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Correlation between log P_{OCT/H_2O} and pK_B estimates for a series of muscarinic and histamine H_2-receptor antagonists

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1 With histamine used as agonist, pK_B values were estimated for seventeen histamine H_2-receptor antagonists on assays involving acid secretion by the mouse isolated stomach and contraction frequency of the guinea-pig right atrium.

2 With the exception of oxmetidine, SK&F 94,826 and SK&F 94,206 on the right atrium assay, the compounds behaved as simple competitive antagonists on both assays. Although the former three compounds produced concentration-dependent, parallel, displacement of the histamine concentration-effect curves, subsequent analysis indicated Schild plot slope parameters significantly less than unity. However, the application of a combined dose-ratio analysis indicated that their antagonistic behaviour did not differ from expectations for simple competition at dose-ratios of approximately 20, and pK_B values were estimated on this basis.

3 In accordance with previously reported data, pK_B values were found to be consistently lower on the stomach than atrial assays. The pK_B value for tiotidine was underestimated to the same extent on the stomach assay when impromidine was used as agonist.

4 The removal of the serosal muscle from the mouse stomach, achieved by using an isolated, perfused, mucosal sheet preparation, did not significantly affect the underestimation of the pK_B value for metiamide.

5 Linear regressional analysis indicated a significant, positive, correlation between lipophilicity (log P_{OCT/H_2O}) of the antagonists and the degree of antagonist pK_B value underestimation on the gastric secretion assay.

Introduction

In a previous paper (Black *et al.*, 1985) we extended the original studies of Angus & Black (1979) and Angus *et al.* (1980) who found that the calculated pK_B values obtained for the histamine H_2-receptor antagonists, burimamide, metiamide and cimetidine were significantly lower in the isolated, lumen-perfused, stomach preparation of the mouse than in the guinea-pig right atrium-assay. Although confirming the original results, our examination of a further three antagonists, ranitidine, oxmetidine and famotidine, indicated that the differences between the pK_B values obtained on the gastric and atrial assays were not constant. Subsequently, we observed a similar phenomenon when pK_B values were estimated for three muscarinic receptor antagonists both on the mouse stomach and guinea-pig trachea assays (Black & Shankley, 1985a). The pK_B value for atropine was found to be underestimated to a greater extent on the gastric acid assay than the pK_B values obtained for pirenzepine and N-methylatropine. We suggested that lipophilicity was a determinant of the degree of pK_B underestimation in the gastric acid assay because atropine exhibits greater lipophilicity than pirenzepine and N-methylatropine. In accord with the original hypothesis of Angus & Black (1979) and Angus *et al.* (1980), the compounds were imagined to partition across the gastric mucosa resulting in a net, lower, concentration of antagonist in the region of the oxyntic cell receptors. The above hypothesis requires that the underestimation of antagonist affin-

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ity in the mouse stomach assay is independent of the class of receptor under investigation. The underestimation should be a simple function of extent of partitioning across the mucosa. The ability of a compound to penetrate biological membranes has been correlated with its oil/water partition coefficients (Hansch & Leo, 1979). Accordingly, in this study, we have examined a series of histamine H_2-receptor antagonists which were selected to allow investigation of the relationship between antagonist lipophilicity, expressed as log P_{OCT/H_2O}, and the underestimation of pK_B value in the mouse stomach assay. In addition, the effect of removing the serosal muscle on pK_B value underestimation was investigated on an isolated, perfused, mucosal sheet preparation of the mouse.

Methods

Acid secretion

Gastric acid secretion was measured in the isolated, lumen-perfused, stomach preparation of the mouse as described previously (Black & Shankley, 1985b). 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.

In a separate series of experiments, gastric acid secretion was studied in an isolated, perfused, mucosal sheet preparation of the mouse. The technique used the same principles applied by Main & Pearce (1978) to the development of the rat mucosal sheet preparation. In brief, the stomach, from the same stock of mice used for the whole stomach assay, was opened by incision along the greater curvature. The glandular part of the stomach was gently stretched and ligated over a perspex disc (10 mm ext. dia.) to produce a water-tight seal. Approximately 0.5 ml of mucosal solution was injected, through a fine needle, between the mucosa and serosal muscle layers and the blister of muscle produced was carefully removed with fine iridectomy scissors. The mucosal surface of the sheet was perfused at 0.5 ml min^{-1} with mucosal solution via two stainless steel tubes (2 mm int. diam.) passing through the centre of the perspex disc. The whole preparation was then lowered into a 40 ml organ bath containing serosal solution (Black & Shankley, 1985b). In other respects the assay was identical to that employed for the whole stomach with the exception that the pH-electrode system was arranged to provide only a 5 cmH_2O pressure which slightly distended the mucosal sheet.

Guinea-pig right atrium preparation

Chronotropic effects were studied in isolated, spontaneously-beating, right atria from male guinea-pigs (for details see Angus & Black, 1980). Briefly, 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 (ΔpH) and guinea-pig right atrium responses as changes in rate (Δ beats min^{-1}). 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, 1985b). Analyses of agonist-antagonist interactions and subsequent estimation of antagonist dissociation constant (pK_B) values were performed as described previously (Black *et al.*, 1985).

Combined dose-ratio analysis

A combined concentration-ratio analysis, based on the technique of Paton & Rang (1965) was performed to determine whether the antagonism observed with oxmetidine, SK&F 94,826 and SK&F 94,206 could be accounted for by a reversible interaction, syntopic with tiotidine, at the histamine H_2-receptor.

i *Additive model* According to the Law of Mass Action, if two competitive antagonists act syntopically the dose-ratio obtained in the presence of both antagonists is predicted to obey the following relation (Paton & Rang, 1965):

$$r_{B+C} = r_B + r_C - 1 , \quad (1)$$

where r_B and r_C are the dose-ratios obtained in the presence of antagonists B and C, respectively. Writing dose-ratios in terms of agonist concentration-effect curve midpoint location parameters ($[A_{50}]$ values), equation (1) becomes,

$$\frac{[A_{50}]_{B+C}}{[A_{50}]} = \frac{[A_{50}]_B}{[A_{50}]} + \frac{[A_{50}]_C}{[A_{50}]} - 1 , \quad (2)$$

with the suffix, as before, denoting the presence of antagonists. However, experimentally, $\log[A_{50}]$ values are determined and these are assumed to be normally distributed. Therefore, taking logarithms and rearranging equation (2) gives,

$$S_A = 0 = \log[A_{50}]_{B+C} - \log([A_{50}]_B + [A_{50}]_C - [A_{50}]) , \quad (3)$$

where S_A is the test-statistic for the additive model. Thus, if the experimental data accord with the additive model, S_A (equation (3)) should have a value of zero.

The standard error (approximation provided by J. Wood, Wellcome Research Laboratories, personal communication) of this test-statistic is given by,

$$\text{s.e. } (S_A) = \sqrt{\sigma^2_{B+C} + \left[\frac{[A_{50}]^2 \cdot \sigma^2 + [A_{50}]^2_B \cdot \sigma^2_B + [A_{50}]^2_C \cdot \sigma^2_C}{([A_{50}]_B + [A_{50}]_C - [A_{50}])^2}\right]} , \quad (4)$$

where σ is the standard deviation associated with the experimental $\log[A_{50}]$ estimates.

ii *Multiplicative model* If two antagonists act heterotopically their dose-ratios are predicted to multiply, as follows (Paton & Rang, 1965),

$$r_{B+C} = r_B \times r_C . \quad (5)$$

Writing equation (5), as for the additive model, in terms of $\log[A_{50}]$ values,

$$S_M = 0 = \log[A_{50}]_{B+C} - \log[A_{50}]_B - \log[A_{50}]_C + \log[A_{50}] , \quad (6)$$

where S_M is the test-statistic for the multiplicative model. Thus, if the antagonists behave in accord with the multiplicative model S_M (equation (6)) should have a value of zero.

The standard error of S_M is given by

$$\text{s.e. } (S_M) = \sqrt{\sigma^2_{B+C} + \sigma^2_B + \sigma^2_C + \sigma^2} . \quad (7)$$

log P determination

Octanol : water partition coefficients for the free base of the histamine H_2-receptor antagonists were determined at 37°C by a shake-flask technique using an aqueous buffer at pH 9.0 unless otherwise indicated in Table 1. The phases were allowed to settle out under gravity. The concentrations of compound in the aqueous phase before and after partitioning were determined spectrophotometrically, and buffer salts were used to control the pH of the aqueous phase. Log P for the free base form was derived from the equation,

$$\log P = \log P_a + \log(1 + 10^{pKa - pH})$$

where $\log P_a$ is the apparent partition measured at the given pH.

Drugs

Drugs were prepared in distilled water. Molar stock solutions of histamine dihydrochloride (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. The following compounds were from Smith Kline and French Research Ltd: burimamide, metiamide, cimetidine, oxmetidine, lupitidine (SK&F 93,479), SK&F 94,826, SK&F 92,363, SK&F 92,629, SK&F 92,857, SK&F 93,162, icotidine (SK&F 93,319), SK&F 92,540, SK&F 92,456, SK&F 94,206. Tiotidine, ranitidine and famotidine were gifts from Imperial Chemical Industries Ltd, Glaxo Group Research Ltd and Merck Sharp and Dohme Ltd, respectively.

Results

Estimation of pK_B values for histamine H_2-receptor antagonists on the whole stomach and guinea-pig atrium assays

All the antagonists produced significant concentration-dependent parallel displacement of histamine concentration-effect curves with no significant change in maximal asymptotes on both the stomach and atrium assays with the exception of SK&F 93,162 on the stomach assay (see below). Analysis of the dose-ratios (see Methods) indicated Schild slope parameters (b) not significantly different

from unity with the exception of the analysis of the data from the following interactions on the guinea-pig right atrium: histamine/SK&F 94,826 (b = 0.79 ± 0.08), histamine/SK&F 94,206 (b = 0.78 ± 0.03) and histamine/oxmetidine (b = 0.81 ± 0.07: Black *et al.*, 1985). The estimates of pK_B are presented in Table 1. On the stomach assay SK&F 93,162 was apparently inactive. Concentrations up to 3 mM did not produce a significant shift of the histamine concentration-effect curve although a pK_B value of 6.00 was estimated on the right atrium.

With impromidine as agonist, tiotidine behaved as a simple competitive antagonist on both the whole stomach and atrium assays. The pK_B values estimated (Table 1) did not significantly differ from those obtained using histamine as agonist.

pK_B determination for oxmetidine, SK&F 94,826 and SK&F 94,206 on the guinea-pig right atrium assay: combined dose-ratio analysis

Concentrations of tiotidine (see Black *et al.*, 1985) and of the test compounds were selected to produce individual dose-ratios of approximately 20. In all cases the antagonists and their combinations produced parallel rightward shift of the histamine concentration-effect curves with no significant change in maximal asymptote. Details of the results and analysis are presented in Table 2. In brief, the analysis indicated that the inhibitory action of SK&F 94,206, SK&F 94,826 and oxmetidine could not be differentiated from simple competition at the concentration investigated. Accordingly, the individual log[A_{50}] values, determined in this analysis, in the absence and presence of antagonist ([A_{50}], [A_{50}]$_B$ – Table 2) were fitted to the Schild equation (see Black *et al.*, 1985) with the slope parameter, b, constrained to unity. The pK_B estimates obtained are shown in Table 1.

Comparison of pK_B values obtained from atrium and stomach

As we found previously (Black *et al.*, 1985a) the pK_B values obtained on the stomach assay were consistently lower than those found on the guinea-pig right atrium. A significant correlation ($r = 0.80$: $P < 0.01$) was found between the difference in pK_B values (ΔpK_B) and the lipophilicity of the compounds, the latter expressed in terms of their octanol/water partition coefficients (log P_{OCT/H_2O}). Examination of the log P_{OCT/H_2O}/ΔpK_B plot (Figure 1) reveals that out of the 16 histamine H_2-receptor antagonists and three muscarinic receptor antagonists included in the analysis, only the value for oxmetidine lies outside the 95% confidence limits of the regression line.

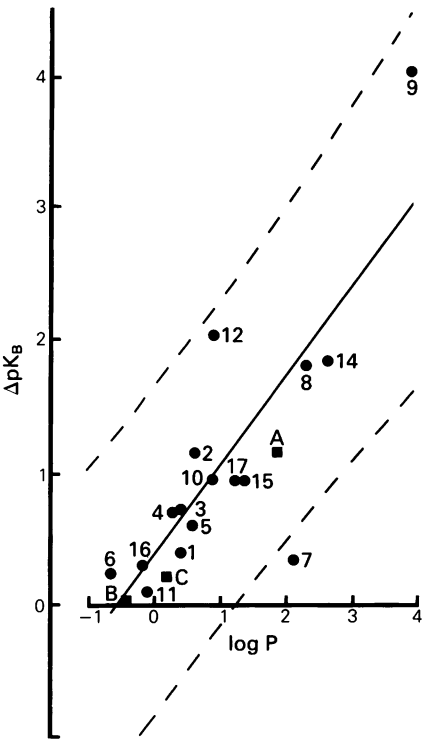


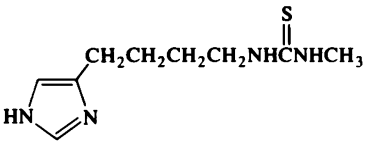
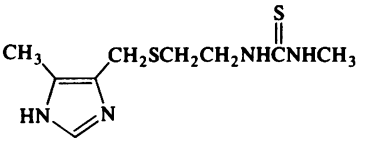
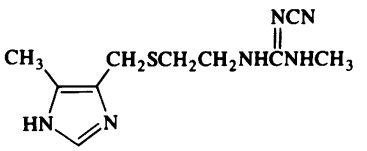
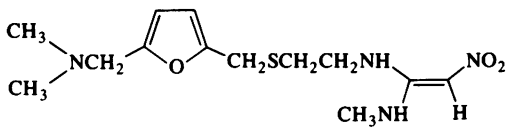
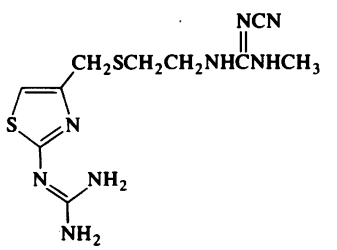
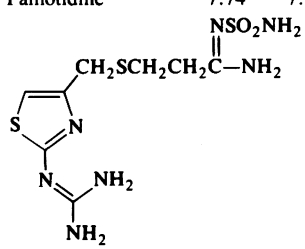
Figure 1 Linear regression analysis of ΔpK_B values and log P_{OCT/H_2O} values for histamine H_2-receptor and muscarinic receptor antagonists. The numbers labelling the data points correspond to those presented in Table 1. A significant correlation ($r = 0.82$: $P < 0.001$) was found between ΔpK_B and log P_{OCT/H_2O}: the equation for the best-fit line was $\Delta pK_B = 0.67 \log P + 0.39$ with the dashed lines representing 95% confidence limits.

Interestingly, SK&F 93,162, which did not produce a significant shift of the histamine concentration-effect curve on the stomach assay, possesses a relatively high log P_{OCT/H_2O} value (Table 1) suggesting that the result is not inconsistent with the relationship found.

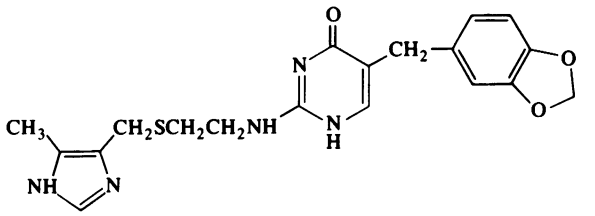
Estimation of pK_B values for histamine H_2-receptor antagonists on the mouse mucosal sheet assay

The isolated, perfused, mucosal sheet preparation of the mouse, like the whole stomach, produced a steady-basal secretion (pH ≈ 4.60) which was not significantly altered by the addition of histamine H_2-receptor antagonists. Histamine produced a sustained, concentration-dependent increase in gastric acid secretion, allowing the full definition of a concentration-effect curve in a single preparation (Figure 2). Both metiamide and famotidine behaved as simple competitive antagonists. The pK_B values

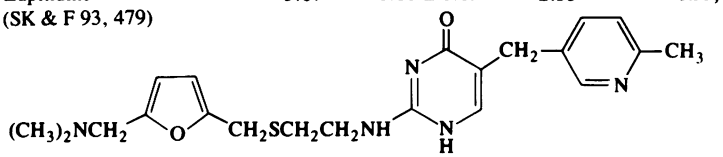
Table 1 pK_B estimates for muscarinic and histamine H_2-receptor antagonists

		pK_B *Atrium*	pK_B *Stomach*	$\Delta pK_B \pm s.e.$	*log P*[7]	pK_a	*Compound reference*
1	Burimamide[3]	4.92	4.51	0.41 ± 0.16	0.40	7.25[16]	
							
2	Metiamide[3]	6.06	4.90	1.16 ± 0.16	0.62	6.80[16]	
							
3	Cimetidine[3]	6.08	5.35	0.73 ± 0.13	0.40	6.80	
							
4	Ranitidine[3]	6.75	6.03	0.72 ± 0.13	0.27[8]	8.18	
							
5	Tiotidine[3]	7.57 (7.64)[5]	6.96 (7.16)[5]	0.61 ± 0.13 (0.48 ± 0.14)[5]	0.60	6.80[17]	
							
6	Famotidine[3]	7.74	7.50	0.24 ± 0.13	−0.67[9]	6.80[17]	
							

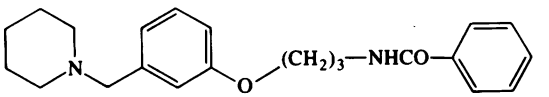
		pK_B Atrium	pK_B Stomach	$\Delta pK_B \pm s.e.$	log P[7]	pK_a	Compound reference
7	Oxmetidine[3]	7.05[1]	6.70	0.35 ± 0.16	2.12[10]	6.80[18]	Durant *et al.*, 1980



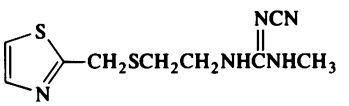
8	Lupitidine (SK & F 93, 479)	7.67	5.87	1.80 ± 0.19	2.33[11]	5.99, 8.18[19]	Blakemore *et al.*, 1981



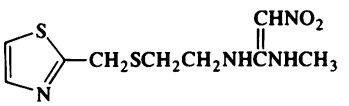
9	SK & F 94, 826	7.44[1]	3.43	4.01 ± 0.20	3.97[12]	9.00[20]	Young *et al.*, 1987



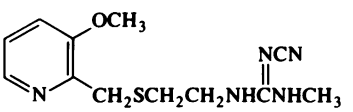
10	SK & F 92, 363	5.85	4.89	0.96 ± 0.12	0.89	1.8[21]	Durant *et al.*, 1975



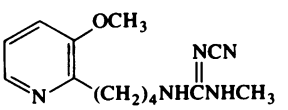
11	SK & F 92, 629	5.30	5.20	0.10 ± 0.13	−0.15	1.8[22]	Durant *et al.*, 1976



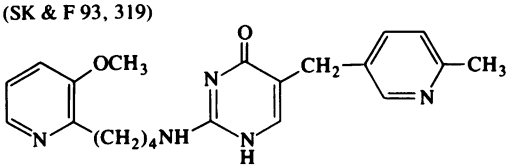
12	SK & F 92, 857	6.66	4.63	2.03 ± 0.16	0.97	4.08	[26]

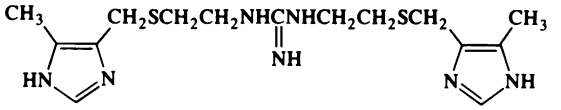
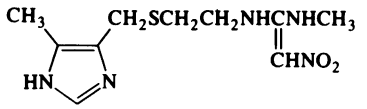
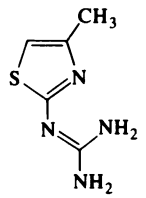


13	SK & F 93, 162	6.00	< 3.52[2]	> 2.50	1.50	5.44	[26]



14	Icotidine (SK & F 93, 319)	7.22	5.38	1.84 ± 0.11	2.64[12]	6.0, 9.78[23]	Ganellin *et al.*, 1986



		pK_B Atrium	pK_B Stomach	$\Delta pK_B \pm$ s.e.	log P[7]	pK_a	Compound reference
15	SK & F 92, 540 	7.24	6.29	0.95 ± 0.13	1.34[13]	6.8, 11.4[24]	Durant *et al.*, 1985
16	SK & F 92, 456 	5.34	5.04	0.30 ± 0.08	−0.40	6.80[18]	Ganellin, 1981
17	SK & F 94, 206 	5.12[1]	3.19	1.93 ± 0.19	1.24[14]	6.80[25]	Button *et al.*, 1985

		Trachea	*Stomach*		
A	Atropine[4]	8.93	7.78 (7.90)[6]	1.15 ± 0.19	1.83
B	N-Me atropine[4]	9.69	9.67	0.02 ± 0.17	−0.40
C	Pirenzepine[4]	6.87	6.67 (6.69)[6]	0.20 ± 0.13	0.20

[1] Low Schild plot slope parameter. pK_B estimate obtained from a combined dose-ratio analysis (see text for details).
[2] No significant shift of the histamine concentration-effect curve was obtained with 3×10^{-4} M SK & F 93, 162. Therefore, the ΔpK_B value was not included in the linear regressional analysis.
[3] Data from Black *et al.*, 1985.
[4] Data from Black & Shankley (1985a) using 5-methylfurmethide as agonist.
[5] Using impromidine as agonist.
[6] Data from Black & Shankley (1985c) using McN-A 343 as agonist.
[7] Octanol: water partition coefficients.
[8] Partition measured at pH 10.5.
[9] Result kindly provided by the Physical Chemistry Department, Wellcome Research Laboratories Ltd.
[10] Partition measured at pH 8.47.
[11] Partition measured at pH 8.26.
[12] Partition measured at pH 7.50.
[13] Value is approximate because of uncertainty in the guanidine pK_a.
[14] Reported by Gilman *et al.*, 1982.
[15] pK_a values measured by M. J. Graham (Smith Kline & French Ltd.) potentiometrically in 0.1M KCl at 25°C and corrected for 37°C unless otherwise indicated.
[16] Black *et al.*, 1974.
[17] pK_a by analogy with SK & F 94, 206 (compound 17).
[18] pK_a by analogy with metiamide (compound 2).
[19] pK_a values for picolyl ring (5.99) and Me_2NH (8.18) respectively. The isocytosine ring has pK_a values of 3.03 (dissociation of cation) and 10.2 (dissociation to anion) at 25°C.
[20] pK_a by analogy with N-(*m*-methoxyphenylmethyl) piperidine determined to be 9.34 at 25°C.
[21] pK_a measured by Dr E. S. Pepper (Smith Kline & French Ltd.) using an n.m.r. method.
[22] pK_a by analogy with SK & F 92, 363 (compound 10).
[23] pK_a values for picolyl ring (5.9) and isocytosine ring (9.78, dissociation to anion) respectively. The methoxypyridyl ring has pK_a 5.5 (Ganellin *et al.*, 1986).
[24] pK_a by analogy with impromidine (Durant *et al.*, 1985).
[25] pK_a 7.05 at 25°C reported by Button *et al.* (1985).
[26] SK & F 92, 857 and SK & F 93, 162 synthesized by Dr G. S. Sach, Smith Kline & French Ltd.

Table 2 Combined dose-ratio analysis: histamine-tiotidine interaction with SK&F 94,206, SK&F 94,826 and oxmetidine in the guinea-pig right atrium assay

Histamine curve Location parameter	*SK&F 94,206*	*SK&F 94,826*	*Oxmetidine*
$\log[A_{50}]$	-5.97 ± 0.06 (5)	-6.23 ± 0.06 (5)	-6.03 ± 0.09 (5)
$\log[A_{50}]_B$	-4.78 ± 0.07 (5)	-4.86 ± 0.12 (5)	-4.66 ± 0.08 (5)
$\log[A_{50}]_C$	-4.96 ± 0.09 (4)	-4.78 ± 0.07 (5)	-4.65 ± 0.06 (6)
$\log[A_{50}]_{B+C}$	-4.63 ± 0.12 (5)	-4.51 ± 0.08 (5)	-4.35 ± 0.14 (5)
Additive model			
S_A ($\pm$s.e.)	-0.05 ± 0.29	0.01 ± 0.45	0.01 ± 0.34
significance	NS	NS	NS
Multiplicative model			
S_M ($\pm$s.e.)	-0.86 ± 0.03	-1.10 ± 0.17	-1.07 ± 0.20
significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

Concentrations of antagonist, tiotidine (B) = 5×10^{-7} M: SK&F 94,206 = 7×10^{-5} M: SK&F 94,826 = 10^{-6} M: oxmetidine = 2×10^{-7} M. (Number in parentheses indicates no. of replicates). S_A and S_M are the test-statistic values for the additive and multiplicative models (see Methods for details).

estimated for metiamide (5.20 ± 0.14) and famotidine (7.74 ± 0.11) were both slightly higher than those obtained in the whole stomach assay (Table 1), although not significantly as tested. Apparently, removal of the serosal muscle did not affect the underestimation of pK_B values (metiamide: $\Delta pK_B = 0.86 \pm 0.18$, famotidine: $\Delta pK_B = 0.00 \pm 0.13$).

Discussion

The estimation of antagonist pK_B values is central to contemporary hormone receptor classification techniques. The estimation is usually considered valid when the basic criteria for competition are satisfied, namely, that the antagonist produces a parallel, rightward, displacement of the agonist concentration-effect curves with no change in maximal response and that the $[A_{50}]$ values subsequently analysed fit the Gaddum-Schild equation. In the present study these criteria were satisfied in the analysis of the interaction between histamine and 14 out of 17 histamine H_2-receptor antagonists on both the mouse stomach and guinea-pig right atrium assays. Additionally, when a secondary, combined dose-ratio analysis was performed with the three exceptional antagonists, the results were consistent with those expected for a simple competitive interaction at the concentrations investigated and pK_B values were estimated on this basis (Table 2). Kenakin (1984), among others, has illustrated how underlying complexity in several experimental situations can in the first instance go undetected due to the apparently simple competitive behaviour of an agonist-antagonist interaction. Complexities such as, for example, agonist uptake processes and two receptor systems are usually revealed by using more than one antagonist and/or agonist. Accordingly, confidence in the conclusion of competitivity in the action of an antagonist, and therefore the validity of the pK_B estimate, increases proportionally with the number of agonist-antagonist interactions investigated which produce simple competitive behaviour. For example, the low Schild plot slope parameters estimated from the analysis of the interaction between histamine and the three compounds, SK&F 94,206, SK&F 94,826 and oxmetidine, on the guinea-pig right atrium assay (Table 1) could be indicative of complexities such as those mentioned above. However, the results of the combined dose-ratio analysis, the apparent simple behaviour of these three compounds on the stomach assay and the simple behaviour of the remainder of the compounds on both assays, when considered together, suggest that histamine does interact with a single receptor population in each assay.

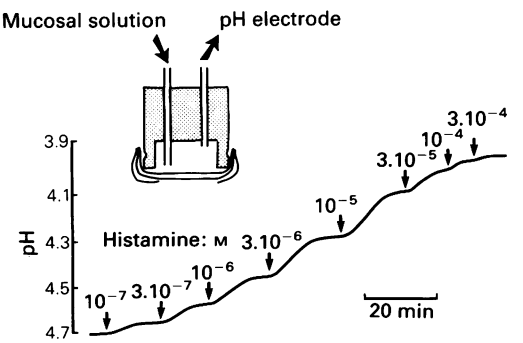


Figure 2 Experimental record of a cumulative histamine concentration-effect curve on the mouse isolated, perfused, mucosal sheet assay. The pH scale refers to the pH of the lumen perfusate. The inset shows, schematically, the mucosal sheet attached to the perspex disc (see Methods).

In the present study the criteria for simple competition were satisfied with a whole range of histamine H_2-receptor antagonists, using histamine as agonist (Table 1). In addition the criteria were also satisfied and similar pK_B values estimated when the selective histamine H_2-receptor agents, dimaprit (Angus & Black, 1979) and impromidine (Table 1) were used as agonists. Thus, if no further information was available the low values of pK_B obtained on the mouse stomach assay could be considered as preliminary evidence for histamine H_2-receptor heterogeneity.

However, in the previous analyses of the underestimation of pK_B values on the mouse stomach assay (Angus & Black, 1979; Black *et al.*, 1985; Black & Shankley, 1985a, c), a more conservative explanation for the results was preferred because similar underestimation of pK_B values was found for muscarinic receptor antagonists on the mouse stomach assay, which was also independent of the agonist used. The antagonists were imagined to partition across the gastric mucosa, resulting in a net lower concentration of antagonist in the region of the oxyntic cell receptors in the mouse stomach assay. Such a process would occur independently of the receptor class involved, as found experimentally. In addition, the difference in antagonist behaviour on the stomach assay compared to the behaviour on the guinea-pig right atrium and trachea assays for histamine H_2- and muscarinic receptor antagonists, respectively, was seen only as a parallel displacement of the Schild plot; that is, no evidence was obtained to indicate that the loss process approached saturating conditions. Furthermore, steady-state agonist responses were obtained which is consistent with the view that steady-state concentrations of the agonist and antagonist were achieved in the region of the hormone receptors. The simplest model to account for all these data involves, firstly, the diffusion of the antagonist from the serosal bathing solution into the receptor compartment and, secondly, the subsequent loss of the antagonist through the mucosal membrane into the gastric acid secretion. The positive, significant correlation found between the lipophilicity of the compounds and the degree of pK_B underestimation on the mouse stomach assay for both muscarinic and histamine H_2-receptor antagonists (Figure 1) supports the general hypothesis that the hormone receptors in the mouse stomach are homogeneous with those found on the guinea-pig right atrium and trachea assays. However, the finding that a single lipophilicity parameter correlates with the antagonist behaviour is perhaps rather surprising. The model involves two processes, diffusion and passage through the mucosal membrane, and we might expect that it is only the rate of the latter process that is related to lipophilicity. The correlation may well be improved if an additional parameter relating to the diffusion process were included. Similarly, the choice of octanol/H_2O coefficients as a measure of lipophilicity was arbitrary, the data were more readily available, and the use of partitioning data using different solvent pairs might also improve the correlation. Interestingly, the results obtained on the isolated, perfused, mucosal sheet preparation indicate that the removal of the serosal muscle layer does not facilitate antagonist concentration equilibrium between the serosal solution and receptor compartment.

The histamine H_2-antagonists originally studied (Black *et al.*, 1985) (compounds 1–7 in Table 1 and Figure 1) are relatively weak bases having pK_a values (6.5–8) near physiological neutrality, thus permitting a substantial proportion of compound molecules to be in the uncharged (i.e. non-protonated) form which is most readily able to cross lipoidal cell membranes. In an attempt to test whether local pH effects could complicate the relationship between lipophilicity and pK_B underestimation, a pair of compounds (SK&F 92,363 and SK&F 92,629, compounds 10 and 11 in Table 1 and Figure 1), were assayed. These compounds have a very low pK_a (1.8) and presumably remain mainly unprotonated even over the pH range likely to be encountered in the mouse stomach assay. The compounds are thiazole analogues of the more basic imidazole compounds, cimetidine (compound 3) and SK&F 92,456 (compound 16) respectively. The pK_B values obtained were similar, indicating that the extent of protonation, for these compounds at least, does not contribute significantly to the process responsible for pK_B underestimation on the mouse stomach assay. By contrast, SK&F 92,540 (compound 15) was selected as a guanidine derivative ($pK_a > 11$) which will be almost exclusively protonated (>99.9%) at pH 7.4 and, therefore, should theoretically possess low ability to penetrate membranes. The fact that the pK_B value was significantly underestimated (Table 1) suggests that the compound is in fact able to penetrate membranes, presumably due to rapid equilibration between the protonated and neutral conjugate base form.

An interesting pair of compounds are the methoxypyridine derivatives, SK&F 92,857 (compound 12) and SK&F 93,162 (compound 13), which differ solely in the side-chain atoms where $-CH_2-$ replaces $-S-$. These compounds, when initially synthesized and tested *in vivo* in the rat were found to differ considerably as inhibitors of histamine-stimulated gastric acid secretion (M.E. Parsons, personal communication: as tested by i.v. administration against a near maximal stimulation of acid secretion produced by histamine infusion in a modified Ghosh-Schild preparation of the lumen-perfused

stomach of the anaesthetized rat). SK&F 93,162 was found to be some 30 fold weaker in activity, although their respective affinities for the histamine H_2-receptor estimated in the guinea-pig right atrium assay in this study only differ by 4.5 fold (Table 1). At the time of initial testing, the reason for the difference was not understood and, in the absence of specific information, was attributed to *in vivo* effects such as a presumed rapid metabolic deactivation of SK&F 93,162. Apparently, there is no need to invoke such effects *in vivo* because the difference in potency is also expressed on the isolated mouse stomach assay.

SK&F 93,319 (compound 14) and SK&F 93,479 (compound 8) are isocytosine derivatives and were tested for comparison with oxmetidine (compound 7, also an isocytosine). They differ from oxmetidine, however, in possessing a methyl picolyl group as a 5-substituent in the isocytosine ring instead of a methylene-dioxybenzyl group, but the lipophilicities of these two groups are similar. The results obtained from the assay of these two compounds were found to lie on the best-fit regression between log P_{OCT/H_2O} and ΔpK_B. At this time, we cannot explain why the data obtained with oxmetidine do not fit the regression.

SK&F 94,206 (compound 17) and SK&F 94,826 (compound 9) were selected simply as examples of compounds previously found to be weak inhibitors of histamine-stimulated acid secretion *in vivo* in the rat (M.E. Parsons, personal communication). They were also found to have very low activity on the mouse stomach assay, although both were potent antagonists in the guinea-pig right atrium assay (Table 1).

In a previous report (Black & Shankley, 1985a) we suggested that the ability of pirenzepine to display selective inhibition of acid secretion, *in vivo*, compared to atropine could simply be due to lack of loss into the gastric acid secretion and hence an effective blocking concentration would occur at much lower plasma levels. The above findings in the rat, using SK&F 94,826 and SK&F 94,206, indicate that the same process could occur for histamine H_2-receptor antagonists and adds support to the notion that the pK_B underestimation observed *in vitro* is relevant to the *in vivo* situation.

In conclusion, it would appear that the inconsistencies among antagonist pK_B values for the muscarinic and histamine H_2-receptors antagonists can be accounted for without the need to postulate heterogeneity of receptor populations. Although log P values measured between octanol and water may perhaps not be expected to be the best indicators of the behaviour of the antagonists in the system, the correlation observed between log P_{OCT/H_2O} and ΔpK_B values does suggest that the degree of underestimation of antagonist affinity is related to the lipophilicity of the antagonists.

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