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A study of the histamine H_2-receptor mediating relaxation of the parenchymal lung strip preparation of the guinea-pig

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1 The relaxation produced by several H_2-receptor agonists and forskolin was investigated on strips of guinea-pig lung parenchyma.

2 Dimparit, 1 μM to 10 mM, 4-methyl histamine, 0.5 μM to 100 μM and impromidine, 10 nM to 1 μM, had no effect on the tone of the unstimulated strips of lung parenchyma but caused a dose-dependant relaxation of strips that were contracted by 2-pyridylethylamine (2-PEA), 15 μM. Forskolin, 10 nM to 4 μM, produced a dose-dependent relaxation of both the stimulated and unstimulated lung strips.

3 The muscarinic antagonist atropine, 1 μM, and the β_2-adrenoceptor antagonist propranolol, 10 μM, had no effect on the dose-response curve for dimaprit-induced relaxation of the lung strip.

4 The dose-response curve for dimaprit was shifted to the right in a dose-dependent manner by increasing concentrations of a variety of H_2-antagonists. Schild plots produced a straight line for all the H_2-antagonists with slopes not significantly different from unity. The equilibrium dissociation constants for the H_2-antagonists on the lung strip preparation were similar to those previously reported for inhibition of the chronotropic activity of histamine on guinea-pig right atria and inhibition of [^{3}H]-tiotidine binding to homogenates of guinea-pig lung parenchyma.

Introduction

Histamine stimulates contraction of the smooth muscle of the guinea-pig ileum and the guinea-pig trachea and these effects are blocked by low concentrations of mepyramine, an H_1-receptor antagonist (Dews & Graham, 1946). However, there are some effects of histamine such as stimulation of acid secretion by the stomach, the positive chronotropic action on the heart and relaxation of the rat uterus, which cannot be antagonized by mepyramine. Ash & Schild (1955) suggested that these actions of histamine were mediated by a different receptor from those actions that are mepyramine-sensitive. The mepyramine-insensitive effects of histamine are antagonized by compounds such as metiamide (Black *et al.*, 1973), cimetidine (Brimblecombe *et al.*, 1975) and ranitidine (Bradshaw *et al.*, 1979), and the receptors interacting with these compounds have been defined as H_2-receptors (Black *et al.*, 1972).

In the lung, histamine contracts smooth muscle *in vitro* and *in vivo* by stimulation of H_1-receptors (Chand & Eyre, 1975). However, histamine stimulates H_2-receptors mediating relaxation of tracheobronchial smooth muscle from a variety of different species such as guinea-pigs (Chand & Deroth, 1979), rhesus monkeys (Chand *et al.*, 1980) and man (Vincenc *et al.*, 1984). Also, antagonists acting at H_2-receptors enhance the response of guinea-pig bronchial smooth muscle to histamine (Eyre, 1977; Dunlop & Smith, 1977; Okpako *et al.*, 1978; Drazen *et al.*, 1980).

Dimaprit, a selective H_2-agonist (Parsons *et al.*, 1977) has been shown to cause reversal of pre-existing anaphylactic contractions of guinea-pig lung parenchymal strips (Chand, 1979) and there is also evidence that, in the guinea-pig, dimaprit has a bronchodilator activity *in vivo* (Drazen *et al.*, 1979; Bottomley *et al.*, 1984). Both these effects were blocked by the H_2-antagonist metiamide (Black *et al.*, 1973). Dimaprit is, therefore, a useful tool to use when studying the effect of histamine H_2-receptors in guinea-pig airways.

In disease states such as asthma, where bronchial hyperreactivity to histamine is one of the characteristic features, the site of action of immunologically released pharmacological mediators such as histamine is mainly in the terminal airways (Drazen & Austen, 1974)

rather than the proximal airways. We have, therefore, used the lung parenchymal strip (Lulich *et al.*, 1976) to study the effect of dimaprit on smooth muscle in the small airways of the lung and characterize the H_2-receptor in lung tissue. The lung strip is known to possess several different contractile elements, the most important being airway smooth muscle and vascular smooth muscle (Clayton *et al.*, 1981; Bertram *et al.*, 1983). Although we believe that 2-pyridylethylamine (2-PEA), a specific H_1-agonist (Durant *et al.*, 1975), and histamine contract only airway smooth muscle, the role of other contractile elements, particularly vascular smooth muscle, cannot be excluded.

Methods

Hartley guinea-pigs of either sex aged 4 to 6 months and weighing 400–600 g were killed by stunning and exsanguination by sectioning of the carotid arteries. The heart and lungs were removed together and placed in Tyrode solution maintained at 37°C. Strips of lung approximately 15 mm × 3 mm × 3 mm were cut from the periphery of each lobe essentially as described previously (Drazen & Schneider, 1978) and mounted under a tension of 0.5 g weight in 2 ml organ baths containing Tyrode solution maintained at 37°C. The strips were gassed continuously with a mixture of 95% O_2 and 5% CO_2. The strips were allowed to rest for 1 h and changes of bathing medium were made every 10 min. During this time period all the strips relaxed until a new steady resting level of tone was produced. All changes in tissue length were recorded isotonically by means of photoelectric transducers connected through a pre-amplifier to Linseis L600 pen recorders. The transducer was calibrated so that actual displacement in mm resulting from tissue contraction could be related to the magnitude of the pen movement on the recorder.

The composition of the Tyrode solution was (mM): NaCl 137, KCl 2.7, CaCl 1.8, MgCl 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6

Addition of 2-pyridylethylamine

Addition of 2-PEA, 100 μM, produced 70 ± 5% of the maximum response to 2-PEA on the lung strip. After the tissue had been incubated with histamine, 100 μM, which produced a maximum contraction, for 20 min and then washed thoroughly, addition of 2-PEA, 100 μM, produced 73 ± 8% of the maximum response to 2-PEA. In all experiments, the tissue was initially contracted with a maximally effective concentration of histamine, 100 μM, and was then washed several times until the resting level of tone was restored. As stated, this did not affect subsequent responses to 2-PEA and was done because it is accepted that 'priming' a tissue with a maximally effective concentration of agonist, in this case histamine, stabilizes the tissue to subsequent doses of the same or a different agonist. Such pretreatment with an agonist can also reduce the variability sometimes seen when dose-response curves are constructed with single doses given in random order rather than being given as progressively increasing doses or by cumulative addition. Dose-response curves to 2-PEA were constructed by giving single doses in randomized order. When the response for a particular dose reached a maximum the bath fluid was replaced and the preparation was allowed to return passively to its resting tension, which took between 10 and 20 min depending on the size of the responses. The dose-response curve was repeated after allowing the tissue to rest for 1 h. All the contractile responses obtained have been expressed as a percentage of the maximum contraction to 2-PEA for that particular strip.

Cumulative addition of an H_2-agonist or forskolin

Doses of an H_2-agonist or forskolin were added in a non-random, cumulative order starting with the lowest dose, either to lung parenchymal strips which had been precontracted with 2-PEA, 100 μM, or to unstimulated strips. 2-PEA at a concentration of 100 μM gave a response that was 70% of the maximum response to 2-PEA. This level of response was chosen to avoid working at the top of the dose-response curve but it gave an adequate level of contraction for study of the effect of drugs inducing relaxation. Cumulative addition of H_2-agonist or forskolin was used because the tissue was pre-contracted with 2-PEA and it was considered undesirable to disturb the tissue or extend the duration of the experiment by repeated changes of bath fluid. The next dose was added when no further relaxation to the first dose was observed and a new steady level of tone had been achieved: this took 10 to 15 min. Cumulative addition of H_2-agonist or forskolin continued until there was no further relaxation of the lung strip. All relaxation responses produced have been expressed as a percentage reduction of the contraction produced by 2-PEA, 100 μM, on that particular strip. The exception was for the relaxation of resting tone produced by forskolin, where the responses have been expressed as a percentage of the maximum relaxation produced by forskolin on that particular strip where 100% maximum relaxation is equivalent to a lengthening of the lung strip of 1.2 ± 0.3 mm (mean ± s.e.mean).

Antagonist experiments

In these experiments lung strips were divided into pairs. A control, cumulative dose-response curve to dimaprit was established on one strip and on the other

strip a cumulative dose-response curve to dimaprit was established in the presence of the concentration of the antagonist being investigated, according to the experimental protocol. After this, the strips were washed thoroughly and allowed to rest for 2 h during which time the resting level of tone was restored. The procedure described above was reversed on the two strips and the results pooled. In the experiments where an H_2-antagonist was present, the lung strips were equilibrated with the H_2-antagonist for between 30 and 45 min before the dose-response curve to dimaprit was determined. From binding studies (Foreman *et al.*, 1985) it is clear that this time period is sufficient for the H_2-antagonist to reach equilibrium with any H_2-receptors in the lung strip. All relaxation responses produced have been expressed as a percentage of the maximum control relaxation to dimaprit on a particular strip.

Evaluation of data

All EC_{50} values were estimated from dose-response curves fitted to the data points by a computerised, non-linear, least squares estimate (Marquardt, 1963).

In experiments involving the histamine H_2-antagonists, the dose-response curves in the absence and presence of the antagonist were constrained to be parallel. The magnitude of the antagonism was calculated using the dose-ratio of Gaddum *et al.* (1955) comparing the EC_{50} values obtained in the presence and absence of antagonist. The dose-ratios were examined according to the method of Arunlakshana & Schild (1959) where least squares regression analysis was used to determine the relationship between log (dose-ratio − 1) and log of the molar concentration of antagonist.

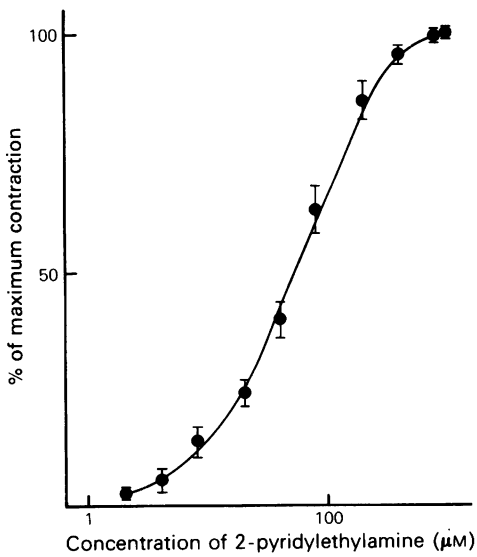

Figure 1 Dose-response curve showing the contraction of the guinea-pig lung strip induced by 2-pyridylethylamine (2-PEA). Responses have been expressed as a percentage of the maximum contraction to 2-PEA for a particular strip. Points are means of five determinations and the vertical bars represent s.e.mean.

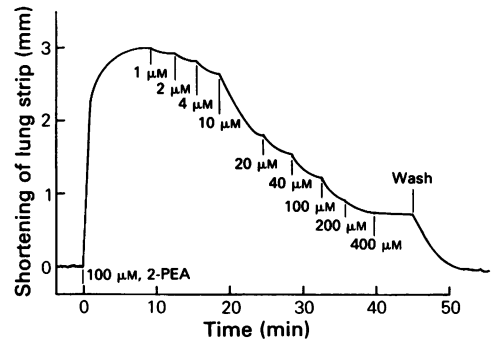

Figure 2 The effect of cumulative addition of dimaprit on the contraction produced by 2-pyridylethylamine (2-PEA), 100 μM, in a lung strip. A single experiment.

Results

The effect of H_2-agonists on the unstimulated lung strip

Dimaprit, 1 μM to 1 mM, 4-methyl histamine, 0.5 μM to 100 μM or impromidine, 10 nM to 4 μM, had no effect on the level of tone in the unstimulated lung strip. Therefore, tone was induced in the lung strips by precontracting them to examine the relaxant effect of these H_2-agonists.

The effect of 2-pyridylethylamine on the lung strip

Carbachol, 0.1 μM to 100 μM and histamine, 1 μM to 1 mM were not used to induce tone in the lung strips because contractions produced by these two compounds were not sustained.

2-PEA, a specific H_1-agonist (Durant *et al.*, 1975), produced dose-related contractions of strips of guinea-pig lung parenchyma at concentrations between 1 μM and 1 mM. A dose-response curve for 2-PEA has been constructed from the results pooled from five experiments (Figure 1). The EC_{50} for 2-PEA was found to be 50 ± 6 μM (mean ± s.e.mean) and the EC_{50} for histamine was 6 ± 2 μM. The maximum response to 2-PEA occurred with a concentration of approximately 800 μM and was equivalent to 98.5% of the maximum response to histamine, which was achieved with a concentration of 100 μM. In a paired *t* test of the individual experimental results, the maximum response to 2-PEA was found not to be

significantly different from the maximum response to histamine ($P > 0.05$). A concentration of 2-PEA of 100 μM produced approximately 70% of the maximum response and this was used to induce tone in the lung strips prior to examining any relaxant effects of the H_2-agonists. Contractions produced by 2-PEA, 100 μM, were sustained for at least 1 h. Taking the contraction produced by 2-PEA, 100 μM at 5 min after addition as unity, the contraction recorded after 20, 40 and 60 min was 1.1, 0.9 and 1.0 times the 5 min value.

The effect of H_2-agonists on the lung strip contracted by 2-pyridylethylamine

Cumulative addition of dimaprit to give concentrations within the range 1 μM to 400 μM produced a dose-dependent relaxation of the lung parenchymal strips which had been precontracted by 2-PEA, 100 μM, (Figure 2). A cumulative concentration of dimaprit of greater than 400 μM produced no further relaxation of the lung strips. After washing, the tissue returned to its original resting tension. A dose-response curve has been constructed from the results pooled from five experiments (Figure 3). The EC_{50} for dimaprit was found to be 17.4 ± 3.3 μM. The maximum relaxation produced by cumulative addition of dimaprit to give a final concentration of 400 μM was a reduction of 89 ± 2% of the contraction produced by 2-PEA, 100 μM and the maximum relaxation produced by a single dose of dimaprit of 400 μM was a reduction of 82 ± 12% of the contraction produced by 2-PEA, 100 μM. A non-paired t test of the individual experimental results showed no significant difference ($P > 0.05$) between the maximum relaxation produced by cumulative addition or single addition of dimaprit to give a final concentration of 400 μM in both cases.

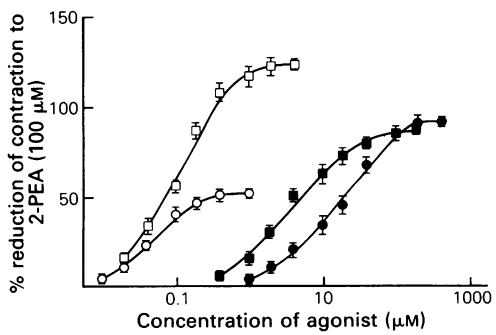

Figure 3 Dose-response curves showing the effect of dimaprit (●), 4-methyl histamine (■), impromidine (○) and forskolin (□) on the lung strip which had been contracted by 2-pyridylethylamine (2-PEA), 100 μM. Responses have been expressed as a percentage reduction of the contraction produced by 2-PEA (100 μM) for a particular strip. Points are means of four to six determinations and the vertical bars represent s.e.mean.

4-Methyl histamine, 0.5 μM to 100 μM and impromidine, 10 nM to 1 μM, also produced a dose-dependent relaxation of the lung strip contracted by 2-PEA, 100 μM. Dose-response curves have been constructed for each of these compounds from the results pooled from four experiments (Figure 3). The mean EC_{50} values for 4-methyl histamine and impromidine were found to be 4 ± 2 μM and 0.04 ± 0.01 μM respectively. The maximum relaxation produced by 4-methyl histamine was equivalent to 85 ± 2% and that by impromidine 52 ± 6% reversal of the contraction produced by 2-PEA, 100 μM. These maximum relations occurred with concentrations of 200 μM and 1 μM respectively.

The effect of forskolin on the lung strip

Forskolin does not combine with histamine H_2-receptors but has a direct stimulatory effect on adenylate cyclase which is the enzyme thought to be activated when H_2-receptors are stimulated. Cumulative addition of forskolin to give concentrations within the range 10 nM to 4 μM produced a dose-dependent relaxation of both unstimulated lung strips and lung strips which had been precontracted by 2-PEA, 100 μM. In each case, dose-response curves have been constructed by pooling results from four experiments (Figures 3 and 4). The mean EC_{50} values for forskolin

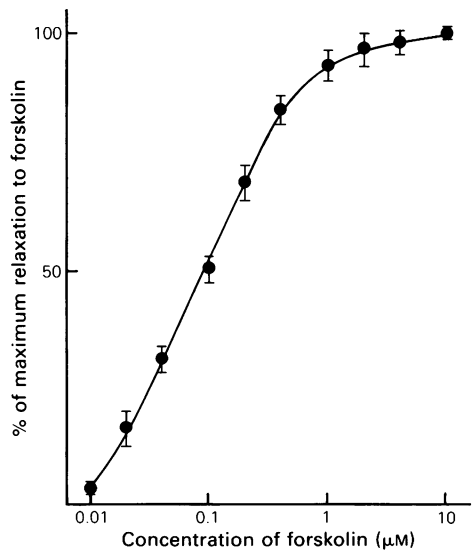

Figure 4 Dose-response curve showing the effect of forskolin on the resting level of tone in the guinea-pig lung strip. Responses have been expressed as a percentage of the maximum relaxation produced by forskolin on a particular strip. This maximum relaxation is equivalent to a mean (± s.e.mean) lengthening of the lung strip of 1.2 ± 0.3 mm.

were found to be $0.11 \pm 0.01\,\mu M$ for relaxation of resting tone and $0.16 \pm 0.04\,\mu M$ for relaxation of lung strips pre-contracted by 2-PEA, $100\,\mu M$.

In the experiments using the lung strips with resting tone, the maximum relaxation produced by forskolin was equivalent to a mean lengthening of the lung strip of 1.2 ± 0.3 mm and occurred with a concentration of $2.5\,\mu M$. When precontracted lung strips were used the maximum relaxation produced by forskolin was a reduction of 130% of the contraction produced by 2-PEA, $100\,\mu M$ and occurred with a concentration of forskolin of $1\,\mu M$. The relaxation produced by forskolin on the 2-PEA-contracted strip (Figure 3) is an effect of forskolin on basal as well as stimulated contraction.

The effect of atropine and propranolol on the response to dimaprit

To establish that dimaprit was not causing relaxation of the lung strip by release of catecholamines we examined the effect of propranolol on the response to dimaprit and to determine whether released acetylcholine was affecting the level of tone in the lung strip and thereby modifying the dimaprit response, we examined the effect of atropine on the dimaprit dose-response curve.

Neither atropine, 0.1 to $10\,\mu M$, nor propranolol, 1 to $100\,\mu M$, had any effect themselves on the level of tone in the unstimulated guinea-pig lung strip or on the lung strip which had been precontracted by 2-PEA, $100\,\mu M$. The effects of atropine, $1\,\mu M$ and propranolol, $10\,\mu M$ have been studied on the cumulative dose-response curve to dimaprit in the lung strip. The mean EC_{50} values for dimaprit in the absence and presence of atropine, $1\,\mu M$ were calculated as $15 \pm 2\,\mu M$ ($n = 6$) and $18 \pm 3\,\mu M$ ($n = 6$) and in the absence and presence of propranolol, $10\,\mu M$ as $25 \pm 5\,\mu M$ ($n = 6$) and $25 \pm 10\,\mu M$ ($n = 6$) respectively. The differences between the EC_{50} values for dimaprit in the absence or in the presence of these drugs were not statistically significant ($P > 0.05$).

The effect of H_2-antagonists on the response to dimaprit

All of the H_2-antagonists examined, at concentrations up to $100\,\mu M$, had no effect on the level of tone in the unstimulated lung strip. Using the experimental design described in the Methods, the effects of several H_2-antagonists on the dose-response curve for dimaprit were studied. Increasing concentrations of the H_2-antagonists caused a graded, parallel displacement to the right of the dimaprit dose-response curve, with no reduction in the maximum response to dimaprit. The effect of the H_2-antagonist burimamide is shown in Figure 5 as an example. As described in the

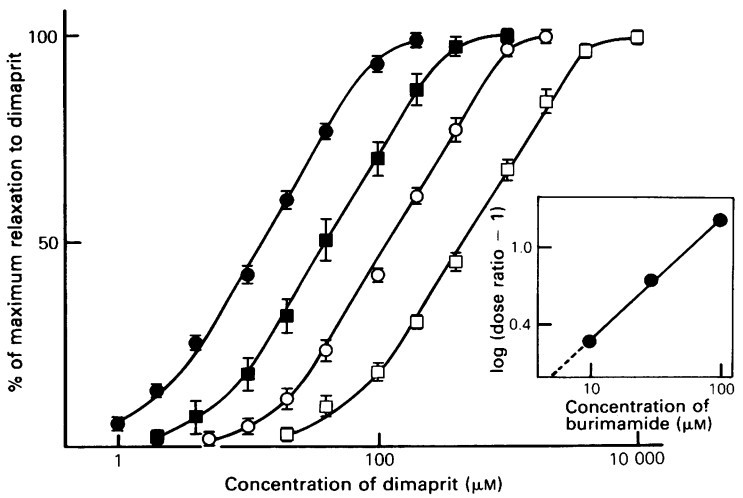

Figure 5 The antagonism of the response to dimaprit by burimamide. Dose-response curves are to dimaprit alone (●) and dimaprit in the presence of burimamide, $10\,\mu M$ (■), $30\,\mu M$ (○) and $100\,\mu M$ (□). The curves have been constrained to be parallel. Responses have been expressed as a percentage of the maximum relaxation produced by dimaprit on a particular strip. Points are means of 65 to 72 determinations in the control curve and 4 to 6 determinations in the other curves. The vertical bars represent s.e.mean. The figure shows the control curve constructed by pooling results of all experiments. However the K_B values and Schild plots have been calculated from analysis of dose-response curves in the absence and presence of antagonist that were obtained in the same experiment. Inset: Schild plot constructed as described in Methods, uses dose-response curves for dimaprit in the presence and absence of burimamide obtained in individual experiments.

Table 1 The effect of H_2-antagonists on the dose-response curve for dimaprit

H_2-antagonist	*Concentration of H_2-antagonist* (μM)	n	*Dose-ratio* (mean with 95% confidence limits)	K_B (μM)	*Slope of Schild plot*
Tiotidine	0.01	4	3.8 (2.5– 5.0)		
	0.03	4	10.5 (7.8–13.2)	0.0037	1.00
	0.1	4	29.0 (24.5–33.5)		
YM11170	0.1	4	2.9 (2.1– 3.7)		
	0.3	6	9.1 (6.8–11.4)	0.5	1.05
	1	4	21.0 (17.5–26.3)		
Ranitidine	1	6	3.5 (2.0– 5.0)		
	3	4	7.9 (5.8–10.0)	0.35	0.90
	10	6	19.1 (15.2–23.0)		
Cimetidine	1	4	2.4 (1.5– 3.3)		
	3	6	5.4 (3.3– 7.5)	0.71	0.98
	10	6	15.1 (11.8–18.4)		
Metiamide	1	6	2.1 (1.8– 2.4)		
	3	6	5.5 (3.4– 7.6)	0.91	1.15
	10	4	18.2 (15.5–20.9)		
Burimamide	10	6	3.3 (2.4– 4.2)		
	30	4	10.0 (6.9–13.1)	5.1	1.10
	100	4	38.0 (31.8–44.2)		

Dose-ratios and equilibrium dissociation constants (K_B) were calculated as described in Methods. *n* indicates the number of experiments from which results were pooled.

Methods, dose-ratios have been calculated for each concentration of each H_2-antagonist used, and these are summarised in Table 1. Schild plots have been constructed for each H_2-antagonist on the basis of the dose-ratios shown in Table 1, and burimamide is again shown as an example in the inset to Figure 5. The equilibrium dissociation constants and accompanying slopes for the H_2-antagonists obtained from these Schild plots are also shown in Table 1. None of the slopes calculated from the Schild plots were significantly different from one ($P > 0.05$).

The highest concentration of each H_2-antagonist used in the experiments described above has also been examined for its effect on the dose-response curve to 2-PEA. The results have been summarised in Table 2. In all cases the H_2-antagonists had no effect on the dose-response curve for 2-PEA, and the EC_{50} values for 2-PEA in the absence and presence of each H_2-antagonist were not significantly different ($P > 0.05$).

Table 2 The effect of H_2-antagonists on the dose-response curve for 2-pyridylethylamine (2-PEA)

H_2-antagonist	*Concentration* (μM)	*Parameters for 2-PEA*			
		Absence of antagonist		*Presence of antagonist*	
		EC_{50} (μM)	*Maximum response* (%)	*EC_{50}* (μM)	*Maximum response* (%)
Tiotidine	0.1	55 ± 10	100	53 ± 7^{NS}	98 ± 4
YM11170	1	50 ± 8	100	48 ± 9^{NS}	103 ± 5
Ranitidine	10	67 ± 14	100	69 ± 17^{NS}	99 ± 3
Cimetidine	10	55 ± 6	100	52 ± 13^{NS}	97 ± 6
Metiamide	10	61 ± 12	100	64 ± 17^{NS}	98 ± 10
Burimamide	100	35 ± 10	100	32 ± 8^{NS}	105 ± 6

EC_{50} values were calculated as described in Methods. The values given are the means ± s.e.mean from six experiments. NS = not significantly different ($P > 0.05$) from the EC_{50} values obtained in the absence of antagonist.

Discussion

The effect of H_2-agonists were studied against contractions produced by 2-PEA because this H_1-agonist gave a sustained contraction of the tissue. It is not known why histamine and carbachol failed to produce sustained contractions but these compounds may be taken up or metabolised by the tissue or histamine may exert H_2-effects (relaxation) in addition to an H_1-mediated contraction.

There is now substantial evidence that H_2-antagonists potentiate the contractile action of histamine on the guinea-pig lung strip (Dunlop & Smith, 1977; Okpako *et al.*, 1978; Drazen *et al.*, 1980) but have no effect on contractions produced by the selective H_1-agonist, 2-PEA (Okpako *et al.*, 1978). It has been suggested that this is the result of histamine having an action on both H_1 and H_2-receptors whereas the effect of 2-PEA is specifically at the H_1-receptor. In this study we have shown that several H_2-antagonists had no effect on the dose-response curve for 2-PEA on the lung strip (Table 2) which suggests that 2-PEA has no agonist activity at histamine H_2-receptors. It is, in fact, unlikely that 2-PEA would be able to produce relaxation of the lung strip by an action on H_2-receptors since its H_1:H_2 selectivity ratio has been reported to be 30:1 (Durant *et al.*, 1975).

The EC_{50} for 2-PEA of 50 μM calculated in this study is in good agreement with that of 40 μM previously reported for 2-PEA-induced contraction of the lung strip (Chand & Deroth, 1979). The maximum response to 2-PEA was approximately equal to the maximum response to histamine in this study and this finding is also in agreement with other work (Chand & Deroth, 1979; Drazen *et al.*, 1979).

In this study, relaxation of the guinea-pig lung parenchymal strip by each H_2-agonist could only be demonstrated in the presence of induced tone. This is in agreement with the work of Vincenc *et al.* (1984) who found that dimaprit could not produce relaxation of unstimulated human lung strips. This may suggest that there is little tone present in the lung strip in its resting state. However, it has previously been shown that β_2-adrenoceptor agonists are able to produce relaxation of guinea-pig lung strips which have not been precontracted (Goldie *et al.*, 1982; Carswell & Nahorski, 1983) and indeed, in this study, forskolin was able to produce a relaxation of unstimulated lung strips suggesting that the tissue has the ability to relax further when in a resting state. Although the low efficacy of impromidine (Durant *et al.*, 1978), which produced only a 50% reversal of the contraction to 2-PEA in this study, could explain why it had no measurable effect on resting tone, this explanation is unlikely because both dimaprit and 4-methyl histamine, which produced approximately 90% reversal of the contraction to 2-PEA, also had no measurable effect on resting tone. Moreover it has previously been reported that dimaprit produces at least the same maximum response as histamine when acting at H_2-receptors (Parsons *et al.*, 1977), and indeed, Bottomley *et al.* (1985) have suggested that both dimaprit and 4-methyl histamine have a two to three times higher efficacy than histamine at H_2-receptors in the guinea-pig right atrium. Hence, it is unlikely that the efficacy of dimaprit and 4-methyl histamine is too low to show a response on the resting lung strip.

Another explanation for the lack of activity of the H_2-agonists on resting tone is that the H_2-receptors in the lung strip may only become activated when the contractile mechanism of the lung strip is stimulated either chemically or mechanically. This argument is supported by the work using forskolin which was able to produce a relaxation of the lung strips under resting tension but this drug activates adenylate cyclase directly without stimulating H_2-receptors. Forskolin produced 130% reversal of the contraction to 2-PEA on the lung strip compared to 90% reversal produced by dimaprit and 4-methyl histamine. This suggests that, in our study, there is some tone present in the unstimulated lung strip. However, it may not be sufficient to cause activation of the H_2-receptors. Previous workers have suggested that dimaprit is able to produce a relaxation of resting tone in the lung strip but in those studies the tissues were maintained at a much higher tension (Yen & Kreutner, 1979; Tomioka & Yamada, 1982). In our study, a much lower tension was applied to the lung strips and they were allowed to relax completely before addition of an H_2-agonist.

Forskolin gave EC_{50} values of 0.11 μM and 0.16 μM for relaxation of lung strips under resting tension and lung strips contracted by 2-PEA respectively. Both these figures are in good agreement with EC_{50} values of 0.2 μM and 0.4 μM reported respectively for relaxation of normal and sensitized guinea-pig lung strips (Burka & Saad, 1984).

The EC_{50} values obtained in this study, for relaxation induced by dimaprit, 4-methyl histamine and impromidine were 17 μM, 2 μM and 0.04 μM respectively. Similar EC_{50} values have been reported for the action of these H_2-agonists on H_2-receptors in the guinea-pig right atrium (Gajtkowski *et al.*, 1983). In the case of dimaprit and 4-methyl histamine, similar values were obtained for relaxation of lung strips precontracted with 2-PEA (Tomioka & Yamada, 1982).

It is unlikely that the action of dimaprit on the lung strip is due to release of catecholamines since propranolol did not affect the response to dimaprit. This result is in agreement with those of Parsons *et al.* (1977) where propranolol had no effect on the positive chronotropic effect of dimaprit in the guinea-pig right atrium.

The order of potency for the H_2-agonists and the

lack of effect of non-H_2-receptor antagonists suggests that dimaprit, 4-methyl histamine and impromidine are stimulating the histamine H_2-receptor to produce relaxation of the guinea-pig lung strip. This is substantiated by the parallel shift of the dose-response curve for dimaprit produced by several concentrations of a variety of H_2-receptor antagonists (Figure 4). There was also no reduction in the maximum response to dimaprit in the presence of the H_2-antagonists and this together with the fact that the slopes of the Schild plots for the H_2-antagonists were not significantly different from one is compatible with surmountable competitive antagonism between dimaprit and the H_2-antagonists at an H_2-receptor within the lung parenchymal strip. However, for a more precise investigation of the receptor type causing the relaxation of the lung strip produced by dimaprit it is necessary to compare the dissociation constants for the H_2-antagonists obtained in this study with their dissociation constants obtained in other pharmacological and biochemical studies and this has been done in the paper which follows.

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