FIG. 4. Representative records of the tachyphylaxis of the coeliac artery to histamine-induced contraction and the lack of cross-tachyphylaxis to the relaxation. Values are log M concentrations of histamine. At the arrow, NA (5 μM) was added to the bath after tachyphylaxis to histamine-induced contraction had been established. At H, histamine 10⁻⁶; 5 × 10⁻⁶ and 10⁻⁵ M was successively added to the bath to elicit relaxation. The vertical bar represents 1 g tension and the horizontal bar denotes time in min.
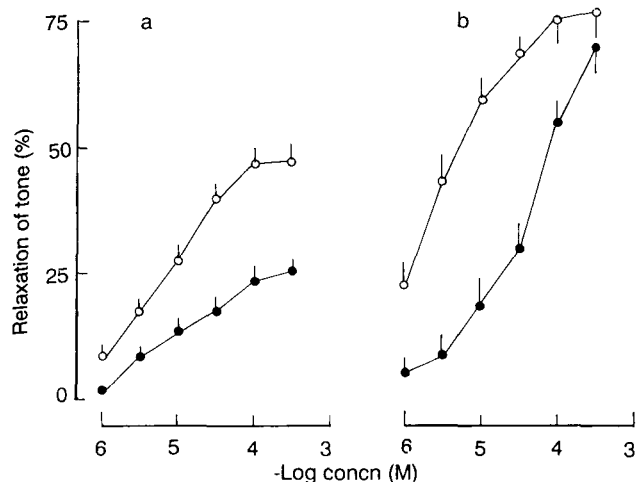
FIG. 5. Effect of metiamide (10 μM) on histamine-induced relaxation of the coeliac artery given (a) "moderate" tone 40–55% and (b) "high" tone 80–85%. In addition (b) had mepyramine (1 μM) to block H_1-receptors. O, control; ●, metiamide (10 μM). Each point represents the mean ± s.e.m. of 5 observations.

Effect of an H_2-receptor antagonist on relaxations

Moderate tone. In vessels with moderate tone, metiamide (10 μM) blocked the relaxations and suppressed the maximum effect of histamine (Fig. 5a). It was not possible to calculate the K_B of metiamide because of the nature of this antagonism.

High tone. When vessels were treated with mepyramine and contracted to a high tone, metiamide reduced histamine-induced relaxations without suppressing the maximal (Fig. 5b). The K_B value obtained was $8 \cdot 13 \pm 0 \cdot 22 \times 10^{-7}$ M (n = 5).

Discussion

The present results show that H_2-receptors of the rabbit coeliac artery are located on the endothelium and on smooth muscle cells. In addition, there is an excitatory but largely indirect action of histamine via NA release. The greater effects of histamine in contracting denuded preparations even after tachyphylaxis suggests that H_2-receptors on the endothelium are probably activated alongside the contraction. This limiting action of histamine would be lost in denuded vessels.

In this tissue, the vascular tone plays an important role in the inhibitory action of histamine. At moderate tone histamine relaxed the vessel even without blocking excitatory H_1-receptors. In rabbit vascular segments H_2-receptors are generally uncovered after blocking excitatory H_1-receptors (Owen 1977; Reinhardt & Ritter 1979; Tsuru et al 1983; Tayo & Bevan 1986). The loss of dilation under moderate tone after rubbing the endothelium indicates the presence of inhibitory H_2-receptors on the endothelium. This observation is noteworthy because histamine H_2-receptor-induced relaxation in rabbit vessel is generally thought to be endothelium-independent. For example, in the renal artery there is an enhanced relaxation (Tayo & Bevan 1986) and in the thoracic and abdominal aorta removal of the endothelium did not influence the relaxation (unpublished observations).

That endothelial H_2-receptors of the coeliac artery could be stimulated at moderate tone without blocking H_1-receptors suggests that these receptors may play a role in maintaining blood flow to the stomach. The loss of relaxation at high tone in the presence of H_2-receptors suggests the importance of the vascular tone on the response. This means that at moderate tone the endothelial H_2-receptors are responsive to stimulation and may intervene to increase blood flow when the tone is about to increase. This effect will be beneficial in conditions where sympathetic activity increases, as vasoconstriction will reduce blood flow to the stomach and will tend to concentrate acid secretagogues such as histamine. In the rat mesenteric circulation, Impicciatore et al (1983) found that H_2-receptors are more sensitive to histamine than H_1-receptors and that the H_2-receptor-dependent dilation was a moderate one. Based on this, they suggested that histamine H_2-receptor-dependent vasodilation may have a role in the physiological regulation of blood flow to the gut by endogenous histamine.

In the present study, there is also evidence that H_2-receptors are not located exclusively on the endothelium. There are some on the smooth muscle cells. However, these muscular H_2-receptors are only activated at high tone level after H_1-blockade. These receptors can be stimulated in the absence of the endothelium. Similar H_2-receptors are found in the rabbit renal artery (Tayo & Bevan 1986), veins (Tsuru et al 1983), aorta and superior mesenteric artery (F. Tayo, unpublished observations). Blockade of both relaxations by metiamide confirms that they are H_2-receptor-mediated. The K_B obtained corresponded to a pA_2 value similar to that reported in the literature ($6 \cdot 18$–$6 \cdot 98$) for H_2-receptor antagonists in the guinea-pig atrium (Brimblecombe et al 1975), gastric acid secretion (Durant et al 1978), rat uterus (Parsons et al 1977), rabbit veins (Tsuru et al 1983) and renal artery (Tayo & Bevan 1986). The two H_2-receptors are probably the same since the IC50 values of histamine are not statistically different.

Inability to demonstrate a reduced relaxation after tachyphylaxis of the contractions to histamine suggests that there was no cross-tachyphylaxis between the contraction and the relaxation. Tachyphylaxis reached a maximum in denuded vessels probably because some contractile agents, the nature of which are not known, are released from the endothelium.

In conclusion, the rabbit coeliac artery has histamine H_2-receptors located at two different sites: the endothelium and the smooth muscle cells. The receptors at both sites are blocked by metiamide. The activation of these receptors depends on the vascular tone; at moderate tone the endothelial receptors are activated and at high tone the smooth muscle receptors. In addition, there are H_1-receptors through which NA release is stimulated to produce a contraction which readily shows tachyphylaxis.

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