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Application of a model to explore interspecies differences in acetylcholine M-receptor-stimulated gastric acid secretion

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1 Concentration-effect curves were obtained, in the absence and presence of histamine H_2-receptor blockade, to 5-methylfurmethide (5-MeF) and McN-A 343, high efficacy and low efficacy acetylcholine (ACh) M-receptor agonists, respectively, in isolated stomach preparations from the mouse and immature rat and guinea-pig.

2 In the immature guinea-pig assay, the responses to 5-MeF and McN-A 343 were abolished by histamine H_2-receptor blockade suggesting that the responses were totally dependent upon gastric mucosal histamine. However, in the mouse and immature rat assays, although the histamine H_2-receptor antagonists produced small but significant rightward shifts and, in some cases, depression of the maximum of the agonist concentration-effect curves, a significant secretory response remained, presumed to be due to direct stimulation of oxyntic cells.

3 Previously, by assuming that the histamine H_2-receptor blockade alters the mode of agonist-stimulated acid secretion from mainly an indirect action mediated by histamine release to direct stimulation of the oxyntic cell, we applied an operational model of agonism to similar data obtained in the mouse preparation. In that study we were able to account for the behaviour of 5-MeF and McN-A 343 by assuming that the agonists expressed 6 fold higher efficacy, τ in the operational model of agonism, at ACh M-receptors on the histamine-releasing cells than on the oxyntic cells. In this study it was possible to account for the variation in the behaviour of the agonists both between and within assays by simply varying the efficacy expressed by the agonists at each of the cells in the model. The efficacy variation could be due to receptor concentration variation.

4 The data and analysis are discussed in terms of contemporary models for the role of histamine in the regulation of gastric acid secretion.

Keywords: Receptor, muscarinic; parasympathomimetics; gastric acid secretion; histamine

Introduction

Although when applied exogenously histamine is a powerful stimulant of gastric acid secretion, its physiological role in the gastric mucosa remains unclear. In particular, the question of how gastrin and acetylcholine (ACh) stimulate gastric acid secretion is unresolved. Do these agents release histamine locally or is a subthreshold background of histamine needed to potentiate their activity at the oxyntic cell? In previous publications, we developed the hypothesis that they act indirectly by releasing mucosal histamine (see Black & Shankley, 1987). In this study, we have investigated the histamine-dependence of the secretory response mediated by ACh M-receptors and have continued to use this hypothesis as an initial basis for interpretation. In a previous paper (Black & Shankley, 1986), we described a study of the neural regulation of gastric acid secretion in isolated, lumen-perfused, mouse stomach preparations. We concluded that the responses produced by electrical stimulation were mediated by ACh released by preganglionic nerve stimulation. The stimulation-frequency responses could be abolished by both histamine H_2-receptor antagonists and ACh M-receptor antagonists. However, it was still possible to obtain a response to the stable ACh muscarinic agonist, 5-methylfurmethide (5-MeF), in the presence of histamine H_2-receptor blockade (Black & Shankley, 1985a). Therefore, we concluded that the neurally-released ACh acted selectively on histamine-releasing cells whereas 5-MeF stimulated ACh M-receptors located on both histamine-releasing and oxyntic cells (Black & Shankley, 1987).

These studies have now been extended to the guinea-pig and rat (Welsh *et al.*, 1994) in an attempt to explore the generality of the model developed for the mouse. In the guinea-pig, as in the mouse, histamine H_2-receptor blockade abolished the vagal response. In the rat, however, the frequency-effect curves could not be abolished by either ACh M- or histamine H_2-receptor blockade alone but were abolished by a combination of the two. This suggested that there was direct cholinergic innervation of the oxyntic cell and a non-cholinergic innervation which either involved histaminergic nerves or nerves containing another transmitter which acted to release gastric mucosal histamine. However, we could not rule out that there was also an indirect cholinergic histamine release, as in the other species (Welsh *et al.*, 1994).

In the isolated mouse stomach assay, we found that the ACh M-receptor agonist, McN-A 343, expressed lower potency than 5-MeF which appeared to be due not only to lower receptor binding affinity, but also to lower intrinsic efficacy (Black & Shankley, 1985b). Subsequently, Eglen *et al.* (1987) also reached the conclusion that McN-A 343 behaves as a low efficacy agonist at ACh M-receptors. By simultaneously applying an operational model of agonism (Black & Leff, 1983) to the 5-MeF and McN-A 343 concentration-effect curve data obtained in the absence and presence of histamine H_2-receptor blockade, we were able to estimate the relative efficacy of the agonists at both the histamine-releasing and oxyntic cells. Using the model we were able to simulate the experimental data by assuming that there was a six fold higher expression of efficacy at ACh M-receptors on the histamine cell than at those on the oxyntic cell in the mouse stomach assay (Black & Shankley, 1985b). We now report the extension of this analysis to the immature guinea-pig and rat isolated stomach preparations, in particular to ask whether there is an ACh M-receptor-mediated release of histamine in the rat assay. As part of this study, we obtained a new data set on the mouse sto-

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mach assay under identical experimental conditions to those used in the original study. Both data sets are presented because the effects of histamine H_2-receptor antagonism appeared to be different.

Methods

Isolated, lumen-perfused, whole stomach preparations

Gastric acid secretion was measured in isolated, lumen-perfused, stomach preparations essentially as described previously for the mouse (Black & Shankley, 1985c). Young adult male mice (Charles River, 22–26 g, fasted 18 h with free access to water) and pre-weaned rat pups (Wistar, 32–38 g corresponding to an age range of 10–23 days) were killed by cervical dislocation. The abdomen was opened and the oesophagus ligated close to the stomach. A polythene cannula (2 mm internal diameter) was inserted into the pylorus via the duodenal bulb and a small incision made in the fundus through which the stomach contents were gently washed. A second cannula was tied into this incision. The stomachs were then transferred into 40 ml organ baths containing buffered serosal solution (mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.14, $NaHPO_4$ 15.9, $CaCl_2$ 0.65, glucose 31.6) maintained at 37°C and gassed with 95% O_2/5% CO_2. The lumen was perfused from the fundic to the pyloric cannulae with warmed unbuffered mucosal solution (mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 0.65, glucose 31.6) gassed with 100% O_2, and the perfusate passed over a pH-electrode system adjusted for height to provide 12 cmH_2O intragastric pressure.

Guinea-pig mucosal sheet preparation

Immature, male guinea-pigs, weighing 180–220 g were killed by cervical dislocation, the abdomen was opened and the stomach removed. The dissection technique, employed previously for the mouse (Shankley *et al.*, 1988), was adapted from that described by Main & Pearce (1978) for the rat. In brief, the stomach was opened by an incision along the greater curvature and a piece of glandular mucosa gently tied to a Perspex tissue holder drilled to allow perfusion of the lumenal mucosal surface. After ligation to produce a watertight seal, the overlying muscle layer was removed from the mucosa by injecting saline between mucosal and muscle layers to create a blister so that the muscle layer could be safely excised. In all other respects the conditions were as for the whole stomach preparations except that the back pressure was reduced to 6 cmH_2O.

Experimental design

Six preparations were used simultaneously and, after a 60 min stabilization period, any not showing stable basal responses were rejected (less than 5%). Thereafter, drugs were added to the serosal solution according to individual experimental protocols. The total volume of vehicle did not exceed 1 ml and had no effect on basal gastric acid secretion in the assays.

A randomized block design was used throughout for allocation of experimental treatments such that, as far as possible, each organ bath received each treatment within the course of an experiment. The effects of histamine H_2-receptor blockade on the two agonists, 5-MeF and McN-A 343, were investigated within separate experiments except in the case of the guinea-pig mucosal sheet assay, when both agonists were studied within the same experimental protocol.

Acid secretory responses were expressed as ΔpH, that is the difference between basal pH, measured immediately prior to experimental intervention, and stimulated pH. This response metameter was chosen in preference to $[H^+]$ as a previous analysis has shown that pH values obtained under basal and stimulated conditions are normally-distributed (Shankley, 1985). Fully-defined agonist concentration-effect curves were obtained by cumulative dosing at 0.25 or 0.5 $\log_{10}$ unit intervals when stable response plateaux were achieved. In preliminary experiments, the validity of the cumulative dosing regimen was confirmed by comparing the curve maxima to the response to single, near-maximal, bolus doses (data not shown).

Data analysis

Where possible, the agonist concentration-effect curve data from individual preparations were fitted, using a computer programme based on the procedure described by Parker & Waud (1971), to a general logistic function to provide esti-

Table 1 Logistic curve-fitting parameters (±s.e.mean) for 5-methylfurmethide (5-MeF) and McN-A 343 concentration-effect curves obtained in the absence and presence of histamine H_2-receptor blockade (100 μM tiotidine or 20 μM famotidine for the guinea-pig assay) in isolated stomach preparations of mouse, and immature guinea-pig and rat

	n	$\log[A_{50}]$	p	$\alpha(\Delta pH)$
Mouse				
5-MeF	6	−7.67 ± 0.07	1.64 ± 0.09	0.54 ± 0.04
5-MeF (+H_2-block)	6	−7.40 ± 0.07	2.38 ± 0.25	0.36 ± 0.04
McN-A 343	6	−5.10 ± 0.10	1.00 ± 0.14	0.63 ± 0.10
McN-A 343 (+H_2-block)	6	−4.66 ± 0.11	2.11 ± 0.32	0.38 ± 0.09
Guinea-pig				
5-MeF	4	−7.23 ± 0.17	1.40 ± 0.36	0.55 ± 0.07
5-MeF (+H_2-block)	5		NF	−0.02 ± 0.08
McN-A 343	5	−4.78 ± 0.12	0.86 ± 0.19	0.34 ± 0.06
McN-A 343 (+H_2-block)	6		NF	0.00 ± 0.09
Rat				
5-MeF	8	−7.36 ± 0.02	3.92 ± 0.38	0.60 ± 0.06
5-MeF (+H_2-block)	8	−7.10 ± 0.05	2.80 ± 0.30	0.37 ± 0.03
McN-A 343	7		NF	0.13 ± 0.05
McN-A 343 (+H_2-block)	9		NF	0.12 ± 0.03

NF = not fitted.

mates of the midpoint location ($\log[A_{50}]$), midpoint slope parameter (p) and upper asymptote (α) as described previously (Black & Shankley, 1985c). For analysis and display purposes the individual computed parameter estimates for each treatment group were expressed as mean ± s.e.mean and single logistic curves simulated shown superimposed upon the experimental data. Computed logistic curve-fitting parameters were compared using Student's t test. Values of $P<0.05$ were considered significant.

Operational model of agonism

Previously, we were able to account quantitatively for the data obtained on the mouse stomach assay (Black & Shankley, 1985b) using an operational model of agonism (Black & Leff, 1983). In the present study, simulations were obtained using the following form of the model which describes agonist concentration-effect data in terms of four parameters,

$$E = \frac{E_m[A]^n\tau^n}{(K_A+[A])^n+[A]^n\tau^n},$$

where E is pharmacological effect, [A] is agonist concentration, K_A is the agonist equilibrium dissociation constant, n is the slope of the transducer function relating occupancy to effect and τ is the efficacy parameter or transducer ratio which is given by the ratio of the total receptor concentration ($[R_0]$) and the midpoint location parameter of the transducer function (K_E). E_m is the maximum pharmacological effect.

The goodness-of-fit of the model simulations to the experimental data was assessed using a chi-squared analysis where chi-squared was calculated by comparing the mean observed data points with the expected values obtained by model simulation using the following equation:

$$\chi^2=\sum\frac{(\text{observed-expected})^2}{\text{m.(expected}^2)+\text{c}},$$

where the denominator is a correction factor applied to account for the fact that an approximately linear relationship was found between the observed variance (σ^2) and the square of the observed effect values for the data from each assay and the values of c and m were estimated from a linear regression analysis of these data. In this way the chi-squared value was corrected to account for any differences in the amplitude of the 5-MeF and McN-A 343 concentration-effect curves within an assay.

Drugs

Tiotidine (a gift from Imperial Chemical Industries Ltd.) and famotidine (a gift from Merck, Sharp and Dohme Ltd.) were dissolved in dilute HCl to give 0.2 mM stock solutions. Nifedipine (Sigma Chemical Co. Ltd.) was dissolved in 100% ethanol to give a 20 mM stock solution; subsequent dilutions were made in distilled water. Isoprenaline hydrochloride (Sigma Chemical Co. Ltd.) was dissolved and diluted in stoichiometric aqueous ascorbic acid to prevent oxidation. All other compounds were dissolved in distilled water and sources were: atropine sulphate (Sigma Chemical Co. Ltd.); 5-methylfurmethide iodide (a gift from Wellcome Foundation Ltd.); McN-A 343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride, (a gift from McNeil Laboratories U.S.A. Ltd.).

Results

Effect of histamine H_2-receptor blockade on 5-methylfurmethide and McN-A 343 concentration-effect curves in isolated stomach assays

Single concentrations of selective histamine H_2-receptor antagonists (100 μM tiotidine or 20 μM famotidine for the guinea-pig assay) were used to obtain H_2-receptor blockade. These concentrations were at least one thousand-fold greater than the K_B values estimated on the stomach assays when histamine was used as agonist (Black *et al.*, 1985a; Welsh *et al.*, 1992). These concentrations had no significant effect on basal acid secretion in any of the assays. We have assumed

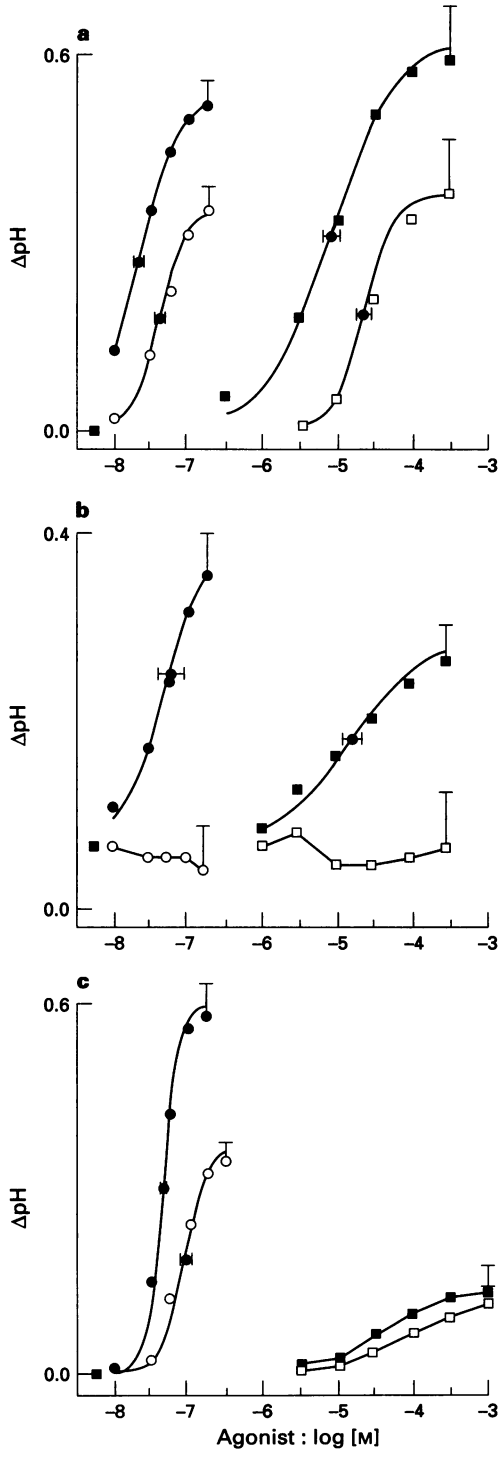

Figure 1 Agonist concentration-effect curves obtained with 5-methylfurmethide (●,○) and McN-A 343 (■,□) in the absence (closed symbols) and presence (open symbols) of histamine H_2-receptor block (100 μM tiotidine or 20 μM famotidine for the guinea-pig assay). Data are shown from (a) the current mouse stomach study, (b) immature guinea-pig and (c) immature rat. The curves shown superimposed on the mean experimental data points ($n=4/9$ ± s.e.mean) were obtained by logistic curve-fitting (for details see methods).

that these concentrations produced essentially the maximum achievable blockade of endogenous histamine. The receptor selectivity of the agents at these high concentrations was evaluated (data not shown) by investigating the effects of tiotidine and famotidine on 5-MeF curves obtained on an ACh M-receptor assay, the guinea-pig isolated gall bladder (Bishop *et al.*, 1992). Tiotidine (100 μM) had no significant effect. Famotidine (20 μM) produced a small, parallel shift (dose-ratio = 3.1) of the 5-MeF concentration-effect curve. However, famotidine was used only in the guinea-pig stomach preparation where its effect could not possibly be confused with this small shift (see below). The switch to famotidine for the guinea-pig assay experiments was made simply because our supply of tiotidine was exhausted.

Mouse stomach The effect of the histamine H_2-receptor antagonism was assessed by its effect on the concentration-effect logistic curve-fitting parameters (Table 1). In the mouse assay, tiotidine (100 μM) produced a small but significant rightward shift and steepening of both the 5-MeF and McN-A 343 concentration-effect curves. The upper asymptote (α) of the 5-MeF curve was significantly reduced by 33%. The mean value of the McN-A 343 curve maximum was also reduced by 40% but because of exceptionally large variances this difference was not statistically significant as tested at the 95% confidence level (Table 1, Figure 1a).

In the original mouse stomach study tiotidine (100 μM) produced a small, but significant, shift (dose-ratio $\sim$6) and significant steepening of the 5-MeF concentration-effect curve with no change in maximum asymptote (Black & Shankley, 1985a; Figure 2a). The McN-A 343 concentration-effect curve was both significantly shifted to the right and depressed by 85% in the presence of 30 μM tiotidine (Figure 2a). In the present study, the curves obtained in the mouse stomach assay were not as steep as their counterparts in the original study.

Immature guinea-pig 5-MeF did not produce consistent, concentration-dependent, acid secretory responses in the guinea-pig whole stomach assay which had been used in previous studies on vagal stimulation of gastric acid secretion (Welsh *et al.*, 1994). The problem was that powerful, 5-MeF-induced, contractions of the fundic smooth muscle interfered with the lumen perfusion. Attempts were made to antagonize functionally the muscle contraction with either the β-adrenoceptor agonist, isoprenaline (100 μM), or the calcium channel blocker, nifedipine (1 μM). Neither was found to improve the assay. Therefore, a mucosal sheet preparation was used in which the serosal muscle layer had been stripped away by the technique described by Main & Pearce (1978). The effect of preparing the mucosal sheet assay on the integrity of the gastric mucosa and, in particular, on the integrity of responses apparently mediated by histamine release, was assessed by comparing pentagastrin and histamine concentration-effect curves obtained on both the sheet and intact stomach assays. We have shown previously that the pentagastrin response is abolished by histamine H_2-receptor blockade in the immature guinea-pig isolated stomach preparation (Shankley *et al.*, 1992). The pentagastrin and histamine concentration-effect curves obtained on the sheet and intact stomach assay apparently differed only in amplitude. Both agonists had a smaller maximum response (α) in the sheet assay as might be expected for a smaller secretory surface area (Table 2).

In the gastric mucosal sheet assay, 5-MeF and McN-A 343, like histamine and pentagastrin, produced concentration-dependent acid secretion. The upper asymptote of the McN-A 343 curve was significantly lower than that obtained with 5-MeF (Table 1) and the responses to both agonists were totally abolished by histamine H_2-receptor blockade (20 μM famotidine, Figure 1b).

Immature rat In the rat assay, the control 5-MeF curve was approximately twice as steep as those obtained in the mouse and guinea-pig assays although 5-MeF was approximately equipotent in the three assays (Table 1, Figure 1c). In the presence of tiotidine (100 μM) the 5-MeF-curve was shifted to the right (dose-ratio $\sim$2) and depressed but, in contrast to the result in the mouse stomach, the curve was significantly flattened. McN-A 343 behaved as a partial agonist with respect to 5-MeF in the absence and presence of histamine H_2-receptor blockade. It was not possible to fit the McN-A 343 data to the logistic function due to the poor definition of some of the individual curves. However, inspection of the mean data (Figure 1c), suggests that histamine H_2-receptor blockade did not produce a large change in the shape or location of the curves.

Application of an operational model of agonism

As in our previous analysis (Black & Shankley, 1985b), it was assumed that histamine H_2-receptor blockade converted ACh M-receptor agonist activity by an indirect action due to histamine release to a direct muscarinic action on the oxyntic cells. For simplicity and in the absence of information to the contrary, the ACh M-receptors were assumed to be identical in all species and on both the histamine-releasing and oxyntic cells. Therefore, the same K_A values were used to stimulate each 5-MeF and McN-A 343 curve. The K_A values for 5-MeF (2.4 μM) and McN-A 343 (15 μM) were fixed at those values estimated by Leff *et al.* (1985) on guinea-pig trachea and Van Rossum (1962) on the rat intestine, respectively. The [A_{50}] values of the 5-MeF concentration-effect curves (Table 1) were all at least 30 fold lower than the K_A value so that, according to the model of agonism, 5-MeF could be considered to behave as a high efficacy agonist in all assays in both the absence and presence of histamine H_2-receptor blockade. Previously, it has been shown that the transducer slope parameter, n, and the maximum effect, E_m, in the operational model of agonism are indistinguishable from the slope (p) and upper asymptote (α), respectively, of the concentration-effect curves obtained with high efficacy agonists (Black *et al.*, 1985b). Therefore, out of necessity, the values of n and E_m were fixed at the values of p and α, respectively, estimated for the 5-MeF curves obtained in

Table 2 Logistic curve-fitting parameters (± s.e.mean) for histamine and pentagastrin curves obtained on immature guinea-pig gastric sheet and whole stomach assays

	n	$log[A_{50}]$	p	$\alpha(\Delta pH)$
Mucosal sheet				
Histamine	5	−5.48 ± 0.22	1.38 ± 0.23	0.54 ± 0.05
Pentagastrin*	6	−8.20	0.76	0.10
Whole stomach				
Histamine	8	−5.67 ± 0.12	1.40 ± 0.19	0.66 ± 0.02
Pentagastrin	7	−8.29 ± 0.08	0.96 ± 0.11	0.36 ± 0.07

* Due to the small responses in some preparations it was not possible to fit all the data. Therefore the parameters shown were obtained by logistic curve-fitting the mean data.

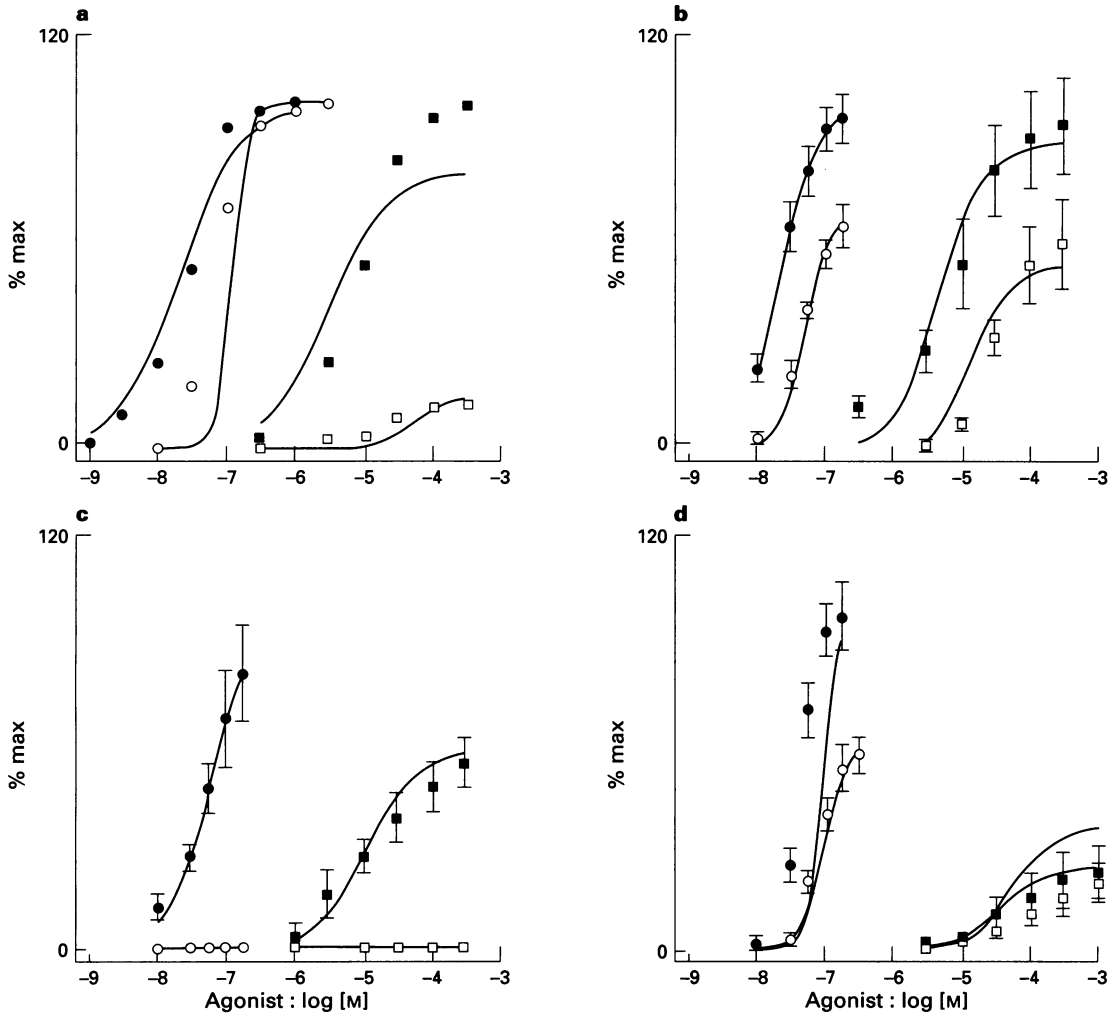

Figure 2 5-Methylfurmethide (5-MeF, ●,○) and McN-A 343 (■,□) experimental concentration-effect curve data obtained in the absence (closed symbols) and presence (open symbols) of histamine H_2-receptor blockade using concentrations of the antagonists tiotidine or famotidine equivalent to at least 300 fold their K_B values. Panels show data from (a) the original mouse stomach study (redrawn from Black & Shankley, 1985b), (b) the current mouse stomach study, (c) immature guinea-pig and (d) immature rat. The curves shown superimposed on the mean experimental data points ($n=4/9 \pm$ s.e.mean) were obtained using an operational model of agonism (see methods). The 5-MeF and McN-A 343 K_A values were fixed at 2.4 and 15 μM, respectively and the following additional parameter values were used in the simulations:

	E_M	n	τ
(a) Original mouse			
5-MeF	100	1.0	120
5-MeF (+100 μM tiotidine)	100	3.8	20
McN-A 343	100	1.0	4
McN-A 343(+30 μM tiotidine)	100	3.8	0.67
(b) Mouse			
5-MeF	100	1.6	110
5-MeF (+100 μM tiotidine)	70	2.4	50
McN-A 343	100	1.6	3.7
McN-A 343(+100 μM tiotidine)	70	2.4	1.7
(c) Immature guinea-pig			
5-MeF	100	1.4	39
5-MeF (+20 μM famotidine)*	0	–	–
McN-A 343	100	1.4	1.3
McN-A 343(+20 μM famotidine)*	0	–	–
(d) Immature rat			
5-MeF	100	3.9	27
5-MeF (+100 μM tiotidine)	60	2.8	27
McN-A 343	100	3.9	0.9
McN-A 343(+100 μM tiotidine)	60	2.8	0.9

* The response obtained in the presence of histamine H_2-receptor blockade was not significantly different from zero and was therefore assumed to be zero for simulation purposes.

the absence and presence of histamine H_2-receptor blockade (Table 1). To facilitate the simulation and comparison of the data obtained across species, the effect data with both agonists were normalized to these E_m values. Furthermore, when there was no significant difference between the control maximum effect obtained with 5-MeF and McN-A 343 both were considered to be equal to 100%. Thus, the constraints introduced by making the assumption that 5-MeF was a high efficacy agonist had the effect of leaving only the efficacy parameter, τ, in the model to account for the variation in the behaviour of the agonists in the absence and presence of H_2-receptor blockade both between and within species. Therefore, values of τ were determined maintaining a constant ratio between the 5-MeF and McN-A 343 values in all experimental data sets as would be expected for a pair of agonists acting at the same class of receptor. The τ ratio was fixed at 30 which was the value previously estimated in the mouse stomach.

The simulations and parameter values determined, including the original mouse stomach assay data and model simulation (Black & Shankley, 1985b), are presented in Figure 2. Chi-squared analysis indicated that there were no significant differences ($P>0.05$) between the observed and expected values for the model simulations of the guinea-pig ($\chi^2=8.96$; d.f. = 6) and new mouse stomach data ($\chi^2=17.58$; d.f. = 12). In contrast, according to the analysis, the model did not provide an adequate description of the data obtained in the rat assay ($\chi^2=255.2$; d.f. = 14; $P>0.05$). For reasons discussed below, the rat stomach data were resimulated with the constraint of the τ ratio between 5-MeF and McN-A 343 removed in the absence of histamine H_2-receptor blockade (Figure 3). By increasing the τ ratio from 30 to 75 we were able to obtain a reasonable description of the data ($\chi^2=15.39$; d.f. = 14; $P<0.05$).

Discussion

Our original hypothesis, which evolved to account for the differential effects of histamine H_2-receptor blockade on 5-MeF and McN-A 343 concentration-effect curves obtained in the mouse stomach preparation, was that ACh M-receptors were present on both histamine-releasing and oxyntic cells in the gastric mucosa. Accordingly, histamine H_2-receptor blockade was assumed to switch ACh M-receptor agonist activity due to an indirect action mediated by histamine release to a direct muscarinic action on the oxyntic cells. The ACh M-receptors on the histamine-releasing and the oxyntic cells were judged to be identical and probably of the M_2- or M_3-receptor subtype because the pK_B estimates (~6.8) made for pirenzepine, the selective ACh M-receptor antagonist, were indistinguishable in the absence and presence of histamine H_2-receptor blockade when 5-MeF was used as agonist and when McN-A 343 was used as agonist in the absence of histamine H_2-receptor blockade (Black & Shankley, 1985a,b). Although we cannot apply the more rigorous methods of simulation and significance testing applied to the current data, nevertheless, at that time we believed that we were able to account for the data using an operational model of agonism (Black & Leff, 1983). In the model we assumed that the agonists expressed 6 fold higher efficacy at ACh M-receptors located on the histamine-releasing cells than at those receptors on the oxyntic cells. In model terms, 5-MeF behaved as a high efficacy agonist at both cells whereas McN-A 343, expressing lower efficacy, behaved as a partial agonist (see Figure 2a) with a τ ratio of 30.

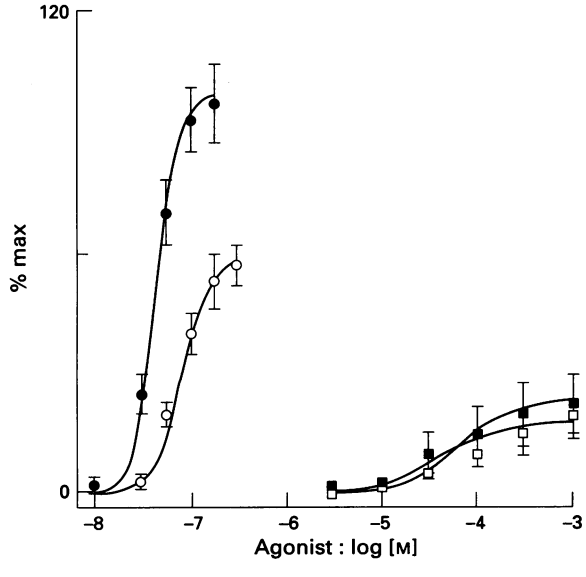

Figure 3 5-Methylfurmethide (5-MeF, ●,○) and McN-A 343 (■,□) experimental concentration-effect curve data obtained in the absence (closed symbols) and presence (open symbols) of histamine H_2-receptor block (100 μM tiotidine) obtained on the immature rat stomach. The curves shown superimposed on the mean experimental data points ($n=7/9\pm$s.e.mean) were obtained using an operational model of agonism (Black & Leff, 1983) as described in the text. In contrast to the simulations shown in Figure 2d, the τ values of the agonists were as follows: 5-MeF, 55; 5-MeF (+H_2-block), 27; McN-A 343, 0.75; McN-A 343 (+H_2-block), 0.9.

In this study, the logistic curve-fitting procedure allowed the comparison of the agonist concentration-effect curves obtained within and between the different experiments (Figure 1 and Table 1). Evidently, there was considerable variation in the data obtained between the species and also, perhaps unexpectedly, between the original and current mouse stomach assay data (Figure 2). The operational model of agonism (Black & Leff, 1983) was applied to determine whether the within and between assay variation in the mouse could be accounted for by the original hypothesis. According to the model, the values of the tissue-dependent parameters (E_m and n) were dictated by the behaviour of the high efficacy agonist, 5-MeF, and values of the agonist equilibrium dissociation constants (K_A) were held fixed at the values used in the original analysis as though the agonists could not discriminate between any of the ACh M-receptors expressed in the assays. Despite the fact that the model simulation presented in the original study was not a particularly good fit, we decided to accept initially the constraint of the τ ratio of 30 chosen in that analysis because a constant ratio across all the data sets is needed to satisfy the hypothesis. The only available variable was the absolute value for τ for the agonists in each tissue. With these constraints, it was possible to simulate the new mouse data by assuming simply that the τ values of 5-MeF ($\tau=50$) and McN-A 343 ($\tau=1.7$) at the oxyntic cell receptor had increased from the original values by 2.5 fold and, in fact, a much better fit was obtained with this new data set (Figure 2).

In the model, τ is defined by $[R_0]/K_E$ and we cannot determine from these data whether the change was due to an increase in ACh M-receptor concentration and/or an increase in the efficiency of receptor-effector coupling. The increase in agonist efficacy between the two mouse stomach experiments is equivalent to a 0.4 log unit leftward shift of a high efficacy agonist concentration-effect curve according to the operational model of agonism. In our experience, this degree of shift in agonist potency is often encountered within the range of replicate $[A_{50}]$ estimates even in the most robust of isolated tissue bioassays. Previously, we expressed a preference for receptor concentration as the source of variation in the action of 5-MeF and McN-A 343 between histamine-releasing and oxyntic cells (Black & Shankley, 1985a,b) on the basis that receptor concentration is the simplest choice in model terms and is being increasingly recognised as a biological variable. We are still not aware of any reported variation in the number of ACh M-receptors on these cells. However, such variation has been reported for other gastrointestinal hormone receptors, for example, Rubin *et al.* (1988) found that rats, both fed and

starved, exhibited a four fold circadian variation in fundic mucosal gastrin receptor concentration expressed in terms of number of binding sites per mg protein.

In the guinea-pig stomach preparation histamine H_2-receptor blockade abolished the effects of both the agonists (Figure 1b). Therefore, it was possible to simulate the data using the model of agonism simply by assuming that the value of τ at the oxyntic cell ACh M-receptors in the model was too low for the expression of agonism (Figure 2c). The response to electrical field stimulation of the vagus, which could be abolished by atropine, was also abolished by histamine H_2-receptor blockade in this species (Welsh *et al.*, 1994). Previously, we have argued (Black & Shankley, 1987) that, in the mouse, ACh was restricted to the region of the histamine-releasing cells by neural configuration and by acetylcholinesterase (AChE) activity. This view was confirmed by the finding that, following inhibition of AChE, frequency-effect curves were still obtained in the presence of histamine H_2-receptor blockade as though ACh could now diffuse to the oxyntic cells (Black & Shankley, 1987). Unfortunately, we were unable to perform a similar test in the guinea-pig preparation because the AChE inhibitor, physostigmine, produced powerful contractions of the guinea-pig fundic smooth muscle which interfered with the lumen-perfusion of the preparation (Welsh *et al.*, 1994). This has still to be examined in the guinea-pig, isolated, gastric mucosal sheet preparation.

In the current data set, the poorest model simulations were obtained with the immature rat stomach assay data (Figure 2d). Evidently, the simple model could not describe the data. Nevertheless, the elements of the simple model must still apply to the rat. The effects of 5-MeF are partially removed by histamine H_2-receptor blockade indicating, presumably, the presence of ACh M-receptors on histamine-releasing cells. The remainder of the 5-MeF-induced secretion is assumed to be due to direct activation of ACh M-receptors on oxyntic cells. If the model is still applicable qualitatively but fails quantitatively, the failure must be due to application of inappropriate assumption-led constraints. For example, the model assumption that the ACh M-receptors are homogeneous across the species with respect to McN-A 343 and 5-MeF could be flawed. This would allow for variation in the values of the model parameters τ and K_A for each agonist. Clearly, this degree of parameter value flexibility would greatly reduce the analytical value of the model. However, we are not aware of any compelling evidence for different ACh M-receptors on oxyntic and histamine-secreting cells within or between species.

We also explored the validity of the primary assumption that histamine H_2-receptor blockade simply converted ACh M-receptor stimulation from a single action on histamine-releasing cells to a single action on oxyntic cells. Inspection of the 5-MeF curves obtained in the rat in the absence and presence of histamine H_2-receptor blockade (Figure 1) reveals that the location of the curves overlap to some extent so that the control curve may have an additional component due to direct oxyntic cell activation. The fact that the simple model provided a reasonable description of the mouse and guinea-pig data suggests that if there was an interaction between the direct and indirect pathways in the control curves then it was not very large. In contrast, there were features of the data obtained in the rat which suggested that the 5-MeF control concentration-effect curve could be the product of a potentiating interaction between released histamine and 5-MeF. First, the curve was approximately twice as steep as that obtained in the mouse and guinea-pig assays (Table 1). Second, the curve slope parameter was reduced in the presence of histamine H_2-receptor blockade whereas in the mouse and guinea-pig the slope increased. In agreement with this observation, we have previously found that histamine H_2-receptor blockade decreased the slope of a frequency-effect curve obtained by electrical stimulation of the vagus nerve in the rat assay (Welsh *et al.*, 1994).

Potentiating interactions at the level of the oxyntic cell were originally conceived by Grossman & Konturek (1974) and subsequently demonstrated directly by Soll (1978). A formal model and, hence mathematical description, of the oxyntic cell potentiating interactions has not been reported. However, intuitively, steep concentration-effect curves might be expected if the stimuli produced by the activation of the two oxyntic cell receptors interacted in a super-additive manner. In terms of the operational model of agonism applied in this study, this would lead to 5-MeF expressing apparently higher efficacy than expected if it was acting solely by the release of histamine. The 5-MeF and McN-A 343 curves obtained in the presence of histamine H_2-receptor blockade would not be expected to be confounded by potentiation because any such interaction was blocked. Indeed, examination of the model simulation (Figure 2d) suggested that the τ ratio of 30 could be maintained for this pair of curves if they were simulated in isolation. A much improved fit was obtained when the data were re-simulated with the τ ratio only allowed to vary for the 5-MeF and McN-A 343 curves obtained in the absence of histamine H_2-receptor blockade (Figure 3). The increase in τ ratio between 5-MeF and McN-A 343 required to account for the data indicates that the degree of potentiation is proportional to the agonist intrinsic efficacy as expected. Thus, it was necessary to increase the absolute values of τ for 5-MeF but not McN-A 343.

In conclusion, the acid secretory response to ACh M-receptor stimulation and the interaction with histamine H_2-receptor antagonists are significantly different between the guinea-pig, mouse and rat preparations. As in previous publications we have interpreted the effects of H_2-receptor blockade in terms of histamine-secreting cells being functionally-coupled to oxyntic cells. The application of the model of agonism to the data suggests that the differences could be due simply to variations in the efficacy of the agonist expressed at ACh M-receptors on histamine-secreting and oxyntic cells and between species. The ability of the model of agonism to account for the data obtained between and within tissues by varying the efficacy (τ) but not the affinity (K_A) parameter values suggests that efficacy and affinity can be treated, in this instance at least, as independent parameters, an assumption which has been challenged (Colquhoun, 1987). In the guinea-pig, the density of ACh M-receptors on the oxyntic cell may be insufficient to enable a direct secretory response to cholinergic stimulation, whereas in the mouse, although vagal stimulation acted only via histamine release, stable ACh M-receptor agonists may act directly on the oxyntic cell to stimulate acid secretion. In the rat, where we have previously shown that there is a non-cholinergic neuronal component which is either histaminergic or acts via release of histamine (Welsh *et al.*, 1994), the current analysis has exposed a significant ACh M-receptor mediated histamine release in this species which may act synergistically with ACh at the level of the oxyntic cell. The modelling-led conclusion is that it appears that both hypotheses for the regulation of gastric acid secretion described in the introduction need to be invoked. Interestingly, we have previously found a potentiating interaction between vagal nerve and pentagastrin stimulation of gastric acid secretion in the immature rat stomach preparation (Shankley *et al.*, 1992).

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