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A study of the H_2-receptor for histamine stimulating adenylate cyclase in homogenates of guinea-pig lung parenchyma

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1 The effect of forskolin and several H_2-agonists was investigated on the activity of adenylate cyclase in homogenates of guinea-pig lung parenchyma.

2 Histamine, 0.1 μM to 1 mM, dimaprit, 1 μM to 10 mM, 4-methyl histamine, 0.1 μM to 10 mM, impromidine, 10 nM to 10 μM and forskolin, 1 nM to 100 μM, all produced a dose-dependent stimulation of adenylate cyclase activity above the basal level.

3 The histamine H_1-receptor antagonist mepyramine, 10 μM, and β-adrenoceptor antagonist propranolol, 10 μM, had no effect on the stimulation by histamine of adenylate cyclase.

4 The dose-response curve for stimulation by histamine of adenylate cyclase was shifted to the right in a dose-dependent manner by increasing concentrations of several H_2-antagonists. Schild plots constructed for each H_2-antagonist produced straight lines with slopes not significantly different from unity. The equilibrium dissociation constants obtained for the H_2-antagonists in this study were similar to those previously reported for inhibition of dimaprit-induced relaxation of the pre-contracted lung strip, inhibition of [^{3}H]-tiotidine binding to homogenates of guinea-pig lung parenchyma and inhibition of histamine-stimulated adenylate cyclase in guinea-pig gastric mucosa.

Introduction

The statement of the second messenger hypothesis by Rall *et al.* (1957) led to a study of the effects of a variety of hormones and neurotransmitters for their possible action on the adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-generating system of various tissues. Sutherland *et al.* (1962) described a particulate adenylate cyclase enzyme in guinea-pig brain that catalysed the formation of cyclic AMP. Histamine was one of the first agents shown to stimulate the activity of this enzyme and thereby raise the intracellular levels of cyclic AMP (Kakiuchi & Rall, 1965).

However, it was the discovery of specific antagonists of the H_2-receptor (Black *et al.*, 1972) that led to a series of studies in different tissues which developed further the idea of coupling between the H_2-receptor and adenylate cyclase. Verma & McNeil (1974) reported that burimamide, a selective H_2-antagonist, caused a parallel shift to the right of the dose-response curve for stimulation by histamine of adenylate cyclase in guinea-pig heart. Similarly, metiamide was found to inhibit histamine-stimulated adenylate cyclase in gastric mucosa (Rouff & Sewing, 1975). More recently, inhibition by several H_2-antagonists has been studied using histamine-stimulated adenylate cyclase in gastric mucosa, and the affinities of the antagonists for the receptor mediating adenylate cyclase activation correlate well with the affinities of these drugs obtained in other biochemical and pharmacological studies of these receptors (Gajtkowski *et al.*, 1983).

In the guinea-pig lung, H_2-antagonists have been shown to enhance the response of the bronchial smooth muscle to histamine (Eyre, 1977; Drazen *et al.*, 1980). Also, dimaprit, a selective H_2-agonist (Parsons *et al.*, 1977) produced reversal of pre-existing anaphylactic contractions of guinea-pig lung parenchymal strips (Chand, 1979) and this effect was blocked by burimamide. Furthermore, we have shown a correlation between equilibrium dissociation constants for several H_2-antagonists as inhibitors of dimaprit-induced relaxation of guinea-pig lung strips, and the inhibition constants of these antagonists obtained in a ligand binding study in guinea-pig lung (Foreman *et al.*, 1985a). Thus, in guinea-pig lung there are H_2-receptors which mediate relaxation of peripheral air-

way smooth muscle. However, although histamine has been shown to stimulate adenylate cyclase in guinea-pig lung (Bhoola & Gadd, 1984), the link between the H_2-receptor and the cyclic AMP system has not previously been examined in this tissue. Therefore, in this study, we have examined the stimulation, by H_2-agonists, of adenylate cyclase in homogenates of guinea-pig lung parenchyma and the inhibition of this stimulation by several H_2-antagonists.

Methods

Preparation of an homogenate of lung parenchyma

Hartley guinea-pigs of either sex aged 4 to 6 months and weighing 400–600 g were killed by stunning and exsanguination through sectioned carotid arteries. The heart and lungs were removed together and placed in a petri-dish on ice. Strips of lung approximately 15mm × 3mm × 3mm were cut from the periphery of each lobe as described previously (Drazen & Schneider, 1978). These lung strips were homogenized in 20 ml of buffer using a Polytron blender at setting 2 for 3 × 10 s bursts. The buffer for homogenization contained Tris 50 mM, sucrose 0.25 M and EDTA 4 mM; the pH was7.4. The homogenate was passed through cheesecloth to remove any large pieces of unhomogenised lung tissue and was then centrifuged at 1500 *g* for 10 min at 4°C in a Sorvall RC-2B centrifuge. The supernatant obtained was discarded and the pellet washed once by resuspension in 20 ml of buffer followed by recentrifugation at 1500 *g* for 10 min. The pellet was finally resuspended in 5 ml of buffer containing Tris 50 mM and EDTA 4 mM, at a pH of 7.8. The protein content of the suspension was determined by a modified Lowry method (Lowry *et al.*, 1951). The preparation was used immediately for the assay of adenylate cyclase.

Adenylate cyclase assay

The method used was that described by Hegstrand *et al.* (1976). In the experiments with agonist drugs, 50 µl of homogenate, containing approximately 125 µg of protein, was incubated in Tris buffer 50 mM, pH 7.8, containing EGTA 0.6 mM, isobutylmethyl xanthine (IMBX) 1 mM, $MgCl_2$ 2 mM, GTP 0.1 mM and various concentrations of agonist between 10 nM and 10 mM in a total volume of 500 µl. Some tubes contained no agonist and these were used to measure the basal level of cyclic AMP. In experiments with antagonist, the incubation medium contained various concentrations of antagonist between 3 nM and 0.3 mM as well as the required agonist concentrations. Agonist and antagonist were added to the incubation medium together and 10 min was allowed to elapse before the reaction was initiated. It was assumed that this time period was sufficient for equilibrium conditions to be achieved.

In all experiments, the reaction was initiated by the addition of ATP, 1 mM, followed by an incubation for 10 min at 30°C. The reaction was stopped by placing the tubes in a boiling water bath for 3 min. The tubes were allowed to cool before the addition of 50 mg of Alumina 90. After mixing, the samples were centrifuged at 700 *g* for 5 min at 4°C.

The cyclic AMP content of each sample was estimated by the method of Brown *et al.* (1971) using a competitive protein binding assay.

Experimental design

Two sets of experiments were carried out with the H_2-antagonists. In the first, the effect of increasing concentrations of the H_2-antagonists on the maximum stimulation produced by a single fixed concentration

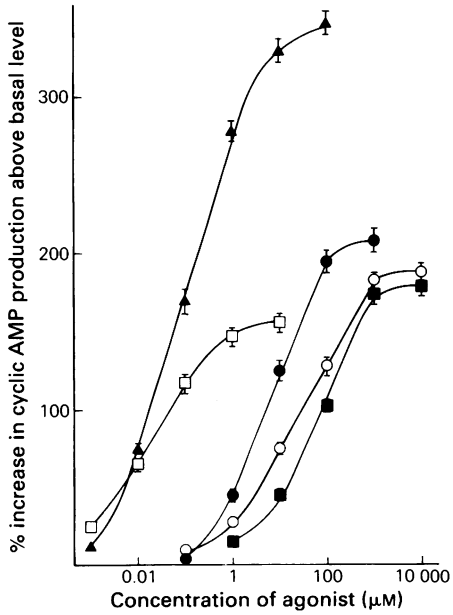

Figure 1 Dose-response curves showing the effect of histamine (●), 4-methylhistamine (○), dimaprit (■), impromidine (□) and forskolin (▲) on adenylate cyclase in homogenates of guinea-pig lung parenchyma. The percentage increase in cyclic AMP above basal level is plotted as a function of agonist concentration. Results have been pooled from four experiments which were carried out in sextuplicate. Therefore, the points are means of 24 determinations and the vertical bars represent s.e.mean. The mean basal activity of adenylate cyclase was equal to 38 ± 6 pmol of cyclic AMP mg^{-1} protein min^{-1}.

of histamine (1 mM) was examined. In the second set of experiments, complete concentration-response curves to histamine were obtained in the absence or in the presence of different concentrations of each H_2-antagonist.

Analysis of data

All EC_{50} values were calculated from dose-response curves fitted to the data points by a computerised, non-linear, least squares method (Marquardt, 1963). In the experiments with 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 analysed by the method of Arunlakshana & Schild (1959) using least squares regression analysis to determine the relationship between log (dose-ratio -1) and log molar concentration of antagonist.

Drugs

The drugs used in this study and the sources of these drugs were: histamine acid phosphate, propranolol hydrochloride (Sigma); mepyramine maleate (M & B); metiamide, burimamide, cimetidine, dimaprit dihydrochloride, impromidine dihydrochloride, 4-methyl histamine dihydrochloride (SK & F); ranitidine, tiotidine, YM11170 (Hoechst); forskolin (Calbiochem).

Results

The effect of forskolin

Concentrations of forskolin within the range of 1 nM to 100 μM produced a dose-dependent stimulation of adenylate cyclase in homogenates of guinea-pig lung parenchyma. A dose-response curve has been constructed from the results pooled from four experiments (Figure 1). A computer-fitted curve (see methods) gave an extrapolated maximum response to forskolin equivalent to an increase of $363 \pm 15\%$ of the basal activity of adenylate cyclase. The EC_{50} for forskolin derived from the fitted dose-response curve was 0.12 μM. The maximum stimulation occurred with a concentration of forskolin greater than 100 μM.

The effect of H_2-agonists

Histamine, 0.1 μM to 1 mM, dimaprit, 1 μM to 1 mM, 4-methyl histamine, 0.1 μM to 1 mM and impromidine, 1 nM to 10 μM, all produced a concentration-dependent stimulation of adenylate cyclase in homogenates of guinea-pig lung parenchyma. Dose-response curves were constructed for each H_2-agonist from the results pooled from four experiments (Figure 1). The EC_{50} values and the maximum stimulation produced by each H_2-agonist is shown in Table 1. Non-paired *t* tests of the individual experimental results showed no significant differences ($P > 0.05$) in the maximum stimulation of adenylate cyclase produced by dimaprit, 4-methyl histamine or histamine. A similar non-paired *t* test showed a highly significant difference ($P < 0.01$) in the maximum stimulation of adenylate cyclase produced by histamine and the maximum stimulation of adenylate cyclase produced by impromidine.

Table 1 The effect of H_2-agonists on adenylate cyclase activity in homogenates of guinea-pig lung parenchyma

Agonist	*EC_{50}* (μM)	*Maximum response* (% increase in cyclic AMP above basal level)
Impromidine	0.02 ± 0.006	163 ± 13
Histamine	6.3 ± 1.8	212 ± 11
Dimaprit	37.5 ± 6.3	185 ± 6
4-Methyl histamine	20.9 ± 5.7	192 ± 9
Forskolin	0.12 ± 0.05	363 ± 15

EC_{50} values ($\pm$ 95% confidence limits) and maximum responses ($\pm$ 95% confidence limits) were calculated as described in the Methods. The mean basal adenylate cyclase activity was 38 ± 6 pmol mg^{-1} protein min^{-1}.

Antagonism of histamine-stimulated adenylate cyclase

The effect of propranolol and mepyramine It is conceivable that histamine could stimulate adenylate cyclase indirectly by releasing catecholamines in the lung homogenate and to exclude this possibility we examined the effect of propranolol on the dose-response curve for histamine and adenylate cyclase activity. Also, to exclude an H_1-receptor-mediated component in the stimulation of adenylate cyclase by histamine, we examined the effect of mepyramine on the response to histamine.

Neither propranolol, 100 μM, nor mepyramine, 100 μM, had any effect by themselves on the basal level of adenylate cyclase activity in homogenates of guinea-pig lung parenchyma. The data shown in Table 2 demonstrate the effect of propranolol, 100 μM and mepyramine, 100 μM, on the basal activity of adenylate cyclase. A non-paired *t* test of the individual experimental results showed no significant difference ($P > 0.05$) in the basal activity of adenylate cyclase in

Table 2 The effect of H_2-antagonists, propranolol and mepyramine on the basal activity of adenylate cyclase in homogenates of guinea-pig lung parenchyma.

Antagonist	Concentration (μM)	Basal activity of adenylate cyclase (pmol mg^{-1} protein min^{-1}) Presence of antagonist	Absence of antagonist
Tiotidine	0.3	46 ± 5	49 ± 3
YM11170	3	52 ± 8	51 ± 6
Ranitidine	30	36 ± 4	39 ± 3
Cimetidine	30	48 ± 2	45 ± 6
Metiamide	30	54 ± 2	54 ± 7
Burimamide	300	43 ± 9	48 ± 6
Propranolol	100	51 ± 3	54 ± 7
Mepyramine	100	33 ± 6	31 ± 2

Values given in the table are means ± s.e.mean obtained from four experiments performed in sextuplicate. The basal activities of adenylate cyclase in the presence and absence of each antagonist were not significantly different.

the presence and absence of propranolol, 100 μM, or mepyramine, 100 μM.

Propranolol and mepyramine also had no effect on the dose-response curve obtained for histamine stimulation of adenylate cyclase in this preparation. Table 3 shows the mean EC_{50} values obtained for histamine and the maximum stimulation produced by histamine in the presence and absence of mepyramine, 10 μM and propranolol, 10 μM. A non-paired t test of individual experimental results showed no significant difference ($P > 0.05$) between the EC_{50} values obtained for histamine in the presence and absence of mepyramine, 10 μM, or propranolol, 10 μM. Similarly, there was no significant difference ($P > 0.05$) between the maximum stimulation of adenylate cyclase produced by histamine in the presence and absence of mepyramine, 10 μM, or propranolol, 10 μM.

The effect of H_2-antagonists None of the H_2-antagonists, when examined at concentrations up to 1 mM, had any effect by themselves on the basal level of adenylate cyclase activity in homogenates of guinea-pig lung parenchyma. The data given in Table 2 demonstrate the effect of the maximum concentration of each H_2-antagonist used in subsequent experiments on the basal activity of adenylate cyclase. Non-paired t tests of the individual experimental results showed no significant difference in the basal activity of adenylate cyclase in the presence and absence of the concentration of each H_2-antagonist shown in Table 2. Increasing concentrations of each H_2-antagonist caused a dose-dependent reduction of the maximum stimulation of adenylate cyclase produced by histamine, 1 mM. At the highest concentrations of H_2-antagonists used, the histamine-stimulated adenylate cyclase activity returned to its basal level. The action of metiamide is shown as an example in Figure 2.

The antagonism produced by the H_2-antagonists was examined in more detail by investigating the effect of various concentrations of each H_2 antagonist on the complete histamine dose-response curve. Increasing concentrations of each H_2-antagonist produced a dose-dependent shift to the right of the dose-response curve for histamine with no reduction in the maximum

Table 3 The effect of propranolol, 10 μM, and mepyramine, 10 μM, on the stimulation produced by histamine of adenylate cyclase in homogenates of guinea-pig lung parenchyma

Antagonist	Parameters for histamine EC_{50} (μM) No antagonist	Antagonist	Maximum response (%) No antagonist	Antagonist
Propranolol	5.8 ± 1.4	6.1 ± 1.8	206 ± 14	210 ± 12
Mepyramine	6.2 ± 2.3	6.0 ± 1.1	221 ± 6	228 ± 8

Values given in the table are means ± s.e.mean from four separate experiments performed in triplicate. EC_{50} values and the maximum stimulation produced by histamine were calculated as described in Methods. The maximum response for histamine has been expressed as a percentage increase of the basal activity of adenylate cyclase. The mean basal activity of adenylate cycase was 42 ± 6 pmol mg^{-1} protein min^{-1} in the experiments with propranolol and 48 ± 9 pmol mg^{-1} protein min^{-1} in the experiments with mepyramine.

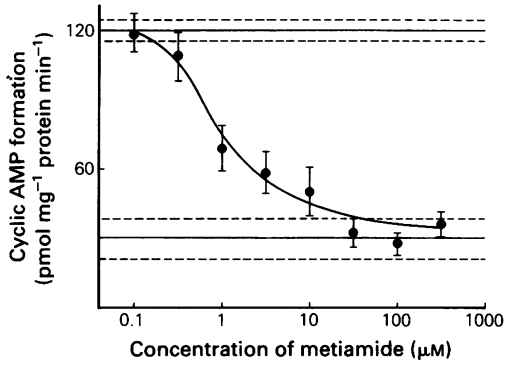

Figure 2 The inhibition by metiamide of the maximum stimulation of adenylate cyclase produced by histamine, 1 mM. Points are means of six determinations from a single experiment and the vertical bars represent s.e.mean. The basal adenylate cyclase activity is equal to 31 ± 6 pmol cyclic AMP mg^{-1} protein min^{-1} and the maximum activity produced by histamine, 1 mM, was 118 ± 4 pmol cyclic AMP mg^{-1} protein min^{-1} (n = 6).

response to histamine. The effect for the H_2-antagonist tiotidine is shown as an example in Figure 3a. For each concentration of each H_2-antagonist, dose-ratios have been calculated as described in Methods, and Schild plots were constructed on the basis of these dose-ratios. The Schild plot for tiotidine is shown in Figure 3b. Equilibrium dissociation constants and Schild slopes have been calculated for each H_2-antagonist from similar pots, and these have been summarised in Table 4. None of the slopes of the Schild plots for the H_2-antagonists was significantly different from one ($P > 0.05$).

Table 4 The effect of H_2-antagonists on histamine-stimulated adenylate cyclase in homogenates of guinea-pig lung parenchyma

H_2-antagonist	K_B (μM)	*Slope of Schild plot*
Tiotidine	0.0055 ± 0.002	1.08 ± 0.04
YM11170	0.019 ± 0.007	1.01 ± 0.1
Ranitidine	0.31 ± 0.07	1.00 ± 0.1
Cimetidine	0.77 ± 0.07	1.02 ± 0.04
Metiamide	0.75 ± 0.09	1.02 ± 0.11
Burimamide	5.7 ± 0.42	0.92 ± 0.02

Equilibrium dissociation constants (K_B) were calculated as described in Methods. In each case, 3 separate experiments were performed in triplicate. The values given are means ± s.e.mean.

Discussion

Forskolin stimulates adenylate cyclase directly without first combining with a receptor on the cell surface in a number of tissues including rat cerebral cortex (Seamon *et al.*, 1981), liver and heart (Metzger *et al.*, 1981), and human platelets (Siegl *et al.*, 1981).

The EC_{50} for forskolin in this study was 0.12 μM which is similar to the EC_{50} value of 0.1 μM reported

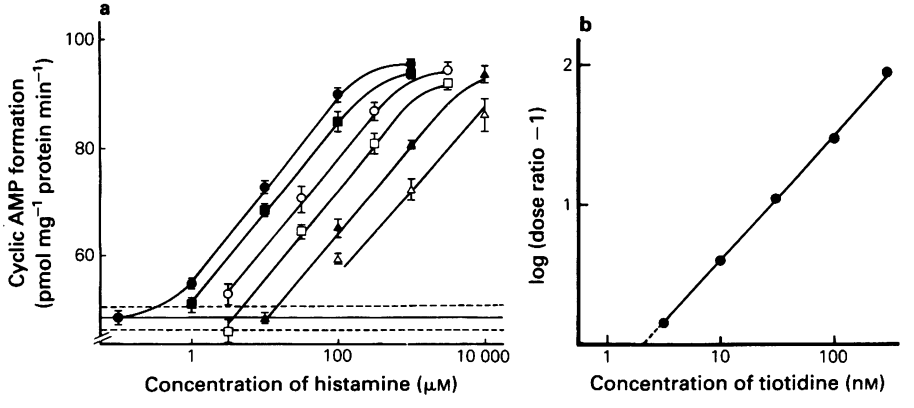

Figure 3 The antagonism by tiotidine of the histamine-stimulated adenylate cyclase activity. (a) Dose-response curves are to histamine alone (●) and histamine in the presence of tiotidine, 3 nM (■), 10 nM (○), 30 nM (□), 100 nM (▲) and 300 nM (△). The curves have been constrained to be parallel. Points are means of three determinations from a single experiment and the vertical bars represent s.e.mean. The basal adenylate cyclase activity was 48 ± 3 pmol cyclic AMP mg^{-1} protein min^{-1}. (b) Schild plot of data from the experiment shown in (a). The slope is 1.03 and the intercept on the abscissa (K_B) is 2.3 nM. Mean values for Schild plot slopes and K_B are given in Table 4.

for guinea-pig lung by Burka & Saad (1984). Moreover, we have recently obtained EC_{50} values of about 0.1 μM for forskolin-induced relaxation of guinea-pig lung strips (Foreman *et al.*, 1985b). The maximum stimulation of adenylate cyclase by forskolin was about a 350% increase in the basal activity, which is similar to the maximum stimulation previously reported by Seamon *et al.* (1981a, b) for stimulation by forskolin of adenylate cyclase in rat cerebral cortex membranes (400% increase in basal activity), and similar to the maximum stimulation in guinea-pig lung membranes (400% increase in basal activity) (Burka *et al.*, 1984). The large increase over basal adenylate cyclase activity observed with forskolin demonstrates that the adenylate cyclase in the homogenate of lung parenchyma is stable and retains its activity after the disruptive procedures used in the preparation stage.

The EC_{50} values obtained in this study for dimaprit- and 4-methyl histamine-induced activation of adenylate cyclase (Table 1) are about an order of magnitude different from those previously reported for relaxation of pre-contracted guinea-pig lung strips (Foreman *et al.*, 1985b) and stimulation of adenylate cyclase in guinea-pig gastric mucosa (Gajtkowski *et al.*, 1983). In contrast, the EC_{50} values obtained in this study for impromidine and histamine (Table 1) are similar to those found in the other systems. The reason for these differences is unknown but may be related to different efficacies of the agonists in different tissues. However, the overall rank order of potency of the H_2-agonists obtained in this study (Table 1) is the same as that reported for stimulation of adenylate cyclase in gastric mucosa (Gajtkowski *et al.*, 1983), relaxation of guinea-pig lung strips contracted by 2-pyridylethylamine (Foreman *et al.*, 1985b) and for the chronotropic effect of the H_2-agonists on guinea-pig right atria (Gajtkowski *et al.*, 1983). We have previously pointed out that the order of potency of H_2-agonists for the biological responses just mentioned is different from the order of potency obtained for inhibition by these drugs of [^{3}H]-tiotidine binding to homogenates of guinea-pig lung parenchyma (Foreman *et al.*, 1985a) and guinea-pig cerebral cortex (Gajtkowski *et al.*, 1983). Such a difference may be expected since displacement of binding gives an estimate of the affinity of the H_2-agonists for the receptor, whereas, the EC_{50} values obtained from biological response measurements also reflect the efficacy of the agonists and the extent of the receptor reserve. In the present study, the maximum stimulation of adenylate cyclase produced by histamine, 4-methyl histamine or dimaprit was not significantly different which suggests that these compounds are all full agonists at the H_2-receptor in this preparation, whereas, the maximum stimulation of adenylate cyclase produced by impromidine was significantly lower than that produced by histamine indicating that impromidine is behaving as a partial agonist.

It is unlikely that the stimulation of adenylate cyclase by histamine is mediated by H_1-receptors since mepyramine had no effect on the response to histamine. It is important to establish that there is no H_1-mediated response because it has been previously reported that mepyramine was more potent than burimamide in antagonizing the stimulation by histamine of guinea-pig heart adenylate cyclase (Weinryb & Michel, 1975). Also, the H_1-antagonist, tripelennamine was reported to inhibit the stimulation of adenylate cyclase produced by histamine in rabbit cortex slices (Palmer *et al.*, 1972). It is also unlikely that catecholamines released by histamine from microsomes in the preparation were acting through β-adrenoceptors to stimulate adenylate cyclase, since propranolol had no effect on the response to histamine.

The order of potency of the H_2-agonists and the lack of effect of non-H_2-receptor antagonists suggests that H_2-agonists are stimulating adenylate cyclase by an

Table 5 Comparison of the activity of H_2-antagonists

	*Lung parenchymal**	*[^{3}H]-tiotidine***	*Adenylate cyclase*	
	strip	*binding*	*Lung*	*Gastric† mucosa*
H_2-antagonist	K_B (μM)	K_i (μM)	K_B (μM)	
Tiotidine	0.0037	0.0064	0.0055	0.02
YM11170	0.05	0.041	0.019	0.005
Ranitidine	0.35	0.8	0.31	0.20
Cimetidine	0.70	0.82	0.77	0.79
Metiamide	0.91	0.76	0.75	0.55
Burimamide	5.1	4.2	5.7	3.2

*Data from Foreman *et al.* (1985b).
**Data from Foreman *et al.* (1985a).
†Data from Gajtkowski *et al.* (1983).

interaction with the H_2-receptor. Further evidence for this is seen in the parallel shift of the histamine dose-response curve produced by increasing concentrations of several H_2-antagonists, with no reduction in the maximum stimulation of adenylate cyclase as the concentration of H_2-antagonist is increased (Figure 3a). Also, the slopes of the Schild plots obtained for the H_2-antagonists are not significantly different from unity, which is compatible with competitive antagonism by the H_2-antagonists of histamine-induced stimulation of adenylate cyclase. However, for more convincing evidence that an H_2-receptor mediates the stimulation of adenylate cyclase produced by histamine, it is necessary to compare the dissociation constants obtained for the H_2-antagonists in this study with their dissociation constants obtained in other biochemical and pharmacological studies (Table 5). There is a highly significant correlation between the dissociation constants obtained for the H_2-antagonists in this study and both the inhibition constants obtained from studies of [^{3}H]-tiotidine binding to homogenates of guinea-pig lung parenchyma ($r = 0.98$, $P < 0.001$) and also the dissociation constants obtained for inhibition of dimaprit-induced relaxation of the pre-contracted lung strip ($r = 0.99$, $P < 0.001$). Thus, the evidence is strongly in favour of the presence of H_2-receptors in the guinea-pig lung parenchyma which mediate raised intracellular levels of cyclic AMP and thereby cause relaxation of peripheral airway smooth muscle.

There is also a significant correlation ($r = 0.92$, $P < 0.005$) between the dissociation constants obtained for the H_2-antagonists in this study and the dissociation constants previously reported for inhibition of histamine-stimulated adenylate cyclase in guinea-pig gastric mucosa (Table 3). Hence, it is likely that the same receptor type (H_2) is mediating raised intracellular levels of cyclic AMP in both guinea-pig lung parenchyma and guinea-pig gastric mucosa.

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