Br. J. Pharmac. (1985), **86**, 581–587

Further analysis of anomalous pK_B values for histamine H_2-receptor antagonists on the mouse isolated stomach assay

J.W. Black[1], P. Leff* & N.P. Shankley

The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5 8RX and Wellcome Research Laboratories*, Langley Court, Beckenham, Kent, BR3 3BS

1 Agonist-antagonist interactions at histamine receptors have been re-examined using improved techniques, on the mouse isolated, lumen-perfused, stomach gastric acid assay.

2 Using histamine as agonist, pK_B values have been estimated for burimamide, metiamide, cimetidine, ranitidine, oxmetidine and famotidine on both the gastric and guinea-pig isolated right atrium assays. With the exception of oxmetidine on the atrial assay, these compounds behaved as competitive antagonists on both assays.

3 Oxmetidine significantly depressed basal rate on the atrial assay and the Schild plot slope parameter (0.81) was significantly less than one.

4 The pK_B values estimated on the gastric assay were lower than those on the atrial assay. However, the difference between the values on the gastric and atrial assays was not constant. The difference between the two assays for famotidine was not significant.

5 We conclude that the apparent varying selectivity of the antagonists for gastric and atrial histamine H_2-receptors may be explained by the differential loss of antagonists into the gastric secretion from the receptor compartment and that there is no need to postulate heterogeneity of histamine H_2-receptors.

Introduction

Angus & Black (1979) and Angus *et al.* (1980) found that the histamine H_2-receptor antagonists, burimamide, metiamide and cimetidine behaved as simple competitive antagonists at histamine receptors coupled to gastric acid secretion in the isolated, lumen-perfused, stomach preparation of the mouse. However, the calculated pK_B values were significantly lower than those found or reported using histamine H_2-receptor assay systems such as the guinea-pig right atrium and rat uterus. Similar differences were found for muscarinic receptor antagonists. Therefore, these differences in pK_B values were attributed to the continuous loss of ligand across the oxyntic cells leading to a steady-state concentration in the receptor compartment which was lower than the concentration applied to the system. This explanation seemed preferable to the more orthodox and less conservative possibility of heterogeneity of histamine H_2-receptors.

Since then, a number of developments has indicated the need to re-examine that hypothesis. Thus Main & Pearce (1981) did not find a low pK_B for metiamide using the rat isolated gastric mucosa preparation for the assay. On the other hand, Arrang *et al.* (1983) have proposed that there is an H_3-subclass of histamine receptors. Therefore the questions to be answered are 'Is there a real difference in pK_B values for antagonists at oxyntic cell receptors?' and 'Is the non-equilibrium hypothesis still tenable?'

Originally Angus & Black (1979) used a single exposure 2 + 2 bioassay design to estimate pK_B values. Although avoiding problems of desensitization, serial correlation and time dependence this method did not permit characterisation of agonist concentration-effect curves and gave values of pK_B's with 95% fiducial limits such that differences of less than approximately 0.5 log units could not be reliably discriminated. Our recent developments of the mouse stomach assay now permit the full definition of agonist concentration-effect curves allowing tests to be made for detecting changes in maximal asymptote and midpoint slope parameters obtained by curve fitting of the experimental data in the presence and absence of antagonist (Black & Shankley, 1985). In addition pK_B

[1]To whom inquiries and reprint requests should be addressed.

values may be determined with greater precision (95% confidence limits approximately 0.2 log units). Thus, the modified assay provides a more reliable instrument for either probing the homogeneity of receptor populations or for detecting non-equilibrium or saturating conditions. In addition to improvements in the precision of the analytical method, we have included in the re-examination of the properties of histamine receptors on oxyntic cells four new compounds classified as competitive histamine H_2-receptor antagonists. For comparative purposes we have also obtained pK_B values for the histamine H_2-antagonists in the guinea-pig right atrium preparation.

Methods

Acid secretion

Gastric acid secretion was measured in the isolated, lumen-perfused, stomach preparation of the mouse as described previously (Black & Shankley, 1985). Briefly, stomach preparations were established with the pH-electrode system arranged to provide a 12 cmH_2O pressure to distend the stomach. Six preparations were used simultaneously and after an initial 60 min stabilization period those not producing a stable basal acid secretion (approximately 5%) were rejected. All drugs were added directly to the organ bath (serosal side) and, following a further 60 min equilibration period in the absence or presence of antagonist, a single cumulative agonist-concentration effect curve was obtained.

Guinea-pig right atrium preparation

Chronotropic effects were studied in isolated, spontaneously-beating, right atria from male guinea-pigs (Dunkin-Hartley, 375–475 g), which were suspended in 20 ml glass organ baths in Krebs-Henseleit buffer (composition, mM: Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 2.2, HCO_3^- 24.9, SO_4^{2-} 1.2, dextrose 10) at 37°C (± 0.3°C) and gassed with 95% O_2, 5% CO_2 (for details see Angus & Black, 1980). Isometric transducer outputs were processed by a ratemeter (ADG Instruments) which gave a direct readout of rate (beats min^{-1}) continuously displayed on a potentiometric recorder (Bryans 28000). Tissues were subjected to 0.5 g resting tension and washed at approximately 15 min intervals during an initial 60 min stabilization period. Krebs-Henseleit solution was routinely prepared containing propranolol 10^{-7}M to inhibit the effects of histamine-stimulated catecholamine release. Six preparations were used simultaneously and following the initial stabilization period and a 60 min equilibration period in the absence or presence of antagonist, a single cumulative agonist concentration-effect curve was obtained.

Experimental design

Experimental treatments were allocated on a block design such that, as far as possible, all organ baths received each treatment during the course of an experiment.

Analysis

Individual responses to drug treatments were measured as changes from basal response levels immediately prior to drug addition. Acid secretion responses were measured as the change in pH of the lumen perfusate (Δ pH) and guinea-pig right atrium responses as changes in rate (Δ BPM). The concentration-effect curve data from individual preparations were fitted to a logistic function which provided estimates of the mid-point location parameter (log $[A_{50}]$), maximal asymptote (α) and mid-point slope (n), as described previously (Black & Shankley, 1985). For display purposes the individual computed parameter estimates for each treatment group were expressed as means and a single logistic curve generated and superimposed upon the experimental data.

Competitive antagonism

Parallelism of agonist concentration-effect curves was tested by one-way analyses of variance, comparing computed midpoint slope parameters and asymptote parameters between and within treatment groups. If no significant differences were found, antagonist equilibrium dissociation constants (estimated as log K_Bs) were calculated by fitting computed log $[A_{50}]$ values obtained in the presence of (log $[A_{50}]'$) and absence of antagonist (log $[A_{50}]$) to the following derivative of the Schild equation (Schild, 1957):

$$\log [A_{50}]' = \log [A_{50}] + \log \left(\frac{1 + [B]^b}{10^{\log K_B}} \right)$$

where b is the order of [B] equivalent to the Schild plot slope parameter. Thus each computed log $[A_{50}]$ is treated as an item of data incorporating the location information of each agonist concentration-effect curve. Therefore analysis of antagonist action according to this equation gives equal weight to each agonist concentration-effect curve unlike conventional Schild plot analysis which, as Stone & Angus (1978) showed, gives undue weight to the control curve. Here the control log $[A_{50}]$ is estimated in the analysis whereas in measuring dose-ratios for the construction of Schild plots the control curve location is assumed to be error-free.

Initially log $[A_{50}]$s were analysed allowing b to vary. If b was found to be not significantly different from unity a second analysis was performed with b constrained to unity allowing the log K_B to be estimated. For display purposes the parameters estimated were used to generate a Schild plot shown superimposed upon calculated dose-ratios.

Drugs

Drugs were prepared in distilled water. Molar stock solutions of histamine dihydrogen chloride (Sigma) were neutralised by addition of sodium hydroxide (Black *et al.*, 1981). The total volume of drug added in any one experiment to the 20 ml (atria) and 40 ml (stomach) organ baths did not exceed 400 μl and 800 μl distilled water, respectively. Histamine H_2-receptor antagonists and their sources are as follows: burimamide (Smith, Kline and French (SKF)), cimetidine (SKF), metiamide (SKF), oxmetidine (SKF), famotidine (Merck, Sharpe and Dohme), tiotidine (Imperial Chemical Industries) and ranitidine (Glaxo).

Results

The effect of histamine H_2-receptor antagonists on basal rate in the guinea-pig right atrium and basal acid secretion in the mouse stomach

The histamine H_2-receptor antagonists, with the exception of oxmetidine on the guinea-pig right atrium, did not significantly affect basal activity on the two assays at the concentrations used in the analyses ($1-1000 \times K_B$ obtained on the guinea-pig right atrium). Oxmetidine produced a significant concentration-dependent decrease in basal atrial rate at concentrations above 10^{-5}M (Figure 1).

Estimation of pK_B values for histamine H_2-receptor antagonists

All seven antagonists produced significant concentration-dependent parallel displacement of histamine concentration-effect curves with no significant change in maximal asymptotes in both the stomach and atrium assays. Analysis of the concentration-ratios (see Methods) indicated Schild slope parameters (b) not significantly different from unity with the exception of the analysis of the data from the histamine-oxmetidine interaction in the guinea-pig right atrium. The estimates of pK_B are presented in Table 1.

The low value of the Schild slope parameter obtained from the histamine-oxmetidine interaction on the guinea-pig right atrium agrees with the results obtained by Blakemore *et al.* (1980) ($pA_2 = 6.7$, Schild

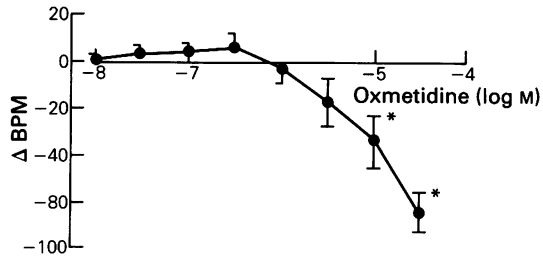

Figure 1 The effect of oxmetidine on basal activity in the guinea-pig right atrium. Results show changes in basal rate (Δ BPM) after 60 min incubation with oxmetidine, presented as mean values from 5/6 observations with vertical lines representing s.e. * Significant decrease from preincubation basal rate ($P < 0.05$).

plot slope = 0.74 (0.52–0.96, 95% confidence limits)) under similar experimental conditions.

Pendleton *et al.* (1983) described famotidine (MK208 or YM11170) as a 'slowly dissociable H_2-receptor antagonist with a unique binding mechanism', having found the H_2-receptor antagonism produced by famotidine to be insurmountable by increasing concentrations of dimaprit in the guinea-pig paired atrium preparation. In this study we could not detect significant deviation from simple competitive behaviour in either assay using histamine as agonist (Figure 2). Pendleton *et al.* (1983) demonstrated a 52% reduction in the maximal asymptote of the dimaprit concentration-effect curve following a 60 min incubation with 3×10^{-7}M famotidine. In this study the histamine concentration-effect curve, with identical famotidine treatment, achieved $86 \pm 8\%$ ($P < 0.05$) of the control curve maximum. Interestingly, in a more recent study Shepherd-Rose & Pendleton (1985) demonstrated a simple competitive interaction between histamine and famotidine in rabbit isolated gastric glands.

The pK_B values obtained on the guinea-pig right atrium agree reasonably well with other published estimates of affinity on this assay using histamine as agonist (Table 1).

Comparison of pK_B values obtained from atrium and stomach

The pK_B values obtained on the stomach assay were, with the exception of the value for famotidine, significantly lower than those obtained on the guinea-pig right atrium (Table 1). Previously Angus *et al.* (1980) and, more recently, Szelenyi & Postius (1984) concluded that the pK_B values estimated for burimamide, metiamide and cimetidine, and metiamide, cimetidine and SK and F 93,479, respectively, were approximately one log unit lower on the mouse stomach assay than

Table 1 pK_B estimates of histamine H_2-receptor antagonists on assays of acid secretion in the mouse isolated, lumen-perfused, stomach and rate in the guinea-pig right atrium

	Guinea-pig right atrium (rate)		*Mouse stomach (acid secretion)*	
	This study (A) pK_B (b)	*Previous* pK_B (95% confidence limits)	(B) pK_B (b)	ΔpK_B
Burimamide	4.92 ± 0.09 (1.00 ± 0.11)	5.11 (5.02–5.19)[1]	4.51 ± 0.13* (0.90 ± 0.13)	+ 0.41 ± 0.16
Metiamide	6.06 ± 0.13 (0.95 ± 0.11)	6.04 (5.93–6.13)[2]	4.90 ± 0.09 (0.99 ± 0.09)	+ 1.16 ± 0.16
Cimetidine	6.08 ± 0.09 (1.03 ± 0.07)	6.10 (6.03–6.17)[3]	5.35 ± 0.09* (0.90 ± 0.07)	+ 0.73 ± 0.13
Ranitidine	6.75 ± 0.07 (0.96 ± 0.07)	7.20 (7.01–7.45)[4]	6.03 ± 0.11* (0.87 ± 0.13)	+ 0.72 ± 0.13
Tiotidine	7.57 ± 0.07 (0.96 ± 0.07)	7.82 (7.72–7.96)[5]	6.96 ± 0.11* (0.98 ± 0.09)	+ 0.61 ± 0.13
Famotidine	7.74 ± 0.07 (0.96 ± 0.07)	pA_2 = 7.80 (7.40–8.10)[6]	7.50 ± 0.11 (0.98 ± 0.09)	+ 0.24 ± 0.13
Oxmetidine	pA_2 = 7.15† (0.81 ± 0.07)	pA_2 = 6.7[7]	6.70 ± 0.11 (0.99 ± 0.09)	—

Data shown are means ± s.e.
*Significantly different from pK_B value estimated on the guinea-pig right atrium assay ($P < 0.05$).
†b (Schild plot slope parameter) significantly different from unity ($P < 0.05$).
[1] Black *et al.* (1972); [2] Black & Spencer (1973); [3] Brimblecombe *et al.* (1975); [4] Daly *et al.* (1981); [5] Yellin *et al.* (1979); [6] Pendleton *et al.* (1983) (using dimaprit as agonist); [7] Blakemore *et al.* (1980).

on the guinea-pig right atrium suggesting perhaps, that the difference in pK_B values (ΔpK_B) was the same for each antagonist. In the present study we found that the ΔpK_B varied significantly between antagonists. The antagonists appear to possess varying selectivities for the gastric and atrial histamine H_2-receptors. For example the difference in pK_B values obtained on the two assays was 1.16 ± 0.16 and 0.24 ± 0.13 ($P < 0.001$) for metiamide and famotidine, respectively. These data indicate that famotidine expresses 8 fold relative selectivity for gastric histamine H_2-receptors compared to metiamide.

Discussion

Hormone receptor classification critically depends on the availability of specific competitive antagonists. The original definition of histamine H_2-receptors (Black *et al.*, 1972) was based on the use of burimamide which was found to behave like a competitive antagonist of histamine in assays using guinea-pig right atrial and rat uterine muscles. The antagonist potency of burimamide was expressed as a K_B on the assumption, *inter alia*, that the concentration of burimamide in equilibrium with the receptors was equal to the applied concentration. Similar K_B values were obtained on both assays suggesting receptor homogeneity in the two tissues. This result has been amply confirmed by others using different antagonists and techniques.

The main interest in the histamine antagonist properties of burimamide was that histamine-stimulated gastric acid secretion was also blocked. However, the attempt to classify the histamine receptors involved was frustrated at that time because no satisfactory *in vitro* bioassay for gastric acid secretion was available. Major advantages of intact tissues *in vitro* for operational pharmacological analysis are that ligand concentration equilibration between bathing fluid and receptor biophase is expected and that a response to receptor activation can be measured unconfounded by hormonal and neural regulatory responses. Since then, a number of *in vitro* assays of gastric acid secretion, suitable for pharmacological analysis, have been developed at several organisational levels between isolated, intact, stomachs and adenylate cyclase activity in broken cell membrane suspensions. However, Black & Shankley (1985) have recently defined the isolated, lumen-perfused, stomach

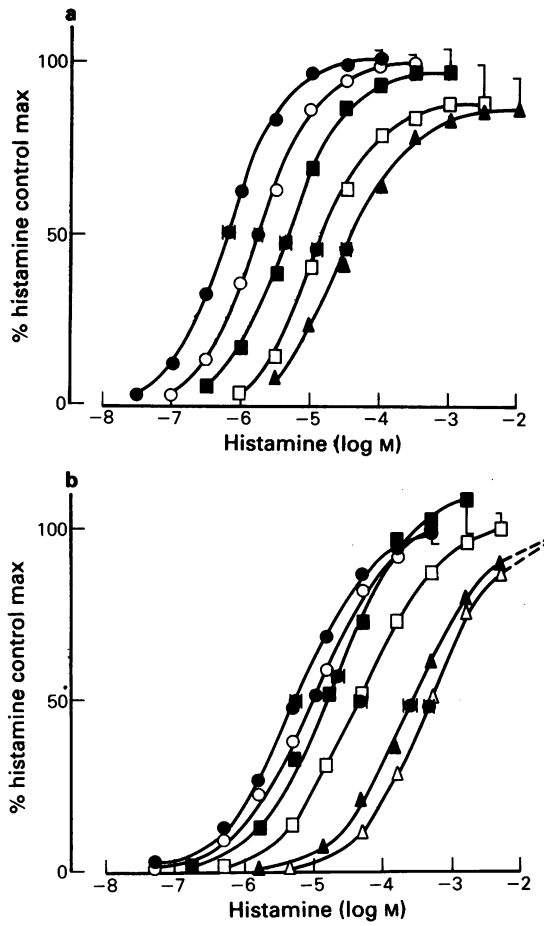

Figure 2 Histamine concentration-effect curves in the absence (●) and presence of famotidine (○) 3×10^{-8}, (■) 3×10^{-7}, (□) 10^{-6} and (▲) 3×10^{-6}M (60 min incubation) on assays of rate in the guinea-pig right atrium (a) and on acid secretion in the mouse stomach (b). Fitted logistic curves are superimposed upon mean experimental data points ($n = 5/6$) expressed as percentage maximum of the histamine control concentration-effect curves. Error bars show standard errors.

preparation as the physiological unit for acid secretion on the grounds that only at this level are all extrinsic regulatory influences eliminated while retaining all the cellular architecture known to be necessary for responses to the physiological agents, nerve stimulation and gastrin. This is the reason why we have persisted with this preparation for the bioassay of histamine H_2-receptor antagonists.

Previous results with this preparation (Angus *et al.*, 1980) found pK_B values for burimamide, metiamide and cimetidine approximately one log unit lower than

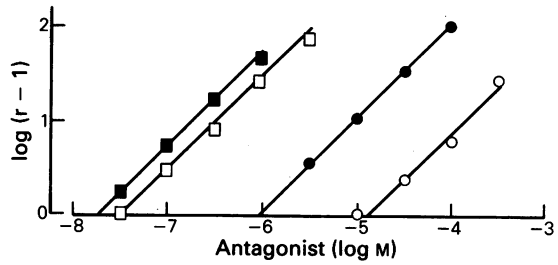

Figure 3 Schild plots for histamine-famotidine (■) and histamine-metiamide (●) assays on the guinea-pig right atrium (closed symbols) and mouse stomach (open symbols). Dose-ratios (r) were calculated from the mean $[A_{50}]$ values estimated for the histamine concentration-effect curves. Estimates of slope parameter (b) and pK_B were obtained by model fitting and are presented in Table 1.

those found with the atrial and uterine assays. Szelenyi & Postius (1984) confirmed these results. So far, no other studies have been reported with this preparation. However, the results found now, using an improved assay with greater precision, have shown not only that low readings occur with all seven antagonists studied but also that the difference between the gastric and atrial assays is not constant. Indeed, the difference between the two assays for famotidine was not significant.

The basic theorem in ligand classification is that when specific assays yield different binding constants, their conjugate systems cannot be homogeneous. Therefore, in this case, if the atrial and gastric assays are giving satisfactory estimates of the relevant pK_B values for the histamine antagonists, then the histamine receptors in the two tissues cannot be homogeneous. Any model of plural receptor populations must not only allow each assay system to have different proportions of each receptor type but also enable each antagonist to have its own unique affinity for these receptors. Plainly, there would be no difficulty in offering a model of H_2-receptor sub-types which could systemize the present results.

Would a model of H_2-receptor subtypes be compatible with the results of other assays of oxyntic cell activity based on different systems? Bunce & Parsons (1976) obtained a pA_2 of 5.91 (4.31–7.51, 95% confidence limits) for metiamide using the isolated, lumen-perfused, stomach of the immature rat, a value not significantly different from the atrial estimate. However, the slope of the Schild regression at 0.73 (0.58–0.88) was significantly less than one. Angus *et al.* (1980) calculated that the pA_2 value was likely to have been overestimated. A similar problem occurs in the finding by Main & Pearce (1981) of a pA_2 for metiamide of 6.49 using isolated sheets of rat gastric

mucosa for the assay. This pA_2 value would not be low compared to atrial assays but as the slope of the Schild regression (= 0.81) was low the comparison, strictly speaking, would not be valid. In general, if low Schild regression slopes are obtained which are nevertheless not significantly different from unity, then refitting the data with the slope constrained to one leads to an estimated pK_B lower than the pA_2 value obtained from the unconstrained regression. Sjostrand *et al.* (1977) also used mucosal sheets (from guinea-pig stomachs) for the bioassay and with Schild regression slopes apparently not different from unity, their pK_B estimates for metiamide (4.82) and cimetidine (5.81) were both comparatively low.

On the other hand, Scholes *et al.* (1976) estimated a pK_B of 6.46 for cimetidine using a histamine-sensitive adenylate cyclase preparation from dog gastric mucosa. Since then, pA_2 values of 6.22 for cimetidine and 5.29 for burimamide were obtained by Berglindh & Öbrink (1979) using a suspension of gastric glands obtained from rabbit mucosa: a pK_B of 6.00 (0.81–1.03), with a Schild regression slope of 0.92, was measured for cimetidine by Soll (1980) using a suspension of oxyntic cells from dog mucosa: and, finally, pA_2 values of 6.33 for cimetidine and 7.65 for famotidine were found by Harada *et al.* (1983) using a histamine-sensitive adenylate cylcase from guinea-pig mucosa. None of these estimates is low compared to the results from atrial muscle assays. Apparently these assays which have not used intact tissues have given pK_B or pA_2 estimates indicative of H_2-receptor homogeneity.

When one set of assays indicate a single class of receptors and another set indicates the need to multiply the number of classes, then the argument using Occam's Razor has to prefer the single class. Angus *et al.* (1980) also reached that conclusion because similar low estimates of pK_B values were found for the interaction between atropine and muscarinic receptors on oxyntic cells. Their argument at that time was that the lumen-perfused stomach preparation had, both literally and figuratively, a hole in it. The oxyntic cell basal membranes, the locus of the histamine and acetylcholine receptors, was imagined to provide an effective partition between the serosal bath fluid and the fluid eventually secreted into the lumen of the stomach and continuously removed by perfusion. Considered pharmacokinetically, the secreted fluid was an open compartment serving continuously to drain the antagonist from the receptor biophase. Therefore, the concentration of ligand in the bathing compartment was assumed to overestimate the steady state concentration of ligand in the receptor biophase. This idea seemed to point to an important source of error peculiar to secretory as distinct from non-secretory assays.

However, the results obtained with isolated gastric glands or oxyntic cells in suspension, giving pK_B estimates not different from those got with functionally reduced systems or with non-secretory tissues, may indicate that the source of error is not due to secretion *per se*. The isolated glands, which become sealed off into closed tubes, are imagined to secrete into the closed lumen: the isolated oxyntic cells also seal off into a spherical shape and secretion takes place into the intracellular maze of canaliculi. However, unlike the isolated stomach and mucous membrane preparations, there is no continuous bulk flow of secretion in the other preparations. Pharmacokinetically, the isolated glands and cells are closed systems which might be expected to end up in ligand concentration equilibrium.

Our conclusion, then, is that there are significant differences in the apparent pK_B values for antagonists at oxyntic cell receptors when the assays are made on pharmacokinetically-open secretory systems. The differences in the pK_B values would then be associated with certain physico-chemical properties of these ligands, such as their lipophilicity and will be considered in more detail in a future paper.

This work was supported by The Wellcome Foundation Ltd. The authors wish to thank Mrs H.D. Williams for help in preparing the manuscript.

References

ANGUS, J.A. & BLACK, J.W. (1979). Analysis of anomalous pK_B values for metiamide and atropine in the isolated stomach of the mouse. *Br. J. Pharmac.*, **67**, 69–65.

ANGUS, J.A. & BLACK, J.W. (1980). Pharmacological assay of cardiac H_2-receptor blockade by amitriptyline and lysergic acid diethylamide. *Circulation Res.*, **46**, Suppl. I, 164–169.

ANGUS, J.A., BLACK, J.W. & STONE, M. (1980). Estimation of pK_B values for histamine H_2-receptor antagonists using an *in vitro* acid secretion assay. *Br. J. Pharmac.*, **68**, 412–423.

ARRANG, J-M., GARBARG, M. & SCHWARTZ, J-C. (1983). Autoinhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature*, **302**, 832–837.

BERGLINDH, T. & ÖBRINK, K.J. (1979). Histamine as a physiological stimulant of gastric parietal cells. In *Histamine receptors*. ed. Yellin, T.O. pp. 35–56. New York: Spectrum.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature*, **236**, 385–390.

BLACK, J.W., GERSKOWITCH, V.P., RANDALL, P.J. & TRIST, D.G. (1981). Critical examination of the histamine-cimetidine interaction in guinea-pig heart and brain. *Br. J. Pharmac.*, **74,** 978P.

BLACK, J.W. & SPENCER, K.E.V. (1973). Metiamide in systematic screening tests. In *International symposium on histamine H_2-receptor antagonists.* ed. Wood, C.J. & Simpkins, M.A. pp. 23–27. London: Smith Kline and French, Ltd.

BLACK, J.W. & SHANKLEY, N.P. (1985). The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis. *Br. J. Pharmac.*, **86,** 571–579

BLAKEMORE, R.C., BROWN, T.H., DURANT, G.J., EMMETT, J.C., GANNELLIN, C.R., PARSONS, M.E. & RASMUSSEN, A.C. (1980). SK F 92994: a new histamine H_2-receptor antagonist. *Br. J. Pharmac.*, **70,** 105P.

BRIMBLECOMBE, R.W., DUNCAN, W.A.M., DURANT, G.J., EMMETT, J.C., GANELLIN, C.R. & PARSONS, M.E. (1975). Cimetidine – a non-thiourea H_2-receptor antagonist. *J. int. Med. Res.*, **3,** 86–92.

BUNCE, K.T. & PARSONS, M.E. (1976). A quantitative study of metiamide, a histamine H_2-antagonist, on the isolated whole rat stomach. *J. Physiol.*, **258,** 453–465.

DALY, M.J., HUMPHRAY, J.M. & STABLES, R. (1981). Effect of a new potent H_2-receptor antagonist 3[[[2-[diaminomethyleneamino]- 4 -thiazolyl] methyl] thiol]-N^2-sulfamoylpropionamidine (YM11170) on gastric mucosal histamine-sensitive adenylate cyclase from guinea-pig. *Biochem. Pharmac.*, **32,** 1635–1640.

HARADA, M., TERAI, M. & HIROO, M. (1983). Effect of a new potent H_2-receptor antagonist 3[[[2-[(diaminomethylene)amino]- 4 -thiazolyl]methyl]thio]- N^2 -sulfamoylproprionamidine (YM-11170) on gastric mucosal histamine-sensitive adenylate cyclase from guinea-pig. *Biochem. Pharmac.*, **32,** 1635–1640.

MAIN, I.H.M. & PEARCE, J.B. (1981). pA_2 determination of muscarinic and H_2-receptor antagonists on gastric acid secretion. *Br. J. Pharmac.*, **74,** 969–970.

PENDLETON, R.G., TORCHIANA, M.L., CHUNG, C., COOL, P., WIESE, S. & CLINESCHMIDT, B.V. (1983). Studies on MK-208 (YM11170) a new, slowly dissociable H_2-receptor antagonist. *Arch. Int. Pharmacodyn.*, **266,** 4–16.

SCHILD, H.O. (1957). Drug antagonism and pA_2. *Pharmac. Rev.*, **9,** 242–246.

SCHOLES, P., COOPER, A., JONES, D., MAJOR, J., WALTERS, M. & WILDE, C. (1976). Characterization of an adenylate cyclase system sensitive to histamine H_2-receptor excitation in cells from dog gastric mucosa. *Agents and Actions*, **6,** 677–682.

SHEPHERD-ROSE, A.J. & PENDLETON, R.G. (1985). Studies on the H_2-receptor antagonism of MK208 in isolated rabbit gastric glands. *Eur. J. Pharmac.*, **106,** 423–426.

SJOSTRAND, S.E., RYBERG, B. & OLBE, L. (1977). Analysis of the actions of cimetidine and metiamide on gastric acid secretion in the isolated guinea-pig gastric mucosa. *Naunyn-Schmiedebergs Arch. Pharmac.*, **296,** 139–142.

SOLL, A.H. (1980). Secretagogue stimulation of [^{14}C]-aminopyrine accumulation by isolated canine parietal cells. *Am. J. Physiol.*, **238,** G366–G375.

STONE, M. & ANGUS, J.A. (1978). Developments of computer-based estimation of pA_2 values and associated analysis. *J. Pharmac. exp. Ther.*, **207,** 705–718.

SZELENYI, I. & POSTIUS, S. (1984). The effect of different histamine H_2 receptor antagonists on the gastric acid secretion of the isolated whole stomach of the mouse and on the heart rate of spontaneously beating atrium of the guinea pig. *Arzneim. Forsch.*, **34,** 787–788.

YELLIN, T.O., BUICK, S.H., GILMAN, D.J., JONES, D.F. & WARDLEWORTH, J.M. (1979). ICI 125,211: a new gastric antisecretory agent acting on histamine H_2-receptors. *Life Sci.*, **25,** 2001–2009.

(Received March 4, 1985.)
Revised June 28, 1985.
Accepted July 8, 1985.)