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Histamine receptors in isolated bovine oviductal arteries

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Abstract

The present in vitro study was designed to evaluate the effect of histamine on isolated rings of bovine oviductal artery and to characterize the histamine receptors involved in the histamine-induced response. Endothelial dependence of the response was also investigated. Cumulative addition of histamine and 2-pyridylethylamine (histamine H_1 receptor agonist) induced a concentration-dependent relaxation in intact arterial segments precontracted with noradrenaline. The histamine H_1 receptor antagonist mepyramine showed non-competitive antagonism in the histamine-induced concentration–response curve. However, when the response to histamine was evaluated in the presence of mepyramine and histamine H_2 and H_3 receptors were blocked, Schild analysis yielded a line with a slope of 1.10 and a pA_2 value of 8.91, indicating simple competitive antagonism of mepyramine at histamine H_1 receptor sites. The histamine H_2 receptor agonist, dimaprit, caused marked dilatation only at high doses. Cimetidine, propranolol and mepyramine failed to inhibit this relaxant effect. In precontracted oviductal arteries, cimetidine did not modify the histamine-induced concentration–response curves. Combined treatment with histamine H_1 and H_2 receptor antagonists did not induce an additional displacement with respect to the isolated effect of mepyramine thus excluding activation of histamine H_2 receptors. Histamine and (*R*)-α-methylhistamine, a selective histamine H_3 receptor agonist, produced a moderate contractile effect on the resting tone of preparations. Pretreatment with the selective histamine H_3 receptor antagonist decreased the (*R*)-α-methylhistamine response but increased the maximal relaxant effect and abolished the contractile effect of histamine, suggesting the presence of a limited population of contractile histamine H_3 receptors. Removal of the endothelium or pretreatment with methylene blue produced a significant inhibition of the relaxant response to histamine. Remaining dilatation was practically abolished by mepyramine and also by indomethacin. The L-arginine analogue, N^{ω}-nitro-L-arginine methyl ester (L-NAME) inhibited the effect of histamine and basal production of nitric oxide. L-Arginine, which on its own induced significant endothelium-dependent vasodilatation, reversed the effect of L-NAME on histamine relaxation. Indomethacin only caused a slight modification of the sensitivity of the vessels to histamine, suggesting that prostacyclin or other cyclo-oxygenase products did not make a significant contribution to the model. The absence of the endothelium did not modify the contractile effect of histamine. The results suggest that the relaxant response of isolated oviductal arteries to histamine is dependent on the functional integrity of the endothelium and is mainly mediated by histamine H_1 receptors. These receptors may mask a minority presence of histamine H_3 contractile receptors located on smooth muscle. The main relaxing factor released from the endothelium by mediation of histamine is nitric oxide, which may also exert an effect on vascular tone.

Keywords: Histamine; Histamine receptor; (*R*)-α-Methylhistamine; Histamine H_1 receptor; Histamine H_3 receptor; Endothelium; Nitric oxide (NO); Oviductal artery

1. Introduction

Despite being regarded as a transport tube, the oviduct is a site of fertilization, embryonic development and blastocyst formation. The earliest stages of embryonic development are influenced by a complex array of hormones and other biological factors which are synthesized locally or which are supplied via the vascular system (Rudolph et al., 1993). Limited information exists on the effects of different vasoactive agents on arteries supplying the oviduct. Forman et al. (1985) reported a transient relaxation of isolated oviductal arteries induced by substance P, while the effects of vasoactive intestinal peptide (VIP) were found to be insignificant. α-Adrenoceptors (predomi-

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nantly α_1-adrenoceptor subtype) were shown to be involved in the contractile activity of oviductal arteries without excluding a possible role for α_2-adrenoceptors (Costa et al., 1992). Recently endothelium-dependent relaxation by acetylcholine has been examined (García-Pascual et al., 1995). However, the effect of histamine, a well known regulator of the microcirculation, and the presence of histamine receptor subtypes in these arteries have not yet been investigated. Histamine which acts on oviductal arteries may originate from histamine stores in the secretory granules of mast cells close to blood vessels or may be contained in basophil leukocytes and platelets.

The biological effects of histamine are mediated by different receptors. Three subtypes (H_1, H_2 and H_3) have been identified. Different responses to histamine, mediated by these receptors, have been reported in isolated vascular segments. These vary considerably depending on the species, type of vessel (Van de Voorde and Leusen, 1984) and vascular area from which the tissue was derived (Tsuru et al., 1987). The heterogeneity of vascular responses to histamine mainly depends on its actions on classical histamine receptor subtypes, H_1 and H_2. The third histamine receptor (H_3) has been identified in presynaptic histaminergic nerve terminals in the brain (Arrang et al., 1983) and in perivascular autonomic nerve endings in the guinea-pig mesenteric artery (Ishikawa and Sperelakis, 1987). The pharmacology of the histamine H_3 receptor displays distinct features with respect to histamine H_1 or H_2 receptors. Activation of histamine H_3 receptors not only inhibits histamine release and synthesis at histaminergic neurons. Stimulation of central histamine H_3 receptors has also been shown to inhibit the release of other central nervous system neurotransmitters such as 5-hydroxytryptamine (Schlicker et al., 1988) and more recently acetylcholine (Clapham and Kilpatrick, 1992). Currently there is evidence for the existence of postsynaptic histamine H_3 receptors in in vitro models of rabbit middle cerebral artery (Ea-Kim and Oudart, 1988) and guinea-pig trachea (Cardell and Edvinsson, 1994).

Irrespective of the receptor subtype involved, it is well known that optimal response to histamine may require a functional and intact endothelium. This is due to the presence of specific receptors on the endothelium and/or to the release of potent vasoactive mediators from this layer. Histamine has been shown to cause the release of nitric oxide in several vascular beds and to stimulate the production of prostacyclin in endothelial or smooth muscle cells (Weksler et al., 1978). Nitric oxide causes smooth muscle relaxation by binding to the haem moiety of soluble guanylate cyclase leading to activation of the enzyme and in turn an increase in cGMP levels (Moncada et al., 1991). Prostacyclin, on the other hand, activates adenylate cyclase to induce the relaxant effect (Gryglewski et al., 1986).

The aim of the present investigation was to determine the effect of histamine on bovine oviductal arteries in vitro, to characterize the histamine receptors involved and to investigate the effect of the endothelial layer on the histamine-induced response.

2. Materials and methods

2.1. Preparation of isolated oviduct arterial rings

Genital tracts with macroscopically quiescent ovaries were removed from heifers killed at a local slaughterhouse and transported to the laboratory in ice-cold physiological salt solution (PSS) to minimize tissue metabolism.

The oviduct and a small portion of the tip of the uterine horn were separated from the rest of the genital tract and fixed in a Petri dish filled with ice-cold PSS. With the aid of a stereomicroscope (Nikon SMZ 2B) the uterine branch of the ovarian artery was identified and segments of its secondary branches (200–500 μm outer diameter) supplying the oviductal isthmus were carefully dissected free from the mesosalpinx and surrounding tissue. Oviductal artery segments of approximately 1–2 mm length were dissected and transferred to 5 ml organ baths containing PSS at 37°C bubbled with 95% O_2 and 5% CO_2 to maintain pH at 7.4.

2.2. Measurement of isometric tension

The arterial rings were gently slid onto the parallel stainless steel legs (75 μm diameter) of two L-shaped steel hooks (Högestätt et al., 1983). One wire was fixed and connected to a displacement unit allowing the fine adjustment of tension. The other was attached to a force transducer (Grass FT03C). Changes in isometric force were recorded on a polygraph (Houston D-5236-5).

Preparations were allowed to equilibrate for at least 60 min and washed with fresh PSS at 15 min intervals. The resting tension was adjusted to 0.75 ± 0.06 g ($n = 23$) or the optimal for inducing maximal contraction.

2.3. Experimental protocol

After the equilibration period, the segments were exposed to a depolarizing potassium solution (119 mM) (K-PSS) until reproducible contractile responses were obtained. The maximal contractile capacity of the vessels (E_{max}) was determined by activation with noradrenaline (10^{-6} M) and 5-hydroxytryptamine (10^{-5} M) in K-PSS. The response obtained was used as the reference contraction for each vessel ring.

The integrity of the vascular endothelium was confirmed by immediate relaxation (80–100%) induced by acetylcholine (10^{-6} M) in vessels precontracted by noradrenaline. Lack of relaxation or even an additional contraction induced by exposure to the same concentration of acetylcholine without loss of response to papaverine (10^{-4}

M), was interpreted as an indication of the absence of functional endothelium.

The relaxant effect of histamine and selective histamine receptor agonists was evaluated in arterial rings partially precontracted with noradrenaline ($(1–7) \times 10^{-6}$ M). These concentrations produced a stable contraction of sufficient duration to permit the analysis of relaxation responses. Cumulative concentration–response curves to various histamine receptor agonists were obtained by increasing the organ bath concentration in half log unit steps.

In order to characterize histamine receptors, the histamine concentration–response curves were repeated in the presence of three increasing concentrations of specific antagonist for each vessel ring. Preliminary experiments showed the absence of histamine-induced tachyphylaxis in this vascular tissue. In all experiments the concentration–response curve served as a control and the different antagonists were added to the bathing media at least 30 min before a new concentration–response curve was obtained.

A second series of experiments was conducted to assess the ability of oviductal arteries to release an endothelium-derived relaxing factor (EDRF). The L-arginine and N^{ω}-nitro-L-arginine methyl ester (L-NAME) concentration–response curves were obtained using arterial rings precontracted with noradrenaline.

2.4. Analysis of results

Relaxation responses were expressed as a fraction of the vascular contraction induced by noradrenaline ($\approx 10^{-6}$ M) just prior to addition of the agonists. Contractile responses were expressed as a percentage of E_{max} for each vessel. The agonist concentration required to give half-maximal response (EC_{50}) was determined for each concentration–response curve by computerized iteration-fitting the responses and logarithmic concentrations to the Hill equation. Sensitivities are given in terms of pD_2 values which are defined as the negative logarithm of the EC_{50} for the agonists used. For the assessment of the effect of the histamine antagonist, three different concentrations were employed (four consecutive concentration–response curves to histamine could be performed per vessel ring). The pA_2 values were calculated from the Schild plot (Arunlakshana and Schild, 1959). In this method the log concentration ratio $(CR) - 1$ of the agonist is plotted against the log concentration of the antagonists and subjected to linear regression analysis. The concentration ratio denotes the ratio of EC_{50} values in the presence and absence of a given concentration of antagonist. The slope of the Schild plot indicates the nature of the antagonism. Ideally the slope should be 1 if the response involves a simple competitive antagonism at one receptor subtype only.

Results were expressed as mean values ± standard error (S.E.M.) or 95% confidence limits. Comparisons were performed using the Student's *t*-test for paired or unpaired data where appropriate. A P value of less than 5% ($P < 0.05$) was accepted as denoting a significant difference.

2.5. Solutions and pharmacological agents

Vessels were dissected and maintained in a relaxed state in PSS with the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10, KH_2PO_4 1.2 and ethylene-diaminetetraacetic acid (EDTA) 0.026. K-PSS was identical to PSS except that NaCl was replaced with KCl on an equimolar basis.

Acetylcholine chloride, cimetidine, atropine sulphate, histamine dihydrochloride, indomethacin, L-arginine hydrochloride, methylene blue, N^{ω}-nitro-L-arginine methyl ester, noradrenaline hydrochloride, papaverine hydrochloride, serotonin creatinine sulphate complex, sodium nitroprusside and tetrodotoxin were supplied by Sigma (USA). Mepyramine maleate was kindly donated by Rhône Poulenc Rorer (UK). Dimaprit dihydrochloride and 2-pyridylethylamine dihydrochloride were kindly donated by Smith Kline and Beecham (UK). (*R*)-α-Methylhistamine dihydrogenomaleate and thioperamide maleate were kindly donated by Bioproject (France). All drugs were added in volumes not exceeding 0.3% of the organ baths to reach the final required concentration. They were dissolved in distilled water with the exception of indomethacin and papaverine which were prepared in ethanol (99%) and thioperamide in dimethyl sulphoxide. Previous experiments showed that these solvents had no effect on preparations. Stock solutions of drugs were stored at −20°C and fresh dilutions were made daily. Concentrations of agents were expressed as the final concentration in the organ bath.

3. Results

3.1. Relaxant effect of histamine on bovine oviductal arteries

Histamine (10^{-9}–10^{-4} M) produced a concentration-dependent relaxant effect on 176 out of 206 vascular segments with intact endothelium precontracted with noradrenaline (0.86 ± 0.06 g). The pD_2 value was 6.48 ± 0.14, corresponding to an EC_{50} of $3.74 \pm 0.49 \times 10^{-7}$ M. Relaxation induced by histamine showed a maximum (MR) of 76 ± 3.52% of the noradrenaline precontraction ($n = 176$).

The mean outer diameter of the oviductal arteries was 372 ± 18 μm ($n = 30$). Microcirculatory studies have consistently demonstrated that vascular responsiveness to topically applied pharmacological compounds varies inversely with arteriolar diameter (Prieto et al., 1991). However, in the present study no differences in the relaxation response of different vascular segments to histamine were detected (200–500 μm) (data not shown).

In order to exclude the involvement of other receptors

Table 1
Estimated pD_2 and maximum response values for the substances indicated

	pD_2 [a]	Maximum response (%) [a]	n
Noradrenaline control [c]	5.31 ± 0.05	102.64 ± 2.89	8
Mepyramine (10^{-8} M) [c]	5.25 ± 0.04 [b]	99.60 ± 2.93 [b]	8
Histamine control	6.59 ± 0.12	76.46 ± 10.01	8
Propranolol (10^{-7} M)	6.41 ± 0.17 [b]	73.13 ± 8.36 [b]	8
Histamine control	6.41 ± 0.12	70.49 ± 8.23	8
Atropine (10^{-7} M)	6.17 ± 0.14 [b]	69.11 ± 6.78 [b]	8

n = number of vessels. [a] Values represent mean ± S.E.M. [b] Value not significantly different from the control response (paired t-test). [c] Values are expressed as percentage reference contraction to E_{max}.

in histamine-induced vasodilation, differences in the histamine response were investigated by stimulation or inhibition of physiologically active receptors in the oviductal arteries. Results showed no variation in maximal relaxation or pD_2 values of the control agonists (Table 1) ruling out any effects of adrenoceptors and cholinergic receptors on the histamine concentration–response curve.

3.2. Histamine receptor characterization

3.2.1. Histamine H_1 receptor

In the presence of noradrenaline-induced tone, 2-pyridylethylamine, a selective histamine H_1 receptor agonist, caused a concentration-dependent relaxation (10^{-9}–3×10^{-4} M) in the arteries supplying the oviduct ($pD_2 = 4.66 \pm 0.18$; $n = 7$). 2-Pyridylethylamine induced a maximum dilatation of $98.99 \pm 0.60\%$, greater than that induced by histamine (Fig. 1).

The selective histamine H_1 receptor antagonist, mepyramine, was also used to determine the receptor subtype responsible for histamine-induced relaxation. The histamine response was tested before and after incubation with mepyramine (10^{-9}–10^{-7} M) which markedly inhibited the relaxant effect, producing a significant shift towards the right of the histamine concentration–response curve (pD_2 value following addition of 10^{-7} M was 4.53 ± 0.23, $n = 10$; $P < 0.001$) (Fig. 2). This antagonism was considered non-competitive due to the depression of the maximal agonist response (MR value following addition of mepyramine (10^{-7} M) was $43.82 \pm 4.37\%$; $P < 0.01$). However, when the response to histamine was assessed in the presence of mepyramine, after blocking histamine H_2 and H_3 receptors, the antagonism acquired competitive properties (Fig. 3A). The resulting pA_2 value was 8.91 ± 0.44 and the Schild plot of mepyramine against histamine yielded a straight line with a slope close to 1 (slope = 1.10 ± 0.1; $r = 0.92$; $n = 7$) (Fig. 3B).

3.2.2. Histamine H_2 receptor

Dimaprit (10^{-9}–3×10^{-4} M), a specific histamine H_2 receptor agonist, showed no relaxant effect on precontracted vessel rings, at low and mid concentrations ($< 10^{-5}$ M). This drug induced significant dilatation only at the highest concentration ($pD_2 = 3.91 \pm 0.10$; MR = $65.49 \pm 4.80\%$; $n = 8$) (Fig. 1). To clarify the specificity of this effect, the possible participation of histamine H_2 receptors

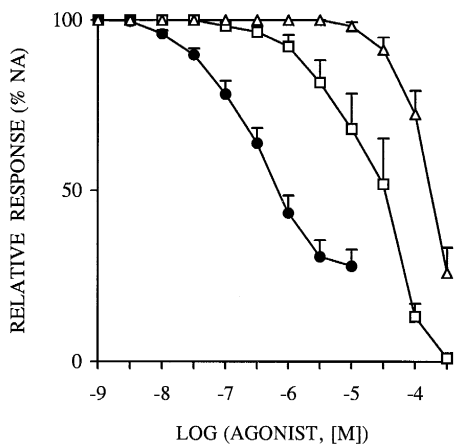

Fig. 1. Concentration–response curves to histamine (●, $n = 15$), 2-pyridylethylamine (□, $n = 7$) and dimaprit (△, $n = 8$) in isolated oviductal arteries. Each point represents the mean ± S.E.M. (vertical lines) of n observations.

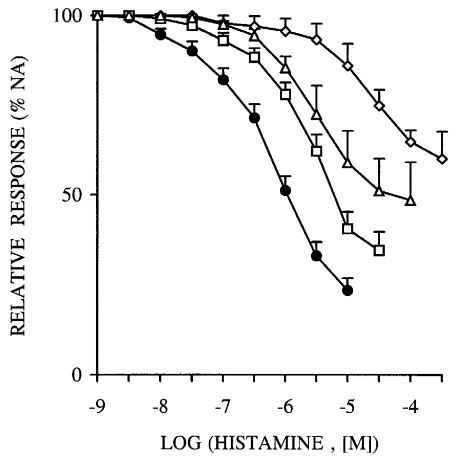

Fig. 2. Concentration–response curves to histamine in isolated oviductal arteries in the absence (●) and in the presence of 10^{-9} M (□), 10^{-8} M (△) and 10^{-7} M (◇) mepyramine. Each point represents the mean ± S.E.M. (vertical lines) of 10 observations.

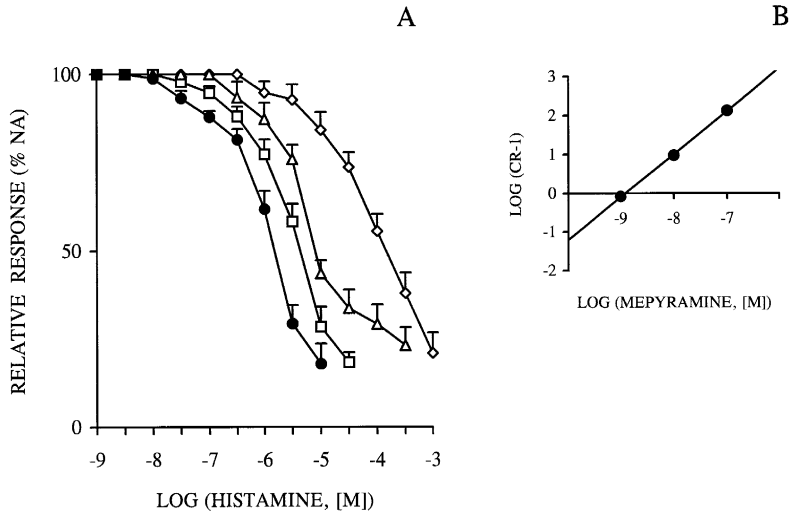

Fig. 3. (A) Concentration–response curves to histamine in isolated oviductal arteries in the absence (●) and in the presence of 10^{-9} M (□), 10^{-8} M (△) and 10^{-7} M (◇) mepyramine. Experiments were carried out in the presence of cimetidine (10^{-5} M) and thioperamide (3×10^{-7} M). Each point represents the mean ± S.E.M. (vertical lines) of 7 observations. (B) Schild plot for the effect of mepyramine on concentration-response curves to histamine. The intercept on the abscissa scale gives a pA_2 value of 8.91 (slope = 1.10 ± 0.1; $r = 0.92$; $n = 7$).

was investigated by incubating preparations with cimetidine (10^{-5} M) before a second stimulation with dimaprit, but this was not effective ($pD_2 = 3.84 \pm 0.05$, MR = 60.05 ± 3.98% before the addition of cimetidine and $pD_2 = 3.92 \pm 0.01$, MR = 55.88 ± 5.92% after the addition of cimetidine; $n = 6$). Further, the relaxant effect was not inhibited by propranolol (10^{-7} M) and mepyramine (10^{-8} M), β-adrenoceptor and histamine H_1 antagonists respectively, since no modification of the dimaprit response was observed (control $pD_2 = 3.95 \pm 0.01$, MR = 71.47 ± 4.9% and $pD_2 = 3.94 \pm 0.01$, MR = 62.40 ± 7.96% after the addition of the antagonists; $n = 9$). These results suggest that the dimaprit response could be the result of indirect actions of the agonist.

Cimetidine did not affect the histamine-induced dose-response curve at concentrations considered to specifically antagonize histamine H_2 receptors (10^{-6} and 10^{-5} M). pD_2 and maximal response values did not vary significantly from controls (Table 2).

Combined treatment with both antagonists, cimetidine (10^{-5} M) and mepyramine (10^{-8} M) produced no additional displacement with respect to the isolated effect of mepyramine (10^{-8} M) (Table 2). These results seem to exclude any participation of histamine H_2 receptors in mediating the responses to histamine in the arteries.

3.2.3. Histamine H_3 receptor

In the presence of mepyramine (10^{-8} M) and cimetidine (10^{-5} M), selective activation of histamine H_3 receptors by the agonist (*R*)-α-methylhistamine (10^{-9}–3×10^{-4} M) induced a small contractile effect in the vascular segments reaching a maximal contraction (MC) of 21.09 ± 1.12% of E_{max}. Vessel sensitivity to (*R*)-α-methylhistamine was 4.22 ± 0.07 ($n = 9$) (Fig. 4). The histamine H_3

Table 2
Effects of histamine receptor antagonists on histamine-induced relaxation in rings of bovine oviductal artery

	pD_2 [a]	Maximum response (%) [a]	*n*
Control	6.43 ± 0.12	79.46 ± 7.28	8
Cimetidine (10^{-6} M)	6.33 ± 0.12 [b]	76.55 ± 6.17 [b]	8
Control	6.42 ± 0.14	81.97 ± 5.81	9
Cimetidine (10^{-5} M)	6.38 ± 0.15 [b]	83.57 ± 4.85 [b]	9
Control	6.50 ± 0.14	88.95 ± 4.47	8
Mepyramine (10^{-8} M)	5.47 ± 0.08 [c]	66.67 ± 10.26 [c]	8
Control	6.37 ± 0.14	79.31 ± 5.42	5
Mepyramine (10^{-8} M) + cimetidine (10^{-5} M)	5.26 ± 0.15 [c,d]	68.48 ± 10.20 [c,d]	5

n = number of vessels. [a] Values represent mean ± S.E.M. [b] Value not significantly different from the control response. [c] Values significantly different from the control response (paired *t*-test, $P < 0.05$). [d] Value not significantly different from the value measured in the group treated with mepyramine (10^{-8} M).

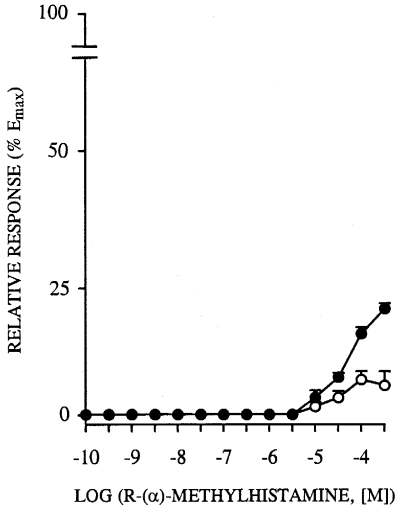

Fig. 4. Concentration–response curves to *R*-(α)-methylhistamine in isolated oviductal arteries in the presence of 10^{-5} M cimetidine and 10^{-8} M mepyramine (●) and in the presence of 10^{-5} M cimetidine, 10^{-8} M mepyramine and 10^{-6} M thioperamide (○). Each point represents the mean ± S.E.M. (vertical lines) of 9 observations.

receptor antagonist, thioperamide (10^{-6} M), decreased the maximal contraction of (*R*)-α-methylhistamine to 8.73 ± 2.12% ($n = 9$; $P < 0.001$) (Fig. 4).

In the presence of mepyramine (10^{-8} M), relaxation induced by histamine was subjected to the action of the histamine H_3 receptor antagonist (10^{-6} M) which enhanced the relaxant effect showing a maximal histamine response value of 97.24 ± 1.38% ($n = 7$) (Fig. 5). This relaxation was significantly greater ($P < 0.01$) than that induced by histamine in the presence of mepyramine only (MR = 69.83 ± 9.73; $n = 7$) (Fig. 5).

High concentrations of histamine ($\geq 10^{-4}$ M) had a contractile effect on the resting tone of vessel rings (pD_2 = 3.61 ± 0.06, MC = 45.87 ± 3.97%, $n = 6$) (Fig. 6). This effect was found to be resistant to mepyramine (10^{-7} M) (pD_2 = 3.54 ± 0.06, MC = 49.50 ± 8.14%; $n = 6$) and tetrodotoxin (10^{-6} M) (pD_2 = 3.43 ± 0.01; MC = 43.36 ± 7.97%; $n = 6$) but was abolished when the rings were incubated with thioperamide (10^{-5} M) ($n = 6$) (Fig. 6). Therefore, these results do not permit the a priori ruling out of the possible presence of an H_3 receptor.

3.3. Effect of endothelium and endothelium-derived relaxing factors on histamine-induced response

The removal of the endothelium produced no variation in the histamine-induced contraction on the basal tone of the segments (with endothelium, pD_2 = 3.61 ± 0.12, MC = 40.71 ± 5.73; without endothelium, pD_2 = 3.60 ± 0.07, MC = 49.88 ± 5.67; $n = 9$).

However, relaxant responses to histamine were markedly and significantly reduced in vessels in which the endothelium had been removed (MR = 21.27 ± 2.83%; $n = 11$; $P < 0.001$) (Fig. 7A). Remaining relaxation was practically

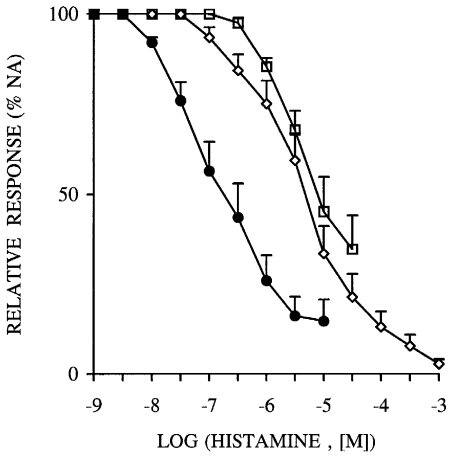

Fig. 5. Concentration–response curves to histamine in isolated oviductal arteries in the absence (●), in the presence of 10^{-8} M mepyramine (□) and in the presence of 10^{-8} M mepyramine and 10^{-6} M thioperamide (◇). Each point represents the mean ± S.E.M. of 7 observations.

abolished by mepyramine (10^{-8} M) (MR = 9.45 ± 1.94%; $n = 6$; $P < 0.05$) (Fig. 7A). Moreover, the relaxation shown by the denuded arteries was substantially reduced by the administration of indomethacin (MR = 8.39 ± 3.42%; $n = 6$; $P < 0.05$).

Maximal dilatation induced by acetylcholine (MR = 61.28 ± 6.28%) and histamine (MR = 60.63 ± 5.23%) showed linear correlation in oviductal arteries with intact endothelium (slope = 0.83 ± 0.17; $r = 0.67$; $n = 35$) (Fig. 7B). In contrast, there was no correlation between relaxation produced by both amines and vasodilation induced by sodium nitroprusside (MR = 93.92 ± 4.36%; $n = 35$).

In the second series of experiments, preparations were incubated with an inhibitor of soluble guanylyl cyclase, methylene blue (10^{-5} M), which also decreased the maxi-

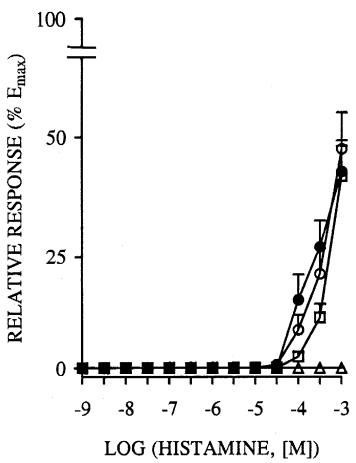

Fig. 6. Concentration–response curves to histamine in isolated oviductal arteries in the absence (●), in the presence of 10^{-7} M mepyramine (○) and in the presence of 10^{-7} M mepyramine and 10^{-6} M tetrodotoxin (□) and in the presence of 10^{-7} M mepyramine and 10^{-6} M tetrodotoxin and 10^{-5} M thioperamide (△). Each point represents the mean ± S.E.M. (vertical lines) of 6 observations.

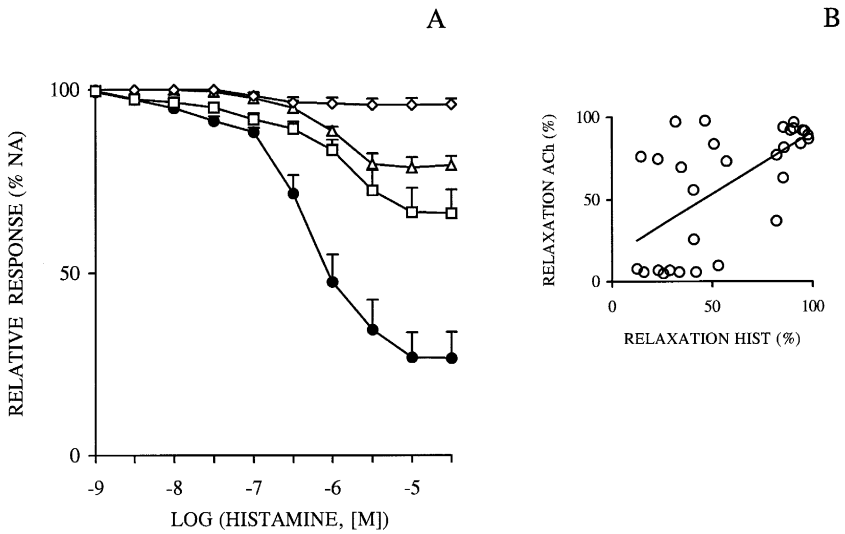

Fig. 7. (A) Concentration–response curves to histamine in isolated oviductal arteries in the absence (●, $n = 13$), in the presence of 10^{-5} M methylene blue (□, $n = 13$), after removal of endothelium (△, $n = 11$) and denuded in the presence of 10^{-8} M mepyramine (◇, $n = 6$). Each point represents the mean ± S.E.M. (vertical lines) of n observations. (B) Plot showing the relation between maximal relaxation induced by acetylcholine (ACh) and histamine (HIST) in isolated oviductal arteries (slope = 0.83 ± 0.17; $r = 0.67$; $n = 35$).

mal response of the arteries to histamine, reaching a relaxation of 33.65 ± 6.48% ($n = 13$; $P < 0.001$) (Fig. 7A).

The endothelium-dependent relaxation produced by histamine in the oviductal arteries (control $pD_2 = 6.44 \pm 0.09$, MR = 53.75 ± 5.78%; $n = 8$) was attenuated by a nitric oxide synthase inhibitor, L-NAME (10^{-6} M). The maximal relaxation produced by histamine after the addition of L-NAME (35.91 ± 7.24%; $n = 8$; $P < 0.05$) and the pD_2 value (5.67 ± 0.08; $P < 0.001$) were significantly different to the control (Fig. 8). This inhibitory effect of L-NAME was reversed in the presence of L-arginine (3×10^{-4} M), a precursor of NO synthesis, returning the pD_2 value to that of the control (6.36 ± 0.21; $n = 8$). The relaxation was significantly greater (MR = 78.33 ± 5.87%; $P < 0.001$) when compared to the histamine control (Fig. 8).

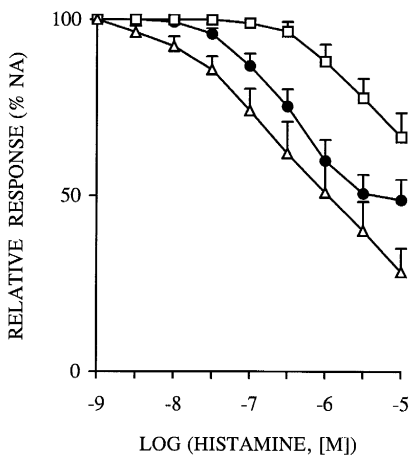

Fig. 8. Concentration–response curves to histamine in isolated oviductal arteries in the absence (●), in the presence of 10^{-6} M N^{ω}-nitro-L-arginine (□) and in the presenceof 3×10^{-4} M L-arginine (△). Each point represents the mean ± S.E.M. (vertical lines) of 8 observations.

The activation of NO synthesis by L-arginine led to the suspicion of a release of NO from endothelium-intact segments. L-Arginine (10^{-5}–10^{-3} M), on its own, induced maximal relaxation of precontracted segments of 54.14 ± 6.77% ($n = 10$) with a pD_2 value of 3.67 ± 0.07. Moreover, this was confirmed when L-NAME (10^{-7}–3×10^{-4} M) caused an increase in vessel tone of precontracted arteries ($pD_2 = 5.02 \pm 0.11$, MC = 43.85 ± 7.27%; $n = 6$).

Incubation of preparations with indomethacin (10^{-6} M), a cyclo-oxygenase inhibitor, slightly reduced the histamine-induced vasodilation at low concentrations but did not modify the maximal relaxation produced by histamine (MR = 73.33 ± 5.25% prior to the addition of indomethacin and MR = 74.73 ± 4.41% after the addition of indomethacin; $n = 12$). However, a slight deviation of the pD_2 value was produced ($pD_2 = 6.53 \pm 0.22$ prior to the addition of indomethacin and $pD_2 = 6.13 \pm 0.14$ after the addition of indomethacin; $P < 0.05$).

4. Discussion

Histamine is thought to play a role in oviductal smooth muscle motility and local vascular permeability. Significant changes in histamine concentration are produced during physiological reproductive processes and mast cells are abundant in female reproductive tissues (Rudolph et al., 1993).

The results obtained in the present investigation demonstrate that histamine acts as a potent vasodilator in isolated bovine oviductal arteries and that this histamine relaxation

is mediated primarily by the activation of histamine H_1 receptors. Any interaction between histamine and adrenoceptors or cholinergic receptors is ruled out in agreement with the work of Krstić et al. (1991) using rat femoral arteries.

In the present study, histamine H_1 receptors seemed to play a major role in mediating responses to histamine. This was supported by the observation that 2-pyridylethylamine, a selective histamine H_1 receptor agonist, mimicked the relaxation elicited by histamine in oviduct arterial segments, showing a greater maximal response but a smaller potency than histamine. However, it is difficult to attribute the effect of histamine to a particular receptor type based only on the action of selective agonists. These often behave as partial agonists in tissues with high receptor density. Verification of histamine H_1 receptor participation requires determination of the effects of antagonists. Antagonism of the relaxant response of precontracted vessels to histamine by mepyramine indicated that vasodilation was mediated by histamine H_1 receptors as postulated. Increasing concentrations of mepyramine caused a shift of the control concentration–response curves to the right but the depression of maximal response suggested that another site was also involved. However, the slope of the Schild plot for mepyramine, in the presence of histamine H_2 and H_3 receptor antagonists, was not significantly different to a value of one, indicating that the parallel shift to the right of the relaxant histamine response can be attributed to participation of histamine H_1 receptors. The pA_2 value was in close agreement with values reported by other investigators in several other tissue preparations where there is a predominance of histamine H_1 receptors. It is also similar to previous reports obtained using other isolated arteries. Histamine-induced relaxation in rat pulmonary artery (Szarek et al., 1992) and in monkey basilar artery (Usui et al., 1992) is also inhibited by histamine H_1 receptor antagonists.

Other arteries relax in response to histamine by activation of histamine H_2 receptors. Examples include rat femoral artery (Krstić et al., 1991), rat middle cerebral artery (Benedito et al., 1991b) and canine spinal artery (Kawai and Ohhashi, 1995). In the present investigation dimaprit, which is known to be a selective histamine H_2 receptor agonist, elicited a relaxant effect but only at higher concentrations. Unexpectedly, when the participation of histamine H_2 receptors was inhibited by the selective antagonist, cimetidine, no displacement with respect to the first stimulation was observed suggesting the activation of receptor types other than histamine H_2 receptors. The receptors that could be involved in the dilatory response were blocked (β-adrenoceptor and histamine H_1 receptor subtypes) without modification of the dimaprit-induced relaxant effect. The mechanism of action of this relaxation remains to be explained. Histamine H_2 receptors did not seem to play an important functional role in regulation of tone in the oviductal circulation since cimetidine showed no inhibition of the histamine control curve at a concentration range considered to be specific for histamine H_2 receptors.

Ottosson et al. (1989) have shown that both histamine H_1 and H_2 receptors participate in the relaxation of the human temporal artery, although it was necessary to increase histamine H_1 receptor blockage to unmask the histamine H_2 receptor-mediated dilatation. In bovine retinal artery (Benedito et al., 1991a) and dog external ophthalmic artery (Wang et al., 1993) the activation of both receptor subtypes was necessary to achieve complete vasodilation using histamine. In the present study, combined treatment with histamine H_1 and H_2 receptor antagonists gave rise to no additional displacement with respect to the effect of the histamine H_1 receptor antagonism alone. These results suggest that histamine H_1 receptors, and not H_2 receptors, play a principal role in the histamine-induced relaxation of bovine oviductal artery.

Histamine H_3 receptors are mainly located presynaptically in the central and peripheral nervous system (Arrang et al., 1983; Ishikawa and Sperelakis, 1987). However, evidence for the existence of postsynaptic histamine H_3 receptors has been reported in guinea-pig trachea (Cardell and Edvinsson, 1994) and rabbit middle cerebral artery (Ea-Kim and Oudart, 1988). The latter authors suggest that the vasodilatory effects of histamine on the cardiovascular system may be mediated by histamine H_3 receptors, in addition to those produced by histamine H_1 and H_2 receptor stimulation.

The presence of histamine H_3 receptors in bovine oviductal arteries seems likely since there was, (a) a change in mepyramine antagonism from non-competitive to competitive after the addition of thioperamide to the organ bath, (b) an enhancement of the relaxation induced by histamine when the preparation was incubated with an histamine H_3 receptor antagonist, (c) a contractile, though modest, effect of (*R*)-α-methylhistamine and (d) vasoconstriction induced by histamine on the resting tone of the preparations which was sensitive to the action of thioperamide.

The present findings may be compared to those published by Cardell and Edvinsson (1994), who also found a drug-receptor interaction where the single agonist histamine interacted with two receptor subtypes in guinea-pig trachea. In the latter model, histamine-induced contraction was mediated by histamine H_1 receptors but the selective stimulation of histamine H_3 receptors caused dilation.

In the present model, histamine H_1 receptors were involved in the relaxant effect and activation of histamine H_3 receptors produced a contractile effect. It is suggested that the slope of the histamine concentration–response curve may be primarily determined by histamine interaction with the histamine H_1 receptor to which it binds with greater affinity. The action of histamine on the contraction-eliciting histamine H_3 receptor may counteract the relaxant effect mediated by the histamine H_1 receptor.

Tetrodotoxin in a concentration known to abolish neuronal transmission did not affect the histamine contractile response. The lack of effect of tetrodotoxin on histamine-induced contraction seems to indicate that the activation of H_3 receptors has a direct effect on vascular smooth muscle as opposed to an indirect mechanism involving sympathetic nerve terminals. Furthermore, mechanisms other than those involving endothelial cells are responsible for the H_3 receptor-induced contraction since response patterns were similar in arteries in the presence and absence of endothelium. This finding differs from that reported by Ea-Kim et al. (1992) who suggest that the endothelium plays an essential role in H_3 agonist-induced response.

The results obtained in the present investigation demonstrate that histamine-induced relaxation in the arteries is largely dependent upon the presence of a functional endothelium.

To date, histamine relaxations have been shown to be endothelium-dependent in most of the vascular beds studied. Histamine, as well as acetylcholine, is known to act on the endothelium of vessels inducing production and liberation of an EDRF (Ignarro et al., 1986). The linear correlation between histamine-induced relaxation and relaxation produced by acetylcholine, in addition to the lack of correlation between relaxation induced by both amines and the vasodilation induced by sodium nitroprusside (which mainly causes direct activation of soluble guanylyl cyclase in vascular smooth muscle), suggest that the relaxation of the oviductal artery produced by histamine is, to a large extent, endothelium-dependent. Additional evidence for the essential role of the endothelium in histamine-induced vasodilation was found when endothelial cells were mechanically removed from the vessel preparations. The absence of endothelium significantly reduced the histamine response and remaining relaxation was practically suppressed by the histamine H_1 receptor antagonist. These results provide evidence for a small population of histamine H_1 receptors located in smooth muscle, which, when stimulated, participate in the relaxant effect. The inhibitory effect of indomethacin on denuded arteries suggests that the subcellular mechanism, responsible for the relaxation produced after activation of smooth muscle histamine H_1 receptors, may involve the action of prostacyclin. Kitamura et al. (1995) are the only authors who report vasodilation induced by stimulation of subendothelial histamine H_1 receptors mediated by prostacyclin. Nevertheless, the main relaxation induced by histamine in oviductal arteries is mediated by the histamine H_1 receptors on the endothelial layer.

The endothelium may modulate the tone of underlying vascular smooth muscle by the synthesis and release of endothelium-derived vasoactive factors and prostanoids. The histamine-induced relaxation in human internal mammary artery (Yang et al., 1989), rat middle cerebral artery (Benedito et al., 1991b) and bovine retinal artery (Benedito et al., 1991a) has been shown, amongst others, to be fully or partially dependent on the release of endothelial factors. However, McCarthy et al. (1994) suggest that histamine relaxation in small placental arteries may not be endothelial dependent.

EDRF stimulates guanylyl cyclase in vascular smooth muscle resulting in an increase in cyclic GMP levels and subsequent relaxation of smooth muscle cells (Moncada et al., 1991). Treatment with methylene blue, a guanylyl cyclase inhibitor, significantly reduced the relaxation produced by histamine in the oviductal arteries. The inhibitor of NO synthase, L-NAME, was used to verify if nitric oxide was responsible for the endothelium-dependent histamine vasodilation. The results suggest a regulation of the relaxant effect of histamine by the EDRF-NO pathway in oviductal segments since the NO inhibitor significantly inhibited histamine-induced relaxation. This inhibitory effect of L-NAME was reversed in the presence of L-arginine.

The concentration of L-NAME used to produce maximal inhibition of histamine-induced vasodilatation (10^{-6} M) was lower than that used by other authors to inhibit NO synthesis (10^{-4} M) (Rees et al., 1990). A possible explanation is that the endothelial enzyme responsible for the synthesis of NO in bovine oviductal artery is more sensitive to inhibition by L-NAME than enzymes from other arterial sources.

Although direct evidence that histamine induces the release of dilatory substances from the endothelium is not provided, two sets of results suggest that endothelial cells possess the biochemical pathway to convert L-arginine to nitric oxide which may participate in the relaxation of this vascular bed by histamine. Concentration-relaxant curves for L-arginine and concentration-contractile curves for L-NAME could be constructed to confirm substantial NO synthase activity in the vascular endothelium of the arteries when activated. Similar effects have been reported by other authors in various vascular beds (Moncada et al., 1991; Cosentino et al., 1993). Furthermore, Jovanović et al. (1994) demonstrated the ability of L-arginine to relax isolated rings of human uterine artery.

Histamine is also able to induce the release of prostanoids from the vascular endothelium. Relaxation of dog superior mesenteric artery caused by histamine is thought to be mediated by prostacyclin release from the arterial wall (Toda, 1984). It has been suggested that prostanoid production by rat aortic endothelial cells induced by histamine constitutes an additional mechanism for EDRF mediation of vasodilatation (Angus and Cocks, 1989). However, the failure of indomethacin, a cyclo-oxygenase inhibitor, to modify the maximal relaxation and the slight modification of sensitivity of the histamine response at the lower concentrations, indicates that the involvement of prostacyclin, or other cyclo-oxygenase products in the relaxation response to histamine, is minor. The present authors and other investigators have also demonstrated that the exposure of rat middle cerebral artery (Benedito et al., 1991b) and common carotid artery

(Krstić et al., 1992) to the action of indomethacin does not affect the concentration–response curve to histamine.

In summary, the principal mechanism for the relaxant response to histamine seems to be the release of nitric oxide through stimulation of histamine H_1 receptors mainly located on the endothelium. The involvement of prostacyclin in the relaxant effect of histamine was found to be minor. The action of histamine on a limited population of contraction-evoking H_3 receptors (located in smooth muscle) may counteract the relaxant effect mediated by histamine H_1 receptors.

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