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Pharmacological analysis of the pentagastrin-tiotidine interaction in the mouse isolated stomach

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1 The pentagastrin-tiotidine interaction has been analysed, using improved techniques, in the mouse isolated, lumen-perfused, stomach assay. For comparison and quantification of the H_2-receptor blocking activity of tiotidine, histamine-tiotidine interactions have also been analysed in the mouse stomach and guinea-pig isolated right atrial preparation.

2 Tiotidine behaved as a competitive antagonist of histamine both in the guinea-pig right atrium (pK_B 7.57) and mouse stomach (pK_B 6.96). The difference in pK_B was attributed to the loss of tiotidine into the gastric secretion.

3 On the stomach assay, pentagastrin concentration-effect curves were significantly flatter with lower maximal responses than those obtained to histamine. In addition the profile of inhibition observed with tiotidine was different in that the pentagastrin curve maxima were depressed with only a small concomitant dextrad shift.

4 A mathematical model has been developed which accounts for the differences in agonist concentration-effect curves and describes in a quantitative manner the expectations for the competitive antagonism of endogenous histamine assumed to be released by pentagastrin. Fitting of the pentagastrin-tiotidine data to this model provided a reasonable goodness-of-fit.

5 The results are discussed in terms of the role of endogenous histamine in gastrin-stimulated acid secretion. We conclude that the results are consistent with the hypothesis that pentagastrin stimulates acid secretion via the release of endogenous histamine under the present experimental conditions.

Introduction

Burimamide, the first substance to be classified as a histamine H_2-receptor antagonist, was found to inhibit pentagastrin-stimulated acid secretion as well as histamine-stimulated secretion (Black *et al.*, 1972). Since then, metiamide (Black & Spencer, 1973), cimetidine (Brimblecombe *et al.*, 1975), ranitidine (Daly *et al.*, 1981b), tiotidine (Yellin *et al.*, 1979), oxmetidine (Blakemore *et al.*, 1980) and famotidine (Pendelton *et al.*, 1983), all of which have been shown to belong to the class of histamine H_2-receptor antagonists, have also been shown to share the property of antagonism to pentagastrin-stimulated acid secretion. This strong correlation between the two classes of property suggests that they are functionally related. However, the interaction between the various antagonists and the two agonists are clearly distinguishable. Histamine log concentration-effect curves are invariably displaced in parallel to the right by H_2-receptor antagonists. This has been shown in physiologically-integrated stomachs (Black, 1973: Daly *et al.*, 1981), as well as in isolated stomachs (Angus *et al.*, 1980), gastric mucosal sheets (Sjostrand *et al.*, 1977) or suspensions of separated oxyntic cells (Batzri *et al.*, 1983: Soll, 1980). However, wherever enough dose-response information has been generated, the maximum response which pentagastrin can produce has been found to be progressively reduced by increasing doses of H_2-receptor antagonists (Black, 1973: Daly *et al.*, 1981). Therefore, the hypothesis which tries to explain the interaction between H_2-receptor antagonists and pentagastrin must account for the reduction of pentagastrin maximum responses. Currently, two distinct hypotheses are on offer to explain the antagonism between H_2-receptor antagonists and pentagastrin. One hypothesis was initiated by MacIntosh (1938), maintained and expanded by Code (1965) and developed by Kahlson & Rosengren (1972) – the MacIntosh-Code-Kahlson (M-C-K) hypothesis. This hypothesis proposes that, physiologically, oxyntic cells are activated by histamine which has been

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secreted locally by histamine cells. Gastrin, reaching the circulation from antral G cells, and acetylcholine, released at parasympathetic postganglionic neurones, are imagined to activate appropriate receptors on the histamine cells. Acid secretion is controlled by histamine secretion and histamine secretion is controlled by the major neural and hormone regulatory systems.

The alternative hypothesis which was formulated by Grossman & Konturek (1974) and developed by Soll (1977) – the Grossman-Konturek-Soll (G-K-S) hypothesis – proposes that the regulatory hormones impinge directly on the oxyntic cells. In this model, histamine indirectly controls the physiological effects of gastrin and acetylcholine by an unspecified series of so-called 'potentiating interactions' at the basement membrane of the acid secreting cells. However, this hypothesis does not explain how, if at all, the histamine secretion/release is regulated.

Both models provide an explanation for the antagonism to pentagastrin by the H_2-receptor antagonists. Both models, intuitively, also explain why H_2-receptor blockade reduces the pentagastrin maximum responses. Thus, in the M-C-K two-cell hypothesis, H_2-receptor blockade contributes indirect competitive antagonism (Black *et al.*, 1978; Black *et al.*, 1980) and so the pentagastrin dose-response curves are expected to be displaced to the right in association with maximum reduction. In the G-K-S one-cell hypothesis, blockade of H_2-receptors is imagined to reverse the 'potentiating' effect of histamine on the pentagastrin responses and so a downward displacement with or without a shift to the right might also be expected.

The possibility of a more rigorous quantitative examination of the implications of these hypotheses has arisen by the recent improvements in the mouse isolated stomach assay system (Black & Shankley, 1985a). The improved method allows full concentration-effect curves to be generated and their operating parameters calculated. The interpretation of the families of concentration-effect curves produced by the assay has been constrained by the development of model systems corresponding to the two-cell hypothesis.

Unfortunately, only the two-cell hypothesis can be modelled free from ambiguity at this time: the one-cell hypothesis still needs to be developed into a more explicit form. Nevertheless, in this paper we examine the attempt to fit a formulation of the two-cell model to data produced on the mouse isolated stomach preparation.

Methods

Acid secretion

Gastric acid secretion was measured in the mouse isolated, lumen-perfused, stomach preparation as described previously (Black & Shankley, 1985a). Briefly, stomach preparations were established with the pH electrode system arranged to provide 12 cmH_20 pressure to distend the stomach, from mice from whom food had been withdrawn 24 h beforehand. Six preparations were used simultaneously. After an initial 60 min stabilization period those not producing a stable basal acid secretion, about 5%, were rejected. All drugs were added directly to the organ bath (serosal side). Following a further 60 min equilibration period in the absence or presence of antagonist a single cumulative concentration-effect curve was obtained to either histamine or pentagastrin (Black & Shankley, 1985a).

Guinea-pig right atrium

Positive chronotropic responses to histamine were recorded in the guinea-pig right atrium preparation (Angus & Black, 1980). Six preparations were used simultaneously and after an initial 60 min stabilization period, during which four changes of the bath fluid were made, those preparations not possessing a stable basal rate of between 170–240 min^{-1} were discarded. Following a further 60 min period in the absence or presence of antagonist a single cumulative concentration-effect curve was obtained to histamine.

Experimental design

Antagonist 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

Logistic curve-fitting experimental concentration-effect curves Acid secretion responses produced by histamine and pentagastrin were measured as the change in pH (ΔpH) of the lumen perfusate referred to that immediately prior to starting the cumulative concentration-effect curve. Concentration-effect curve data from individual preparations were fitted, by an iterative least squares computer programme, to a logistic function of the form.

$$E = \frac{\alpha [A]^n}{[A_{50}]^n + [A]^n}, \qquad (1)$$

In which E is effect and α, $[A_{50}]$ and n are the maximal asymptote, midpoint location and slope parameters, respectively. The location parameters were actually estimated as base 10 logarithms (log) by making the substitution $[A_{50}] = 10^{\log[A_{50}]}$. The parameters α, $\log[A_{50}]$ and n are assumed to be distributed normally

and are presented as means ± s.e. For graphical purposes the individual computed parameter estimates for each treatment group were expressed as means and a single logistic curve generated, superimposed upon the experimental data and displayed on a log scale in the usual way.

Competitive antagonism The midpoint slopes and asymptotes were tested by one-way analysis of variance, comparing computed parameter estimates between and within drug treatment groups. If no significant differences were found the log $[A_{50}]$ estimates in the presence ($\log[A_{50}]'$) and absence of antagonist ($\log[A_{50}]$) were used to estimate the logarithm of the antagonist equilibrium dissociation constant ($\log K_B$). This was achieved by direct fitting of the individual $\log[A_{50}]$ estimates to the following derivation of the Schild equation (Schild, 1957):

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

where b is equivalent to the slope of the Schild plot. If the latter was found to be not significantly different from unity the data were refitted with b constrained to unity. This direct fitting method avoids the overweighting of the control $[A_{50}]$ information as occurs with linear regression of dose-ratio/antagonist concentration in the Schild plot (see Black *et al.*, 1985).

For display purposes the parameters estimated were used to generate a Schild plot shown superimposed upon calculated dose-ratios.

Model fitting A BMDP Module AR (Dixon, 1981) computer programme was used to perform derivative-free non-linear regression of experimental data to the models detailed in the theoretical section. The parameters estimated from the model fitting were used to simulate full concentration-effect curves and are displayed superimposed upon the experimental data.

Drugs

Drugs were freshly prepared in distilled water. The total volume added to the 40 ml organ bath did not exceed 800 µl. Molar stock solutions of histamine dihydrogen chloride were neutralized by the addition of sodium hydroxide (Black *et al.*, 1981). Drugs and their sources were as follows: N-methylatropine nitrate (Sigma), histamine di-HCl (Sigma), pentagastrin ('Peptavlon' ampoules, ICI) and tiotidine (ICI 125, 211) which was a generous gift from ICI.

Results

Pentagastrin and histamine concentration-effect curves

Pentagastrin and histamine both produced concentration-dependent sustained increases in basal gastric acid secretion (Figure 1). Logistic curve fitting of individual experimental data gave the mean parameter estimates presented in Table 1. These parameters were used to simulate the logistic curves superimposed upon experimental data, presented in Figure 2.

Competitive antagonism of histamine

Tiotidine has been classified as a potent competitive histamine H_2-receptor antagonist with high selectivity: 10^{-4} M has been found to have no effect on the

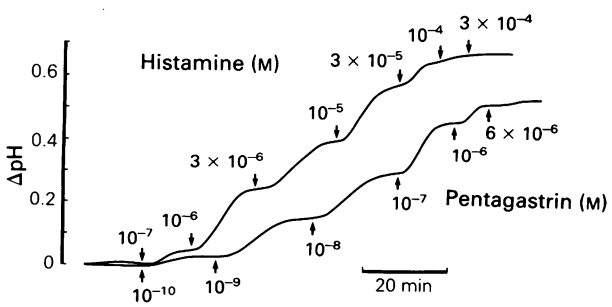

Figure 1 Cumulative concentration-effect curves in mouse isolated stomach to histamine and pentagastrin. In this and subsequent figures, ΔpH (ordinate scale) refers to the change in pH of the lumen perfusate (1 ml min^{-1}). Histamine and pentagastrin doses were added to the organ bath upon attainment of a steady-state response to produce the concentrations indicated.

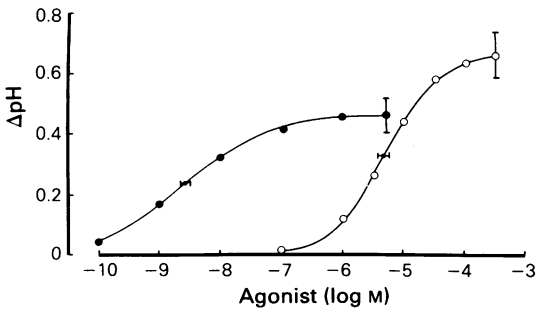

Figure 2 Logistic concentration-effect curves to pentagastrin (●) and histamine (○) in the mouse stomach. The logistic curves are superimposed upon the mean experimental data points (n = 5/6) expressed as the mean decrease in pH (ΔpH) of the lumen perfusate upon the addition of histamine or pentagastrin. Error bars show standard errors.

concentration-effect curves to noradrenaline in the guinea-pig atrium, or to histamine, acetylcholine and 5-hydroxytryptamine in the guinea-pig ileum assays (Yellin *et al.*, 1979). Tiotidine produced a significant concentration-dependent parallel displacement of the histamine log concentration-effect curves with no change in maximal asymptote in both the guinea-pig right atrial and mouse stomach assays (Figure 3). Competitive analysis (see Methods) indicated Schild slope parameters (b) not significantly different from unity (Figure 4). However, in common with other histamine H_2-receptor antagonists on the mouse isolated lumen perfused stomach preparation (Angus & Black, 1979) the pK_B estimated for tiotidine ($pK_B = 6.96 \pm 0.11$) was significantly lower than that obtained on the guinea-pig isolated right atrium ($pK_B = 7.57 \pm 0.07$). Possible explanations for this phenomenon have been analysed by Angus & Black (1979) Angus *et al.*, (1980) and Black *et al.* (1985). Essentially it was concluded that the concentration of antagonist achieved in the H_2-receptor compartment was lower than that in the serosal bathing solution due to a steady-state loss from the receptor compartment into the lumen perfusate.

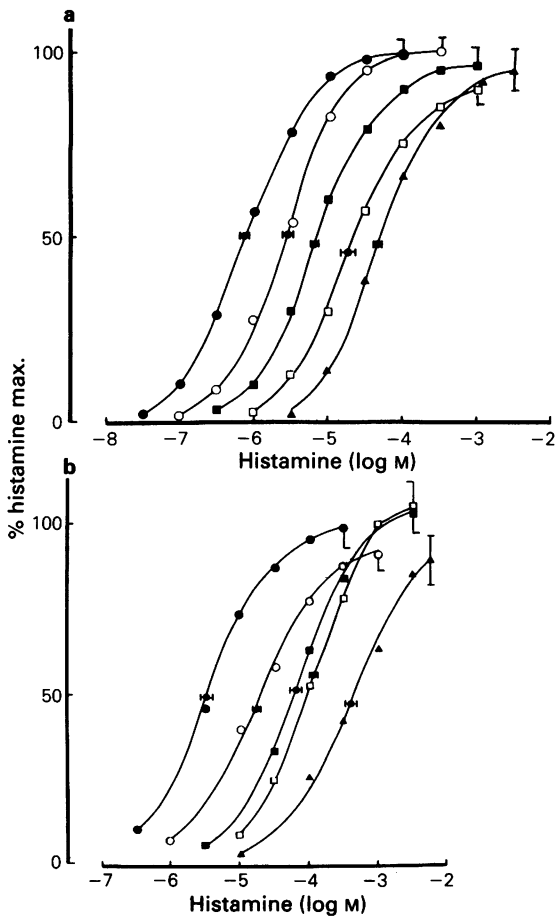

Figure 3 Antagonism of histamine by tiotidine in (a) the guinea-pig right atrium and (b) mouse stomach. (a) Concentration-effect curves for histamine in the absence (●) and presence of tiotidine, (○) 7×10^{-8} M, (■) 2×10^{-7} M, (□) 7×10^{-7} M and (▲) 2×10^{-6} M. (b) Concentration-effect curves for histamine in the absence (●) and presence of tiotidine, (○) 3×10^{-7} M, (■) 1.5×10^{-6} M, (□) 3×10^{-6} M and (▲) 8.5×10^{-6} M.

Pentagastrin concentration-effect curves in the presence of tiotidine

Tiotidine at concentrations within the range which competitively antagonized the histamine effect in the mouse stomach, produced a significant concentration-dependent rightward displacement and depression of the maximal asymptote of the pentagastrin concentration-effect curves (Figure 5). Due to almost maximal inhibition of some of the pentagastrin curves in the presence of 0.75 and 5 μM tiotidine it was not possible to fit individual logistic curves to the experimental data.

A model of indirect agonism

The MacIntosh-Code-Kahlson hypothesis proposes that gastrin (or pentagastrin) acts by stimulating the

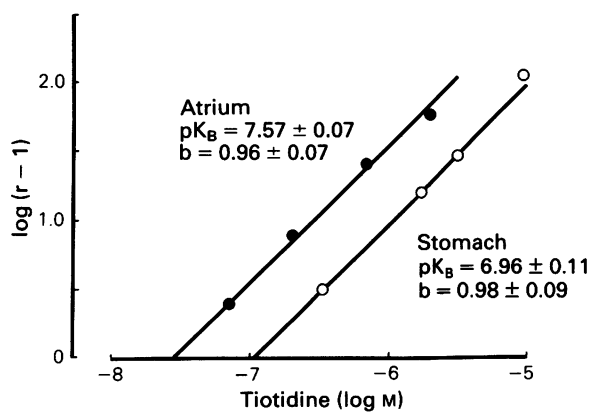

Figure 4 Schild plot representations of antagonism of histamine by tiotidine in the guinea-pig right atrium and mouse stomach preparations. Dose-ratios (r) were calculated from mean $[A_{50}]$ estimates. pK_B and slope (b) estimates were obtained by direct fitting of $[A_{50}]$ data in the absence and presence of antagonist to the form of the equation describing competition presented in the Methods. With both b values insignificantly different from unity, pK_B estimates were obtained by refitting with n constrained to unity.

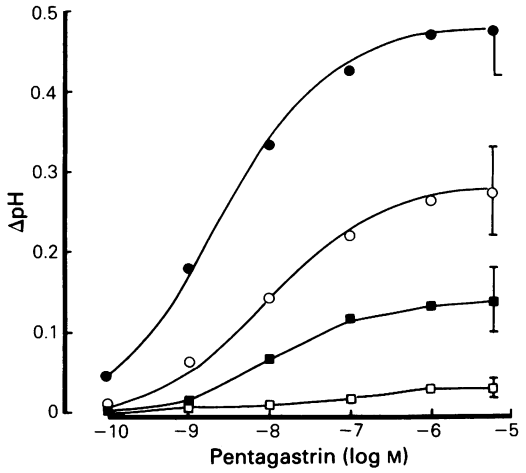

Figure 5 The effect of tiotidine, (○) 0.25, (■) 0.75 and (□) 5 μM, on pentagastrin concentration-effect curves (●) control curve in the absence of tiotidine). Fitted logistic curves are superimposed upon mean experimental data points ($n = 5/6$) obtained in the absence and presence of 0.25 μM tiotidine. Due to almost maximal inhibition of some of the pentagastrin curves in the presence of 0.75 and 5 μM tiotidine it was not possible to fit individual logistic curves to these data. Error bars show standard errors.

local release of histamine: histamine then acts to stimulate the oxyntic cells.

Schematically:

G → H → E

where G, H and E represent gastrin, histamine and pharmacological effect. Here we attempt to model this qualitative mechanistic description in order to determine whether it can account quantitatively for the experimental observations in this study. The results which challenge interpretation are the low slope parameter ($n = 0.67$) and the low maximum parameter ($\alpha = 71\%$ histamine maximum) of the pentagastrin concentration-effect relation and the effect on these parameters of exposure to tiotidine, namely displacement of the location parameter combined with reduction of the maximum parameter. The M-C-K hypothesis can be classified as a system expressing indirect agonism. A model of such a system has been developed by Black *et al.* (1980). In present terms this model assumed a rectangular hyperbolic function between E and H. These assumptions lead to a hyperbolic function between E and G. As the experimental pentagastrin concentration-effect function is evidently not rectangular hyperbolic this model does not apply in the present system. Here we require a more general formulation of the hypothesis which allows flexibility in pentagastrin concentration-effect curve shape. In order to develop such a model we apply the same operational approach as that used previously by Black & Leff (1983) to analyse pharmacological agonism.

The approach begins by characterizing the agonist concentration-effect function and then proceeds by identifying an input function and a transducer function which originally produced that output function. These input, transducer and output functions can then be displayed graphically on a set of three orthogonal spaces (Figure 7). Such an approach is clearly appropriate to the present analysis of indirect agonism in which the problem is to identify the nature of the pentagastrin-histamine and histamine-effect functions which, in sequence, provide the pentagastrin-effect function. One or other of the input or transducer functions must be known or assumed in order to deduce the other because, in practice, a particular output function can be produced by any number of types of two-sequentially arranged functions. Therefore, the analysis begins by characterising the pentagastrin-effect function.

Pentagastrin concentration-effect curves

Curve fitting showed that experimental pentagastrin concentration-effect curves were adequately fitted by a logistic function (Figure 5), that is:

$$E = \frac{\alpha\,[G]^n}{[G_{50}]^n + [G]^n} \qquad (1)$$

Where α is the maximal asymptote of the pentagastrin concentration-effect curve, $[G_{50}]$ is the value of [G] for 0.5α and n is the slope parameter. n was found to be 0.67, indicating that the pentagastrin-effect function is 'flat' compared with a rectangular hyperbola (for which n = 1).

Histamine concentration-effect curves

Curve fitting of experimental histamine concentration-effect curves showed that, at least for exogenous histamine, the relationship was rectangular hyperbolic. Therefore, it can be written,

$$E = \frac{E_m\,[H]}{[H_{50}] + [H]} \qquad (2)$$

Where E_m is the maximal histamine-inducible effect and $[H_{50}]$ is the value of [H] for half E_m. So long as H released *in situ* by G obtains a steady-state, it is reasonable to assume that equation 2 which describes the effects of exogenous H also applies to endogenously-released H.

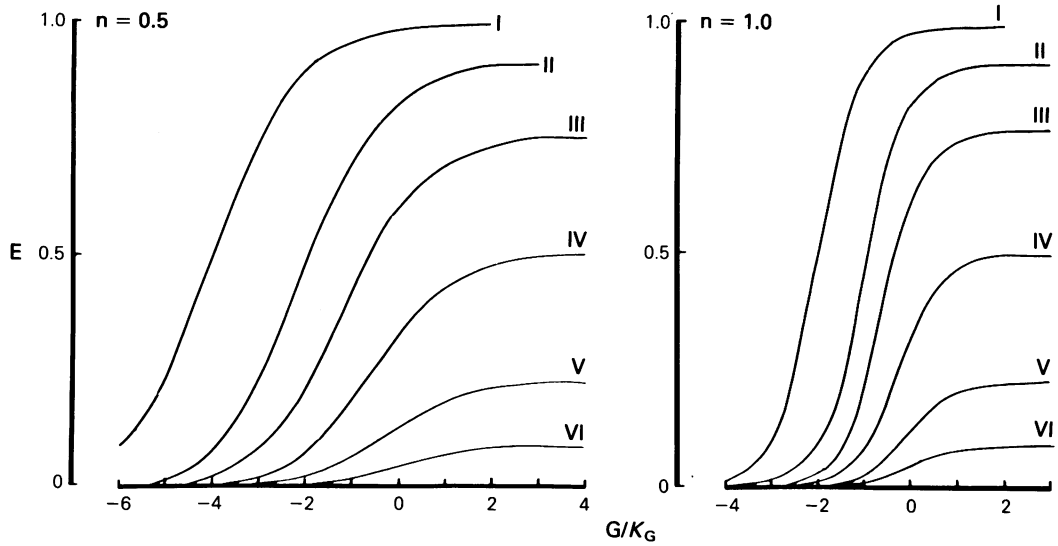

Figure 6 Effect of varying τ and n on the expression of indirect agonist activity in the indirect agonism model. The diagram shows a computer simulation of pentagastrin concentration-effect curves in which τ was varied with n = 1 and n = 0.5. E_m was fixed at 1 and [G] expressed in analytical units of K_G. τ was varied as follows: (I) 100, (II) 10, (III) 3, (IV) 1, (V) 0.3 and (VI) 0.1. For explanation of abbreviations used see text (Results).

Pentagastrin-histamine relation

Knowing the form of the pentagastrin-effect and histamine-effect relations (equations 1 and 2, respectively) allows the pentagastrin-histamine relation to be deduced.

Equating 1 and 2 to eliminate E and rearranging gives:

$$[H] = \frac{\alpha\ [H_{50}]\ [G]^n}{E_m\ [G_{50}]^n + (E_m - \alpha)\ [G]^n} \quad (3)$$

which is the form of the general equation of a logistic function and so is equivalent in form to equation 1. Therefore, the pentagastrin – histamine function may be written,

$$[H] = \frac{[H_m]\ [G]^n}{K_G{}^n + [G]^n} \quad (4)$$

Where $[H_m]$ is the maximum [H] that G can produce and K_G is the [G] for half $[H_m]$.

Characterization of the pentagastrin-effect function

Having established the form of the input, pentagastrin-histamine, and transducer, histamine-effect, functions, the output, pentagastrin-effect, function can be defined by substituting equation 4 in equation 2 as follows:

$$E = \frac{E_m\ [H_m]\ [G]^n}{K^{Gn}\ [H_{50}] + ([H_m] + [H_{50}])\ [G]^n} \quad (5)$$

Therefore the operational parameters α and $[G_{50}]$ which, together with n govern the output pentagastrin-effect function, can be defined as:

$$\alpha = \frac{E_m\ [H_m]}{[H_m] + [H_{50}]} \quad (6)$$

$$[G_{50}] = \frac{K_G}{\left(\frac{1 + [H_m]}{[H_{50}]}\right)^{1/n}} \quad (7)$$

These equations show that when there is a large maximum achievable concentration of released histamine, that is, for large $[H_m]$, or when the potency of H in eliciting an effect is high, that is, for small $[H_{50}]$, α will be indistinguishable from E_m. Also, under these conditions, $[G_{50}]$ will be a much smaller number than K_G. When $[H_m]$ is small or $[H_{50}]$ is large α will only be a fraction of E_m and $[G_{50}]$ will approach K_G in value. In fact, it is the ratio between $[H_m]$ and $[H_{50}]$ which governs these operational parameters. This ratio is analogous to the transducer ratio, 'τ', which governs the efficacy of an agonist in a system, as defined previously (Black & Leff, 1983). Here, the efficacy of

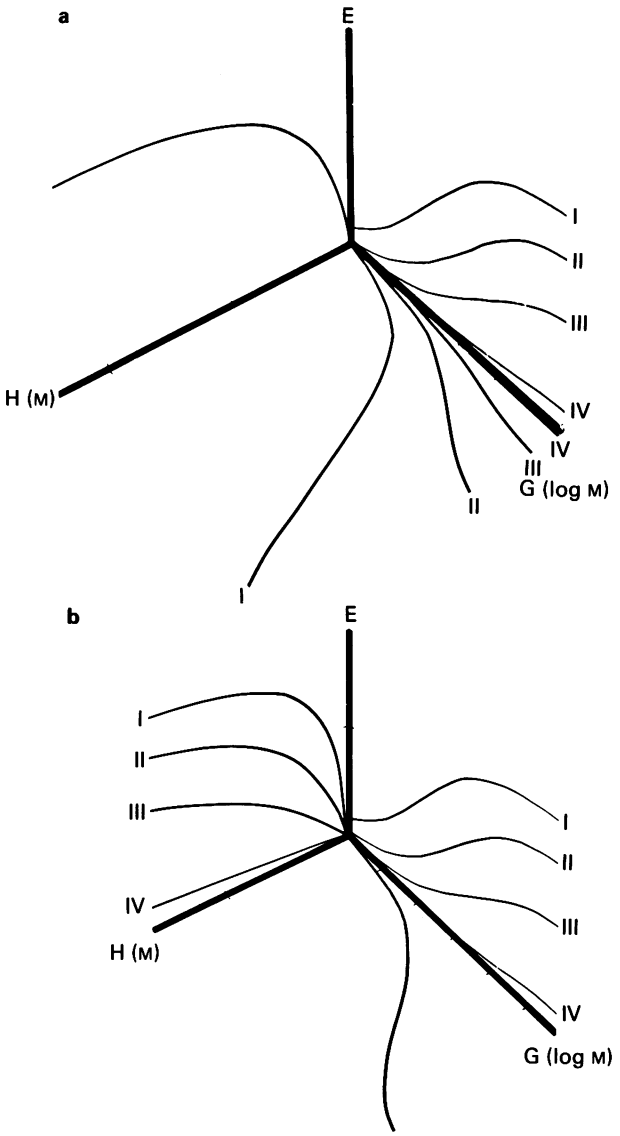

Figure 7 (a) Effect of varying $[H_m]$ on the expression of indirect agonism activity in the indirect agonism model. The diagram shows a computer simulation of model equation 5 in which $[H_m]$ was varied keeping $[H_{50}]$ fixed. K_G was fixed at 10^{-8} M, n at 0.5, E_m at 1 and $[H_{50}]$ at 3×10^{-6} M. $[H_m]$ was varied as follows: (I) 3×10^{-5} M, (II) 9×10^{-6} M, (III) 3×10^{-6} M and (IV) 3×10^{-7} M. (b) Effect of varying $[H_{50}]$ on the expression of indirect agonist activity in the indirect agonism model. The diagram shows a computer simulation of model equation 5 in which $[H_{50}]$ was varied keeping $[H_m]$ fixed, K_G was fixed at 10^{-8} M, n at 0.5, E_m at 1 and $[H_m]$ at 3×10^{-5} M. $[H_{50}]$ was varied as follows: (I) 3×10^{-6} M, (II) 10^{-5} M, (III) 3×10^{-5} M and (IV) 3×10^{-4} M.

G is operationally dependent on the combination of the maximum concentration of released H and the potency of H and we make the definition $\tau = [H_m]/[H_{50}]$ which expresses this.

The operational pentagastrin concentration-effect curve parameters can now be written:

$$\alpha = \frac{E_m \tau}{(1 + \tau)} \quad (8)$$

$$[G_{50}] = \frac{K_G}{(1 + \tau)^{1/n}} \quad (9)$$

The effect of n in the model can be appreciated from Figure 6 which shows simulations of hyperbolic (where n = 1) and non-hyperbolic (n = 0.5) pentagastrin concentration-effect curves, with E_m and K_G fixed and τ varying. While in both cases reduction of τ over the range used causes a rightward shift and depression of the semilogarithmic pentagastrin concentration-effect curves, there is a quantitative difference between the two cases in the degree of right-shift for the same amount of depression. Figure 7 illustrates the model using the three-dimensional display adopted previously (Black & Leff, 1983) showing the relation between the intermediate pentagastrin-histamine and histamine-effect functions and the parameters which govern them. E_m, K_G, and n are fixed in the diagram and $[H_m]$ (Figure 7a) and $[H_{50}]$ (Figure 7b) are varied so that $\tau = 10$, 3, 1 and 0.1. The same family of pentagastrin concentration-effect curves could be generated by either fixing $[H_m]$ and varying $[H_{50}]$ or fixing $[H_{50}]$ and varying $[H_m]$ to give the same τ values.

The quantitative relationships between α and $[G_{50}]$ and the changes in the operational parameters that accompany τ are inbuilt in the model due to the particular assumptions made, namely that the histamine concentration-effect relation is hyperbolic and that the non-hyperbolic nature of the experimental pentagastrin concentration-effect curves resides in a non-hyperbolic pentagastrin-histamine function. In accepting this model as a reasonable description of the system under study it is necessary that the theoretically predicted effects of τ changes can be tested experimentally. This can be achieved by considering the effects of a competitive histamine H_2-receptor antagonist because, as shown in the following section, the model predicts that such antagonism should produce a quantitatively defined effect on τ, and, therefore, on pentagastrin concentration-effect curve displacements.

Competitive antagonism of histamine

Competitive antagonism is predicted to affect the histamine concentration-effect function as follows,

$$E = \frac{E_m\,[H]}{[H_{50}]\left(\frac{1+[B]}{K_B}\right) + [H]} \quad (10)$$

Which is equation 2 with the inclusion of the competition fraction, in the usual way, as a multiple of the location parameter $[H_{50}]$, where [B] is the concentration of the competitor and K_B its equilibrium dissociation constant. We define $[H_{50}]'$ as the location parameter in the presence of B so that the dose-ratio, $[H_{50}]':[H_{50}]$, may be expressed in the form of the Gaddum-Schild equation,

$$\frac{[H_{50}]'}{[H_{50}]} = 1 + \frac{[B]}{K_B} \quad (11)$$

Therefore assuming that B does not affect the value of $[H_m]$ the ratio of values in the presence and absence of B, $\tau:\tau'$, equates to the dose-ratio as follows,

$$\frac{\tau}{\tau'} = \frac{[H_m]}{[H_{50}]} \cdot \frac{[H_{50}]'}{[H_m]} = \frac{[H_{50}]'}{[H_{50}]} \quad (12)$$

This relationship provides a means of testing the model because in fitting experimental pentagastrin concentration-effect curves in the presence of a histamine antagonist, the estimated values of τ should show this dependence on B. Furthermore, they should provide a correct estimate of K_B. Accordingly substitution of equation 12 into 11 provides an equation relating [B] and K_B to τ in the form of the Gaddum-Schild equation.

$$\frac{\tau}{\tau'} - 1 = \frac{[B]}{K_B} \quad (13)$$

which allows comparison with independently obtained competitive data for the histamine antagonism.

Model fitting of experimental data

The data shown in Figure 5 which illustrates the effect of tiotidine on pentagastrin concentration-effect curves have been simultaneously fitted to the equation:

$$E = \frac{E_m\,\tau\,[G]^n}{K_G{}^n + (1+\tau)\,[G]^n} \quad (14)$$

which is equation 5 rewritten using the definition $\tau = [H_m]/[H_{50}]$. The fit provided estimates of E_m, K_G and n and separate estimates of τ at each concentration of tiotidine. The parameter values obtained from the fitting procedure were used to simulate the curves shown superimposed on the experimental data in Figure 8a. The τ values obtained were manipulated according to equation 13 and the result graphically presented in Figure 8b. Also in Figure 8b is the Schild plot for the antagonism of histamine by tiotidine in the mouse stomach. The coincidence of these two lines indicates that the changes in τ for the pentagastrin concentration-effect curves with tiotidine express the apparent affinity of the antagonist in a quantitatively accurate way: K_B for the indirect antagonist can be estimated from these τ changes.

Also estimated in model-fitting was E_m, the parameter defining the maximal effect of the histamine assumed to be released by pentagastrin. The estimated value of E_m was 0.66 which is not significantly different from the value of 0.69 found for the maximal asymptote for exogenously applied histamine (see Figure 2 and Table 1).

Therefore, as judged by its capacity to estimate the independently measurable parameters, K_B and E_m, the model is an adequate description of the system.

Discussion

The ability to obtain full concentration-effect curves on the improved mouse stomach assay (Black & Shankley, 1985a), and the subsequent calculation of their operating parameters using a pragmatic curve fitting procedure (Table 1, Figures 1 and 2), reveals significant differences between the secretogogue activity of histamine and pentagastrin. Histamine concentration-effect curves are observed to be indistinguishable from rectangular hyperbolae, (i.e. n = 1)

Table 1 Histamine and pentagastrin logistic curve fitting parameters

	No. of replicates	*log [A_{50}]*	*Maximal asymptote(α) (ΔpH)*	*Slope (n)*
Histamine	6	− 5.36 ± 0.10	0.69 ± 0.08	1.02 ± 0.03
Pentagastrin	5	− 8.59 ± 0.10	0.49 ± 0.06	0.67 ± 0.07*

The parameters are expressed as means (± s.e.) of computed estimates from individual experimental concentration-effect data.
* Significantly different from unity, $P < 0.01$.

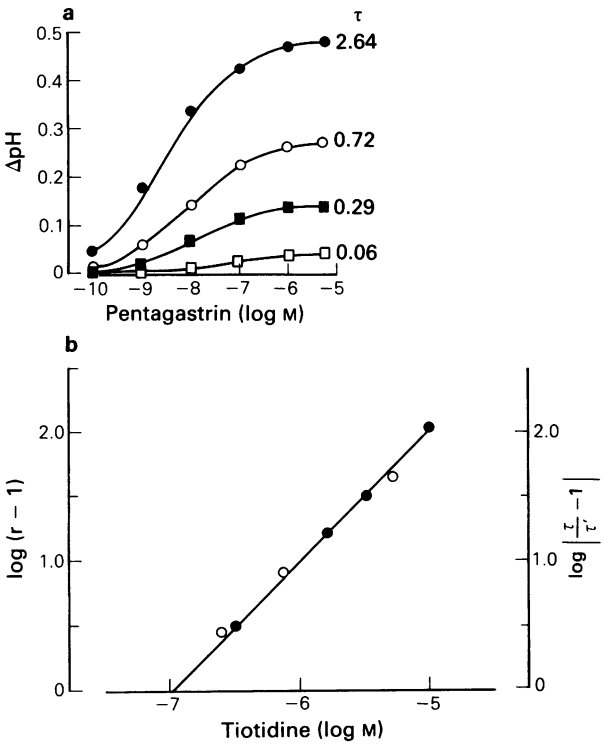

Figure 8 Model fitting of experimental data: (a) the effect of tiotidine on pentagastrin concentration-effect curves. The experimental data shown in Figure 5 have been fitted to the model equation 14. The lines drawn through the data are simulations using the parameters obtained by the model fitting. These parameters were as follows: $n = 0.60$: $K_G = 2.2 \times 10^{-8}$ and $E_m = 0.66$. (b) The τ values obtained with each concentration of tiotidine were then transformed according to equation 13 and are shown (○) on the Schild plot obtained from the antagonism of histamine by tiotidine (●) in the mouse stomach (Figure 4).

whereas pentagastrin concentration-effects curves are significantly flatter (i.e. $n < 1$) than rectangular hyperbolae with the maximal asymptote significantly lower than that attained with histamine. In addition the profile of inhibition following H_2-receptor blockade is seen to be both qualitatively and quantitatively different. Histamine concentration-effect curves are displaced by tiotidine in a manner consistent with expectations for competitive antagonism (Figures 3 and 4) whereas the pentagastrin curve maxima were reduced with only a small concomitant dextrad shift of the curve location (Figure 5). The two-cell, MacIntosh-Code-Kahlson (M-C-K) hypothesis under investigation in this paper requires that a selective, competitive, antagonist action by tiotidine at histamine H_2-receptors will account for the different inhibitory profiles observed with the histamine-tiotidine and pentagastrin-tiotidine interactions.

In the development of the model of the M-C-K hypothesis which predicts the behaviour of pentagastrin concentration-effect curves in the presence of H_2-receptor blockade, we have assumed that pentagastrin-released endogenous histamine acts to stimulate gastric acid secretion in the same way as exogenously applied histamine. Having made this assumption the model logically requires that tiotidine expresses the same inhibitory activity, that is its expressed affinity for the histamine H_2-receptor against both histamine- and pentagastrin-stimulated acid secretion. The estimate of K_B for the histamine-stimulated interaction was obtained using conventional competitive analysis (Figures 3 and 4). An estimate of the K_B for tiotidine from the pentagastrin-tiotidine interaction was obtained from the relationship between the concentration of tiotidine and the model parameter τ (equation 13).

In the model the parameter τ, the ratio of the maximum concentration of released histamine to the potency of histamine ($[H_m]/[H_{50}]$), was shown to provide a measure of the operational efficacy of pentagastrin in the same way as the 'transducer ratio' governs the efficacy of the agonist in a system (Black & Leff, 1983). Thus, in the model, high values of τ imply that pentagastrin is able to release sufficient histamine to produce an effect not significantly different from the maximum effect achievable with exogenously applied histamine while lower values of τ imply that pentagastrin is no longer able to release sufficient histamine to produce a maximal stimulation of the oxyntic cell (Figure 6). Under the present experimental conditions it would appear that pentagastrin behaves as a 'partial agonist' in the system producing only 71% of the histamine maximum secretion (Table 1). It is not possible from the experimental data to elucidate the level at which pentagastrin is behaving as a 'partial agonist' because the model parameter $[H_m]$ is the product of both the binding of pentagastrin to the gastrin receptor and the subsequent expression of its intrinsic activity at the histamine cell. Pentagastrin could be a 'partial agonist' in the system due to low intrinsic efficacy with respect to, for example, gastrin, or, in the mouse stomach preparation under the present experimental conditions, the 'partial agonism' may be due to the inability of even a full agonist at the gastrin receptor to release sufficient histamine to produce a maximum response.

In the model, increasing H_2-receptor blockade reduces the value of τ (equation 13). Therefore the profile of inhibition, that is the degree of dextrad shift and depression of the pentagastrin concentration-effect curves in the presence of tiotidine, is dependent on the initial value of τ. Thus if pentagastrin behaved as 'full agonist' in the system the effect of H_2-receptor

blockade is predicted to produce an initial parallel dextrad shift of the pentagastrin concentration-effect curves (see Figure 6). The depression of the concentration-effect curves, as observed in this study (Figure 5), occurs as τ is reduced to a value so that the maximum concentration of released histamine is insufficient to surmount the H_2-receptor blockade. Therefore, in other experimental systems the pentagastrin (gastrin) concentration-effect curves in the presence of H_2-receptor blockade, may be subject to further rightward shift than observed in this study. However Black (1973) and Daly *et al.* (1981a) demonstrated a similar profile of inhibition as observed here with pentagastrin-metiamide and pentagastrin-ranitidine interactions, respectively, in dogs fitted with Heidenhain pouches. The same analytical approach may be used with their data, although the assumption that released endogenous histamine behaves in a similar way to exogenously applied histamine is probably less valid due to the increased complexity of the experimental systems.

The coincidence of the inhibitory data obtained from the histamine-tiotidine and pentagastrin-tiotidine interactions (Figure 8b) indicated that tiotidine was behaving as a competitive antagonist in both experiments and expressing similar affinity ($pK_B = 6.96$). We conclude that the model developed from the M-C-K hypothesis, provides a reasonable description of the experimental data.

The 'goodness of fit' of the experimental data to the M-C-K model does not alone provide grounds for rejecting the G-K-S hypothesis as the basis for the observed inhibition of pentagastrin/gastrin responses by H_2-receptor antagonists. In the absence of an explicit description of the G-K-S hypothesis we might intuitively expect the same profile of inhibition if one proposes that H_2-receptor blockade effectively produces inhibition at a post-gastrin receptor site of action in the oxyntic cell (Soll, 1980). However, for the H_2-receptor blockade to produce such an effect requires the presence of a basal histamine stimulant effect, the removal of which produces the inhibition of the gastrin (pentagastrin) activity. In the mouse isolated stomach assay we have demonstrated that tiotidine 10^{-4} M does not affect basal secretion (Black & Shankley, 1985a). Therefore, in these experiments pentagastrin appears to stimulate gastric acid secretion in the absence of suprathreshold concentrations of free endogenous histamine. Under these conditions the G-K-S hypothesis does not predict an inhibitory effect of H_2-receptor blockade on pentagastrin-stimulated acid secretion which was observed in this study.

We conclude that the results are consistent with the hypothesis that pentagastrin stimulates gastric acid secretion via the release of endogenous histamine in the present experimental system.

The possibility of an additional direct stimulation of oxyntic cells by pentagastrin is not obfuscated by the present experimental data due to the failure to demonstrate total inhibition of the pentagastrin response with H_2-receptor blockade. However, such an action would appear to produce at the most 10% of the effect achieved by the activity of pentagastrin sensitive to H_2-receptor antagonism (Figure 5) and probably considerably less as evidenced by the 'goodness of fit' of the experimental data to the indirect agonism model (Figure 8). Interestingly, Soll (1980) demonstrated that pentagastrin could only produce about 12% of the maximum histamine stimulation of canine isolated parietal cells. In a subsequent analysis of muscarinic receptors coupled to acid secretion in the mouse stomach preparation we suggest how a difference in the density of muscarinic receptors on histamine and oxyntic cells may account for the apparent selectivity of McN-A 343 (Black & Shankley, 1985b). Similarly, a higher density of gastrin receptors on histamine cells than on oxyntic cells may account for a selective action of pentagastrin/gastrin to stimulate acid secretion via the release of histamine under the present experimental conditions.

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