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Effect of histamine and histamine analogues on human isolated myometrial strips

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1 The effect of histamine and histamine H_1- and H_2-receptor agonists on isolated myometrium strips of premenopausal women has been examined. The effect of acetylcholine was also determined.

2 Histamine, 2-pyridylethylamine, 4-methylhistamine and acetylcholine, but not dimaprit, produced a concentration-related contractile response in human isolated myometrial strips. Histamine also produced a further contraction in human isolated myometrial strips precontracted with KCl (55 mM).

3 The contractile response to histamine was antagonized by the histamine H_1-receptor angatonist, clemizole (0.1 μM) but was potentiated by the histamine H_2-receptor antagonist, ranitidine (10 μM). Clemizole (0.1 nM to 10 nM) competitively antagonized the contractile effect of 2-pyridylethylamine ($-\log K_B = 10.5 \pm 0.5$). The concentration-response curve for acetylcholine was displaced to the right by atropine 0.1 μM.

4 Atropine (0.1 μM), propranolol (0.1 μM), prazosin (0.1 μM) and indomethacin (1 μM) failed to modify the contractile response to histamine.

5 In human isolated myometrial strips precontracted with KCl (55 mM), clemizole at 1 μM completely abolished the contractile response to histamine and revealed a concentration-dependent relaxation. Dimaprit alone and 4-methylhistamine (in the presence of clemizole), produced concentration-related relaxation with a magnitude similar to that in response to histamine. The relaxant response to dimaprit was antagonized by ranitidine.

6 It is concluded that human isolated uterine strips possess histamine H_1- and H_2-receptors: the former mediating contraction and the latter relaxation. The predominant response to histamine in this tissue is contraction.

Keywords: Histamine; histamine receptors; histamine H_1-receptor agonists; histamine H_2-receptor agonists; human isolated myometrium

Introduction

Responses of uterine smooth muscle to histamine vary widely between species. Thus, histamine produces contractions of guinea-pig uterine horns, but causes relaxation of the rat uterus. The receptors involved in these two effects are classified as histamine H_1- and H_2-receptors respectively (Black *et al.*, 1972; Tozzi, 1973; Goyal & Verma, 1981; Rubio *et al.*, 1982; Cortijo *et al.*, 1984). However, there is little information on the effect of histamine on the human uterus, and previous studies reported in the literature have come to differing conclusions. Farmer & Lehrer (1966) reported that histamine had a contractile effect on uteri *in vitro*, but in contrast Dai *et al.* (1982) failed to observe any effect of histamine on spontaneous activity in isolated myometrium. We have recently reported a contractile response to histamine in human myometrial strips (Martínez-Mir *et al.*, 1990) and the object of the present investigation was to establish the role of histamine H_1- and H_2-receptors in this response.

Methods

Tissues

Myometrial tissues were obtained from 60 non-pregnant women who underwent hysterectomy for various pathological gynaecological conditions. All patients were pre-menopausal and were 27–57 years of age. These women were operated on for gynaecological conditions that did not affect the uterus ($n = 16$), for uterine myoma ($n = 33$) and for uterine adenomyosis ($n = 11$). The pathological diagnosis and the phase of menstrual cycle were checked by histological examination and the final diagnoses were as described previously (Martínez-Mir *et al.*, 1990).

Isolated strips of uterus

Immediately after abdominal hysterectomy, muscle strips from an intermediate layer were cut longitudinally from the anterior part of the corpus of the uteri. Care was taken to use only specimens from muscle tissue which appeared to be non-pathological. The tissue was rapidly immersed in Jalon solution of the following composition (in mM): NaCl 155.17, KCl 5.68, $CaCl_2$ 0.41, $NaHCO_3$ 5.95 and glucose 2.78. Samples were then transported to the laboratory. The tissue samples were stored at 4°C until the next day. Longitudinal muscle strips measuring approximately 2 × 3 × 35 mm were dissected out and then suspended under a load of 1 g in a 20 ml organ bath containing Jalon solution aerated with 5% CO_2 in O_2. The temperature was maintained at 31 ± 1°C in order to prevent spontaneous contractions. Isometric tension was recorded by a Ugo Basile C 40 7010 transducer connected to a Ugo Basile mod Gemini 7070 recorder. The preparations were allowed to equilibrate in Jalon solution for 1 h before any drugs were added. At the end of the equilibration period, two different experiments were performed: (i) concentration-response curves to histamine or other agonists were obtained by adding the drugs cumulatively; (ii) a submaximal well-maintained plateau-contraction was obtained by adding KCl (55 mM) and then a cumulative concentration-response curve to agonists was performed as in (i). Only one complete dose-response curve to histamine was constructed for each myometrial strip, in view of the tachyphylaxis to histamine that has been reported for the rat uterus (Tozzi,

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1973; Cortijo *et al.*, 1984). Therefore, separate strips from the same uteri were used in parallel experiments, one strip acting as the control for the other. Strips were incubated with antagonists for 15 min, before the addition of agonists. Immediately after completion of the experiment, the tissue was removed from the bath, heated at 65 ± 1°C for 24 h and then weighed on a precision balance.

Analysis of data

Contractile responses to agonists were determined as changes in isometric tension and transformed into tension (i.e. force (g).g^{-1} tissue dry weight (g_F/g_W)). Relaxant responses to agonists were expressed as the percentage inhibition of the KCl-induced contraction.

Maximum response (E_{max}) and the half-maximal effective concentrations (EC_{50}) of agonists were calculated from concentration-response curves by fitting the experimental data to a logistic equation by non-linear regression analysis (Graph Pad Software; San Diego, California, U.S.A.). The pA_2 value was calculated according to the method of Arunlakshana & Schild (1959) as previously described (Aguilar *et al.*, 1986).

All data are given as mean ± s.e.mean. Significance of differences was assessed with either a paired or unpaired Student's *t* test at a 5% significance level.

Drugs

The following drugs were used: acetylcholine chloride, atropine sulphate, histamine dihydrochloride, propranolol hydrochloride and ranitidine hydrochloride (Sigma Chemical Co St. Louis. MO U.S.A.), dimaprit, 4-methylhistamine dihydrochloride and 2-pyridylethylamine dihydrochloride (Smith-Kline Beecham R & D), clemizole hydrochloride (Schering España SA), prazosin hydrochloride (Pfizer), indomethacin (Liade). All drugs were prepared in Jalon solution before being added to the bath. The histamine stock solution was adjusted to pH = 7.4 with sodium hydroxide. Stock solutions of indomethacin were prepared in absolute ethanol; the final concentration of ethanol in the organ bath has been shown previously not to alter either the baseline tension or the drug-induced responses (Fuchs & Fuchs, 1973).

Results

Contractile effects of agonists on human isolated myometrium in the resting state

Concentration-response curves for histamine, 2-pyridylethylamine, dimaprit, 4-methylhistamine and acetylcholine are shown in Figure 1. With the exception of dimaprit, all agonists produced concentration-related contractions of human uterine strips.

Histamine increased the force of contraction with a maximum of 48.9 ± 3.6 g_F/g_W. The EC_{50} value was 40.2 ± 10.6 μM ($n = 60$). The histamine receptor agonists, 2-pyridylethylamine and 4-methylhistamine also produced a contractile effect of isolated myometrial strips. 2-Pyridylethylamine appeared to be a partial agonist (E_{max} = 30.1 ± 7.2; EC_{50} = 14.5 ± 6.6 μM), while 4-methylhistamine produced an appreciable response only at concentrations ⩾ 0.1 mM and even at 1 mM the response was much less than that to histamine. The selective histamine H_2-receptor agonist, dimaprit, up to 0.1 mM, had no apparent effect.

The myometrial force of contraction was also increased in a dose-related manner by acetylcholine. It reached a maximum effect of 31.3 ± 6.7 g_F/g_W. The EC_{50} value was 4.1 ± 1.5 μM. The response to 55 mM KCl was 53% of the maximum induced by histamine (Figure 1).

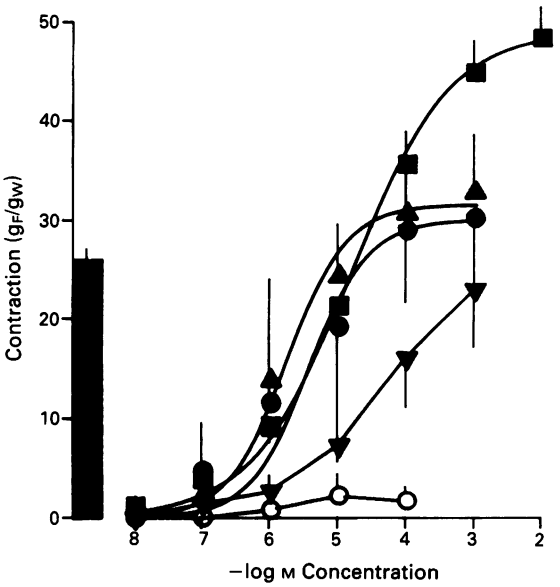

Figure 1 Concentration-response curves to histamine ($n = 60$) (■), 2-pyridylethylamine ($n = 6$) (●), 4-methylhistamine ($n = 6$) (▼), dimaprit ($n = 6$) (○) and acetylcholine ($n = 8$) (▲), and contractile response to K^+ 55 mM ($n = 15$) (histogram) in human isolated myometrial strips. g_F/g_W = force(g).g^{-1} tissue dry weight. *n* is the number of experiments. The curves for histamine, 2-pyridylethylamine and acetylcholine are the best-fit lines calculated as described under Methods.

Effect of antagonists

The histamine H_1-receptor blocker, clemizole (0.1 μM), shifted the concentration-response curve for histamine-induced contraction to the right without any significant change in the maximum response. Ranitidine (1 μM), a selective histamine H_2-receptor antagonist, did not modify the contractile effect of histamine, but 10 μM ranitidine displaced the histamine concentration-response curve to the left (Figure 2).

The contractile response to histamine was not significantly altered by propranolol (0.1 μM), prazosin (0.1 μM), atropine (0.1 μM) or indomethacin (1 μM). The EC_{50} values were: 49 ± 39 μM; 20 ± 7 μM; 42 ± 4 μM and 22 ± 12 μM, respectively; the EC_{50} value for the control group was 25 ± 10 μM.

The histamine H_1-receptor antagonist, clemizole (0.1 nM to 10 nM), produced a parallel shift of concentration-response curves for 2-pyridylethylamine to the right, without affecting the maximal response. A Schild plot is shown in Figure 3. The slope of regression line was not significantly different from unity (0.96 ± 0.10) and the $-\log K_B$ value obtained for clemizole was 10.5 ± 0.5.

The muscarinic receptor blocker, atropine (0.1 μM), shifted the concentration-response curve for acetylcholine to the right increasing the EC_{50} 333 fold ($n = 6$), without any modification in the maximal effect. The dissociation constant calculated for atropine was 0.6 nM.

Relaxant effects of agonists on human isolated myometrium precontracted with KCl

Relaxant effects of histamine were investigated by cumulative addition to isolated myometrial strips precontracted with KCl. As shown in Figure 4a, histamine at low concentrations (0.01, 0.1 and 1 μM) did not modify the K^+-induced contractions, but higher concentrations of histamine (10 μM to 10 mM) produced a further contraction.

Clemizole, 1 μM, acting alone, had a relaxant effect (21.6 ± 7.2%) on the contraction produced by 55 mM KCl. After blockade of the histamine H_1-receptor with clemizole, the contractile effect of histamine was abolished and a relaxa-

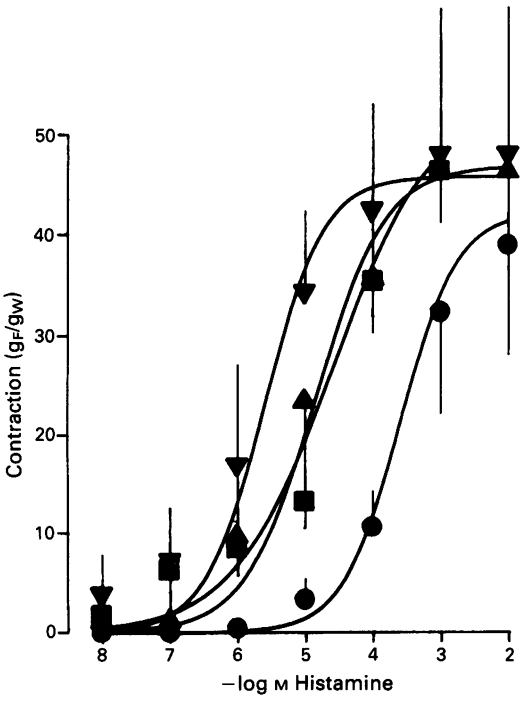

Figure 2 Concentration-response curves to histamine in human isolated myometrial strips. Control ($n = 24$) (■); in the presence of 1 μM ($n = 8$) (▲) and 10 μM ($n = 8$) (▼) ranitidine; in the presence of 0.1 μM clemizole ($n = 8$) (●). g_F/g_W = force(g).g^{-1} tissue dry weight. n is the number of experiments. The curves for histamine, 2-pyridylethylamine and acetylcholine are the best-fit lines calculated as described under Methods.

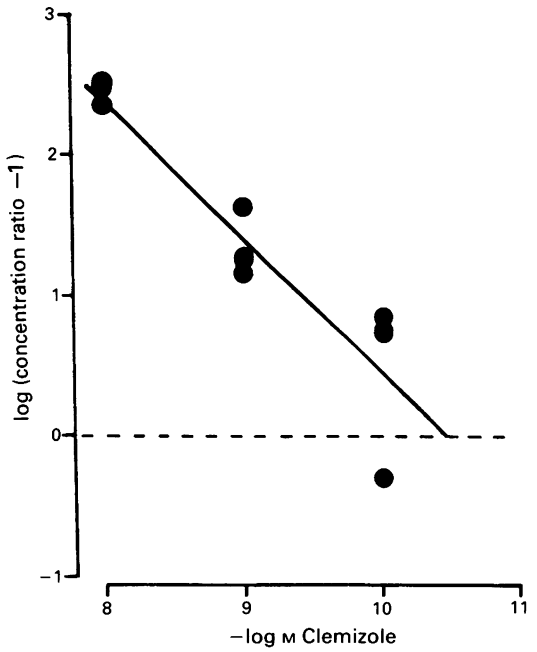

Figure 3 Schild plot of the antagonism by clemizole of the response to 2-pyridylethylamine. The line drawn was calculated by linear-regression analysis.

tion response observed (Figure 4a). 4-Methylhistamine in the presence of 1 μM clemizole also relaxed K^+-precontracted strips ($E_{max} = 24.5 \pm 6.1\%$; $EC_{50} = 9.9 \pm 8.6$ μM).

Dimaprit produced a concentration-related relaxation of isolated uterine strips depolarized with K^+. The EC_{50} value was 18.2 ± 10.3 μM and the maximal effect was $31.7 \pm 8.1\%$. Ranitidine 1 μM produced a significant decrease in the relaxant response to dimaprit and 10 μM rantidine abolished it (Figure 4b).

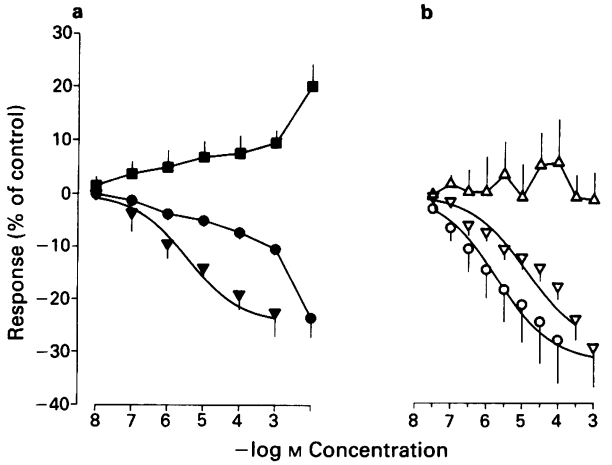

Figure 4 (a) Concentration-response curves to histamine alone ($n = 6$) (■), histamine in the presence of 1 μM clemizole ($n = 6$) (●) and 4-methylhistamine in the presence of 1 μM clemizole ($n = 6$) (▼) in human isolated myometrial strip precontracted with KCl (55 mM). The response is expressed as a percentage of the response to KCl alone or KCl plus clemizole. (b) Concentration-response curves to dimaprit alone ($n = 6$) (○), dimaprit in the presence of 1 μM ($n = 6$) (▽) and 10 μM ($n = 6$) (△) ranitidine in human isolated myometrial strip precontracted with KCl (55 mM). n is the number of experiments. The curves for 4-methylhistamine in the presence of 1 μM clemizole, dimaprit alone and dimaprit in the presence of 1 μM ranitidine are the best-fit lines calculated as described under Methods.

Discussion

We have demonstrated that histamine causes a dose-dependent contractile effect in human isolated myometrial strips both in the resting and submaximally precontracted states of the tissue. However, after blockade of histamine H_1-receptors, histamine produced a slight inhibitory effect in strips precontracted by KCl. The contractile effect of histamine observed in this study resembles that described by Goyal & Verma (1981) in guinea-pig isolated uterus, where histamine produced a dose-related contraction with a similar potency. Our results also confirmed those described by Farmer & Lehrer (1966) in human isolated myometrium, although a comparative analysis of the findings is difficult because the authors did not give the details of their experimental procedure and used only single doses of histamine. In contrast, our results do not accord with the observations of Dai *et al.* (1982), who showed that histamine did not influence the spontaneous activity of human isolated myometrial strips. Isolation of the strips from uteri in different phases of the oestrus cycle does not explain the discrepancy, since we have previously found no significant changes in the contractile uterine effect of histamine during the menstrual cycle (Martínez-Mir *et al.*, 1990). On the other hand, the contractile responses to acetylcholine and potassium that we observed agree well with previous findings in human uterus (Sandberg *et al.*, 1957; Nakanishi & Wood, 1971; Sanger & Bennet, 1981). Moreover, the antagonism of the response to acetylcholine by atropine is in accord with binding studies in this tissue (Vauquelin *et al.*, 1984). The lack of modification by muscarinic, α- and β-adrenoceptor blockade and prostaglandin synthesis inhibition of the contractile response to histamine, argues against the participation of endogenous acetylcholine, catecholamines and prostaglandins and indicates that this effect is probably due to a direct stimulation of histamine receptors. The fact that the dose-response curve to histamine was shifted to the right by clemizole (0.1 μM) but not modified by ranitidine (1 μM) suggests that the receptor involved in the contractile effect of histamine in human isolated uterine strips is the histamine H_1-receptor subtype. However, the concentration-ratio observed with clemizole is lower than expected; moreover, ranitidine (10 μM) shifted to

the left the dose-response curve to histamine. All this suggests some participation of histamine H_2-receptors. To characterize further the receptor subtype mediating contractile responses to histamine, we examined the effect of 2-pyridylethylamine, 4-methylhistamine and dimaprit. The selective histamine H_1-receptor agonist, 2-pyridylethylamine, produced a concentration-dependent contractile effect with a similar EC_{50} to histamine. Although 2-pyridylethylamine appears to be a partial agonist (Figure 1), paired experiments showed that the maximal effects of both compounds were similar. This finding agrees with previous results in various tissues, including the guinea-pig isolated uterus, although in this latter study 2-pyridylethylamine was shown to be less potent than histamine (Duncan *et al.*, 1980; Goyal & Verma, 1981; Schmidt *et al.*, 1987). The contractile effect of 4-methylhistamine agrees with a similar result in guinea-pig uterus and confirms previous findings were 4-methylhistamine was a histamine H_1-agonist (Goyal & Verma, 1981; Black *et al.*, 1972). This underlines the importance of the numbers of histamine H_1-: histamine H_2-receptors in a tissue for the observed effect of agonists which are not very selective (Barker & Hough, 1983).

The $-\log K_B$ value (pA_2) for clemizole, 10.5 ± 0.5, agrees closely with those obtained by Aguilar *et al.* (1986) (10.45 ± 0.44) and Martínez-Mir *et al.* (1988) (10.54 ± 0.44) in other tissues and supports the existence of histamine H_1-receptor in human isolated myometrium.

In human isolated uterus submaximally contracted with KCl, histamine still caused a contractile effect. A weak histamine-mediated relaxation occurred only in the presence of the histamine H_1-receptor antagonist clemizole (1 μM). Moreover, dimaprit and 4-methylhistamine (in the presence of H_1-blockade) also produced a similar relaxant effect. Although 4-methylhistamine and dimaprit have been reported to be less potent than histamine (Hill, 1990), we found that both were more potent. However, in guinea-pig airways, for example, both agonists were equipotent and the EC_{50} calculated with dimaprit and 4-methylhistamine (in the presence of H_1-blockade), were similar to those obtained in the present study. No comparison with histamine was made by the authors (Tomioka & Yamada, 1982). A complicating factor in the analysis of the response to histamine is the relaxation produced by clemizole. Given that studies in our laboratory have shown that clemizole is a selective histamine H_1-receptor antagonist (Aguilar *et al.*, 1986; Martínez-Mir *et al.*, 1988), a possibility that needs investigation is that KCl might release endogenous histamine. The inhibition by ranitidine of the relaxant response to dimaprit, taken together with the potentiation of the histamine-induced contraction by 10 μM ranitidine, is evidence for histamine H_2-receptor-mediated relaxation. Similar results have been described by Ginsburg *et al.* (1980) in human arteries. A predominance of the histamine H_1-receptor-mediated contraction over the histamine H_2-receptor-mediated relaxation can be explained by the balance of apparent affinities for histamine H_1- and H_2-receptors. Consistent with this is the fact that the selective histamine H_2-receptor agonist, dimaprit, relaxed the precontracted uterine strip in the absence of H_1-blockade.

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