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The isolated stomach preparation of the mouse: a physiological unit for pharmacological analysis

J.W. Black & N.P. Shankley

The Rayne Institute, King's College Hospital Medical School, Denmark Hill, London SE5 8RX

1 Although oxyntic cell secretion can be studied at many organisation levels between isolated cell suspensions and non-invasive techniques in animals, the isolated, lumen-perfused, stomach preparation of the mouse represents a hierarchical level which eliminates extrinsic regulatory influences but retains all the cellular architecture known to be necessary for physiological responses and so can be defined as the physiological unit of acid secretion.

2 The feeding pattern before and the distending pressure during an experiment have been identified as the main determinants of basal secretion: the combination of an intragastric pressure of 12 cmH_2O and the fasted state generated a stable basal secretion over 2 h providing a satisfactory basis for bioassays.

3 Basal acid secretion was lowered by treatment with omeprazole and sodium thiocyanate but not with tetrodotoxin, N-methylatropine or tiotidine, suggesting that basal secretion does not involve nervous stimulation or the local release of histamine under these experimental conditions.

4 The improved assay permitted the full characterization of cumulative agonist concentration-effe curves in single stomach preparations to histamine, 5-methylfurmethide, pentagastrin and isobutyl-methylxanthine.

5 Interestingly, pentagastrin produced sustained stimulation of gastric acid secretion under conditions when there was no pharmacological evidence that histamine secretion was taking place. This finding is discussed in relation to the role of histamine in the control of gastric acid secretion.

Introduction

Quantitative pharmacological analysis is based on operational models of molecular interactions. Methods based on these models are only feasible using isolated, operator-controlled, biological systems because, in practice, these systems must equilibrate with ligand concentrations which are constant, known, and assumed to be error free.

Until quite recently, gastric acid secretion could only be studied using intact animals so that descriptive pharmacological studies were all that was possible. However, in the last few years or so, it has become possible to measure the secretion of acid by the intact stomachs of mature mice (Wan, 1977; Angus & Black, 1978); by the intact stomachs of immature guinea-pigs (Spencer, 1974) and rats (Parsons, 1975); by the mucous membrane, separated as a sheet, from kittens (Tepperman *et al.*, 1975) and piglets (Forte *et al.*, 1975); and by mixed but enriched suspensions of separated oxyntic cells (Soll, 1977). In addition, some of the biochemical elements subserving acid secretion, such as the hormone-receptor coupled systems on the basal membranes (Scholes *et al.*, 1976) and the H^+/K^+-ATPase located on the apical membranes (Fellenius *et al.*, 1981) are now available for pharmacological studies. Indeed, the first problem facing the analyst today is the embarrassment of making his first choice.

Important pharmacological problems to be analysed are the interactions of drugs with the known physiological regulators of gastric acid secretion. Gastrin, acetylcholine and histamine are of basic significance so that a bioassay system which responds to each of these agonists in ways which are compatible with their behaviour in intact animals is highly desirable. Only systems in which the mucosal architecture is intact realise this objective: gastrin was reported as inactive in suspensions of separated gastric glands (Berglindh *et al.*, 1976) or relatively inactive, less than 12% of the histamine maximum, in suspensions of isolated oxyntic cells (Soll, 1980); carbachol, though acting in these preparations, had nevertheless lost about 90% of its activity. In addition, only the isolated stomach preparations contain intrinsic innervation. Therefore, we consider the isolated stomach preparation not only to be the physiological unit for acid

secretion but also suitable for first-line studies in pharmacological analysis of drugs which modify the control of this function.

In developing the isolated stomach of the mouse as a preparation suitable for bioassay, Angus *et al.* (1980) found that desensitization with time restricted the amount of information which could be got from a single tissue and settled for a single measurement from each preparation. Although parallel-line assays using 3-point concentration-effect curves were successful in evaluating simple histamine/antagonist interactions (Angus *et al.*, 1980), the inability to define the operating parameters of the full concentration-effect relation, that is the, mid-point, slope and maximum, meant that critical signs of complexity could not be observed.

As preliminary observations indicated that analysis of drug interactions with agonists related to acetylcholine and gastrin could not be achieved using 3-point assays, we have tried to develop the method so that full, cumulative, concentration-effect curves could be generated. This paper describes attempts to analyse and control basal secretion, and to establish the concentration-effect parameters for histamine, pentagastrin and 5-methylfurmethide, the latter two agonists being used as surrogates for the native hormones gastrin and acetylcholine.

Methods

Acid secretion

Gastric acid secretion was measured in the isolated, lumen-perfused, stomach preparation of the mouse, essentially as described by Angus & Black (1978). Briefly, youg adult male mice (Charles River, 22–26 g) were housed in suspended cages 24 h prior to experimentation and, according to experimental protocol, either fed on standard laboratory chow or fasted (water *ad libitum*). Isolated, whole stomachs were suspended in organ baths containing 40 ml buffered serosal solution at 37°C gassed with 95% O_2 and 5% CO_2. The stomachs were continuously perfused through the fundic and out of the pyloric cannulae with unbuffered mucosal solution at 1 ml min^{-1} gassed with O_2 and the perfusate passed over a pH-electrode system adjusted to provide 0, 12 or 18 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 discarded. All drugs were added directly to the organ bath (serosal side) and, where appropriate, following a further 60 min period a single response or single cumulative concentration-effect curve was obtained to histamine, pentagastrin or 5-methylfurmethide.

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

Acid secretion was expressed as the pH of the lumen perfusate. Individual responses to drug treatments were measured as the change in pH (ΔpH) from that immediately prior to drug addition. Concentration-effect curve data from individual preparations were fitted by means of 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 α, $[A_{50}]$ and n are the maximal asymptote, midpoint location and midpoint slope parameters respectively. The location parameters were actually estimated as base 10 logarithms (log) by making the substitution $[A_{50}] = 10^{\log [A50]}$. These parameters were assumed to be normally distributed.

For display purposes the individual computed parameter estimates for each treatment group were expressed as means and a single logistic curve generated and superimposed upon experimental data.

Drugs

Drugs were freshly prepared in distilled water with the exception of omeprazole (a gift from A.B. Hässle Ltd, Sweden) which was initially dissolved in methanol. The total volume added to the 40 ml organ bath did not exceed 800 μl of distilled water and 200 μl of methanol. Molar stock solutions of histamine dihydrogen chloride (Sigma) were neutralised by the addition of sodium hydroxide (Black *et al.*, 1981). Other drugs and their sources were as follows: N-methylatropine, tetrodotoxin (Sigma), isobutylmethylxanthine (IMX) (Sigma), 5-methylfurmethide (Wellcome Research Laboratories), pentagastrin ('Peptavlon' ampoules, Imperial Chemical Industries Ltd (ICI)) and tiotidine, which was a generous gift from ICI.

Results

Basal acid secretion

Angus *et al.* (1980) found that the outflow pH from perfused, isolated stomachs of mice reached a basal steady state condition after about 1 h. The outflow pH

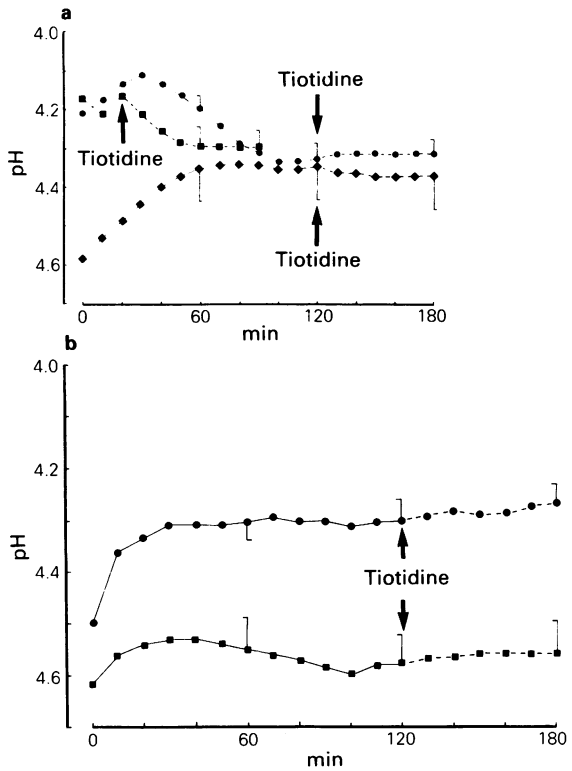

Figure 1 Effects of food withdrawal and intragastric pressure on basal acid secretion. (a) Basal acid secretion in stomach preparations with 18 cmH_2O intragastric pressure from fed (●, $n = 24$) and fasted (◆, $n = 6$) mice and the effect of tiotidine 10^{-4} M at 120 min. The results from an additional experiment show the effect of tiotidine 10^{-4} M at 20 min on basal acid secretion in stomach preparations with 18 cmH_2O intragastric pressure from fed mice (■, $n = 6$). Hatched lines indicate the presence of tiotidine. (b) Basal acid secretion in stomach preparations with 12 cmH_2O intragastric pressure from fed (●, $n = 6$) and fasted (■, $n = 6$) mice and the effect of tiotidine 10^{-4} M at 120 min. In (a) and (b) vertical lines represent s.e. when larger than the symbol.

under these conditions is invariably lower than the inflow pH. Although the fall in pH must be due to the addition of acid by the stomach, this acid need not be of oxyntic cell origin. We have assumed that the oxyntic cells, packed with mitochondria, would be particularly sensitive to the effects of sodium thiocyanate (NaSCN) and we have arbitrarily proposed that any differences in outflow pH after NaSCN treatment must be due to acids of non-oxyntic cell origin. The inflow pH was held constant at 6.6. The outflow pH under the standard basal conditions adopted here (Figure 1) averaged 4.63 ± 0.07 (mean ± s.e. $n = 29$ at 60 min). After NaSCN treatment the outflow pH rose to 5.22 ± 0.04. Although this value is nearly 1.4 log units lower than the inflow pH the acid added to produce this change is only 3% of the basal acid output calculated before NaSCN treatment when basal acid responses are expressed as $[H^+]ml^{-1}$. Therefore, in this paper the whole of the ΔpH found under basal conditions is defined as basal acid secretion. *A fortiori*, no corrections for non-oxyntic secretion have been made in using ΔpH values to express pharmacologically-evoked oxyntic cell secretion.

Factors affecting basal acid secretion

A number of potential sources of variation were investigated: sex, weight, breed, antral pH determined by direction of flow through the stomach, dissection handling, feeding and intragastric pressure. Of these, only fasting and intragastric pressure were found, using suitably controlled experiments, to be significant determinants of basal secretion.

Effects of food withdrawal and intragastric pressure on basal acid secretion

In the methods described by Angus *et al.* (1980) mice had free access to food and water until they were killed. In that experiment, the basal secretion, at an intragastric pressure of 18 cmH_2O, generated an outflow pH of 3.95 ± 0.03 ($n = 12$). After an initial 20 min stabilization period this pH did not change significantly for at least a further 100 min. As no attempt was made at that time to estimate the relative contributions of recent feeding and intragastric pressure to basal secretion, a balanced design experiment has been carried out to assess the effects of food withdrawal and intragastric pressures of 12 cmH_2O and 18 cmH_2O. Angus *et al.* (1980) found that addition of histamine H_2-receptor antagonists produced a fall in basal secretion suggesting the local release of histamine under these basal conditions. In this study, therefore, basal secretion was recorded for 120 min at which time tiotidine (10^{-4} M) was added to the organ bath and secretion recorded for a further 60 min. Tiotidine was chosen due to its classification as a highly selective histamine H_2-receptor antagonist (Yellin *et al.*, 1979) with a pK_B of 7.57 (Black *et al.*, 1985). Concentrations of 10^{-4} M, more than 3,000 × greater than the K_B, had no effect on the noradrenaline interaction with β-receptors in the guinea-pig right atrium or on the effects of histamine (H_1), acetylcholine (muscarinic) or 5-hydroxytryptamine (5-HT_2) on guinea-pig ileum (Yellin *et al.*, 1979). In the present experiments, tiotidine had no effect on basal acid secretion at 120 min from stomachs with 12 cmH_2O or 18 cmH_2O intragastric pressures from fed or fasted mice (Figure 1a,b). Presumably, basal secretion at 120 min did not involve the local release of histamine under these

experimental conditions. The outflow pH recorded at 120 min from stomach preparations with 18 cmH_2O intragastric pressure from fed mice (pH = 4.32 ± 0.04, $n = 24$) was, however, higher than that obtained by Angus *et al.* (1980) under the same conditions when a significant effect of H_2-receptor antagonists was recorded. However, in their study antagonists were added at 20 min and examination of the basal secretion-time curve in this study (Figure 1a) revealed a significantly lower outflow pH within the first 60 min compared to that in the later period of the experiment. Therefore, in additional experiments, tiotidine 10^{-4} M was added at 20 min and was found to raise significantly the outflow pH (Figure 1a). We conclude that the basal secretion in this study from stomachs with 18 cmH_2O intragastric pressure from fed mice involves the transient release of local histamine.

Basal secretion from stomachs with 18 cmH_2O intragastric pressure from fasted mice (Figure 1a) and from stomachs with 12 cmH_2O intragastric pressure from fed mice (Figure 1b) did not show the initial high rate observed from stomachs with 18 cmH_2O intragastric pressure from fed mice. However, after approximately 60 min, similar stable outflow pH values were recorded from each of these treatment groups (pH 4.30). Lowering the intragastric pressure to 12 cmH_2O in stomachs from fasted mice, however, produced a significantly lower basal secretion (Figure 1b) with a mean outflow pH of approximately 4.56 over 180 min.

Interestingly, the basal secretion from stomachs with 12 cmH_2O intragastric pressure from fasted mice appeared to achieve a constant level after approximately 30 min (Figure 1b) compared to 60 min for basal secretion from stomachs with 18 cmH_2O intragastric pressure from fasted mice (Figure 1a). If the back pressure exerted on the preparation results initially in increased tension in the smooth muscle of the stomach, the increased latency in reaching a stable basal secretion, with the higher intragastric pressure may, in turn, be a reflection of the increased time required for this tension to be dissipated to allow distension and thinning of the stomach to occur. Direct observation suggests that feeding and intraluminal pressure are not independent variables. Without fasting, the stomachs of mice are greatly distended with food and, after washout, remain larger/more relaxed than stomachs from fasted mice.

Effects of intragastric pressure on histamine-stimulated acid secretion

Wan (1977) found that some distension of the mouse isolated stomach greatly enhanced the acid secretory response to agonists. Angus *et al.* (1980) obtained satisfactory secretory responses by distending the stomach with an intragastric pressure of 18 cmH_2O but no controlled attempt was made to find an optimum pressure.

Using stomachs from fasted animals, a balanced-design experiment has now been used to evaluate the effects of intragastric pressure on histamine-stimulated acid secretion. The intragastric pressure was varied by adjusting the height of the outlet of the flow-type pH-electrode system. Pressures of 0, 12 and 18 cmH_2O were chosen. Basal acid secretion was monitored for 120 min and then the effects of histamine, 3×10^{-4} M, a concentration known to produce a maximal secretory response, were recorded. When the response to histamine reached a plateau, after about 15 min, the change in pH from basal was noted. The results are shown in Table 1. One-way analysis of variance showed that raising intragastric pressure produced a significant increase in both basal ($P < 0.01$) and histamine-stimulated ($P < 0.05$) secretion. The increase in basal acid secretion observed with increased intragastric pressure accords with the results of Wan (1977). However, the histamine incremental secretory response was significantly greater with an intragastric pressure of 12 cmH_2O than that observed with intragastric pressures of 0 cmH_2O ($P < 0.01$) and 18 cmH_2O ($P < 0.05$) (simultaneous multiple t test, Bonferroni method, Wallenstein *et al.*, 1980). Accordingly 12 cmH_2O intragastric pressure was chosen for standardized conditions for studying basal secretion.

Pharmacological analysis of basal acid secretion

The analyses were carried out using the standardized conditions of 12 cmH_2O intragastric pressure in stomachs from fed and fasted animals. In an attempt to determine the extent of endogenous secretogogue activity in stomach preparations under basal conditions the effects of a neurotoxin, a histamine H_2-receptor antagonist, an acetylcholine muscarinic-receptor antagonist and an inhibitor of the proposed gastric proton pump, H^+/K^+-ATPase, were investigated. Following an initial 60 min stabilization period the inhibitors were added directly to the organ bath (serosal side). The effect on basal secretion was

Table 1 Effect of intragastric pressure on basal and histamine-stimulated gastric acid secretion in stomach preparations from fasted mice

	Intragastric pressure (cmH_2O)		
	0	*12*	*18*
Basal pH	5.06 ± 0.09	4.66 ± 0.18	4.40 ± 0.12
ΔpH with histamine (3×10^{-4} M)	0.29 ± 0.06	0.63 ± 0.06	0.44 ± 0.03

Values shown mean ± s.e., $n = 6$.

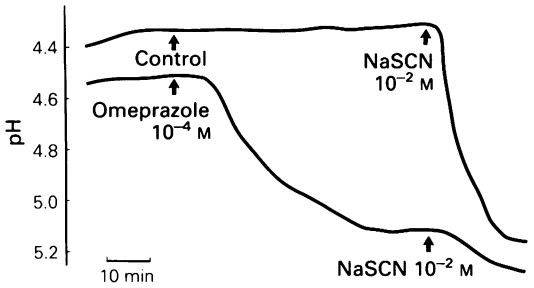

Figure 2 Pharmacological analysis of basal acid secretion. Experimental records of basal acid secretion in two stomach preparations with 12 cmH_2O intragastric pressure from fasted mice and the effect of omeprazole and sodium thiocyanate (NaSCN).

noted after a further 60 min and then 10^{-2} M NaSCN was added (Figures 2 and 3).

Tetrodotoxin Angus & Black (1982) showed that tetrodotoxin blocked the acid secretion induced by electrical field stimulation of the mouse isolated, stomach without blocking the effects of histamine stimulation. Therefore, tetrodotoxin can be used as a probe to elucidate the involvement of nerve networks in the acid secretory activity of this preparation. In this study, as in that of Angus & Black (1982), tetrodotoxin had no effect on basal acid secretion. We conclude that the basal secretion is not due to nervous stimulation.

N-methylatropine N-methylatropine has been shown (Black & Shankley, 1985) to be a competitive antagonist of muscarinic receptors with an estimated pK_B of 9.69. In this study, 3×10^{-7} M N-methylatropine had no effect on basal secretion, confirming that cholinergic nerves are not involved.

Tiotidine As in the previous study of the effects of food withdrawal and intragastric pressure (Figure 1a,b) on basal secretion, mouse stomachs incubated with 10^{-4} M tiotidine secreted basal acid like untreated stomachs, presumably establishing that basal secretion does not involve the local release of histamine under these standardized experimental conditions.

Omeprazole Omeprazole has been described as an inhibitor of the proposed gastric proton pump, H^+/K^+-ATPase (Fellenius *et al.*, 1981: Beil & Sewing, 1984). Omeprazole 10^{-4} M produced a significant inhibition of basal secretion in stomachs from both fed and fasted mice. The inhibition observed with omeprazole, under these standardized experimental conditions, probably provides a measure of basal oxyntic cell activity in the same way as the inhibition produced by NaSCN 10^{-2} M, discussed previously.

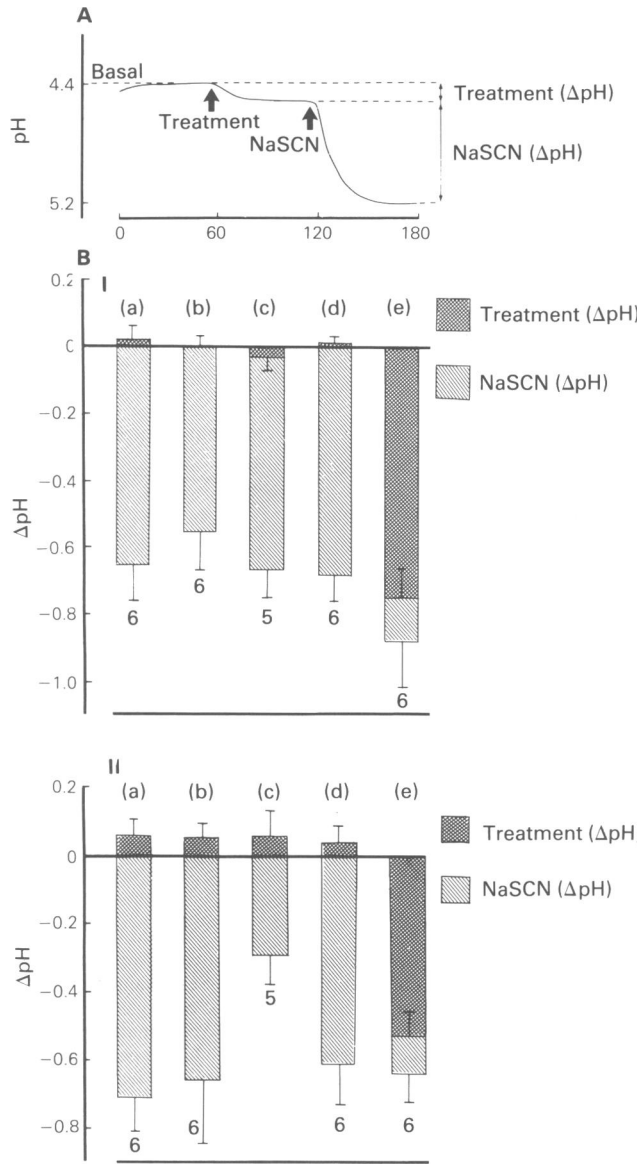

Figure 3 Pharmacological analysis of basal acid secretion. (A) Diagram showing the experimental protocol. (B) The effect of inhibitors ((a) control, (b) tetrodotoxin 10^{-7} M, (c) tiotidine 10^{-4} M, (d)N-methylatropine 3×10^{-7} M, (e) omeprazole 10^{-4} M) on basal acid secretion in stomach preparations with 12 cmH_2O intragastric pressure from fasted (I) and fed (II) mice. Results are expressed as mean changes in pH (ΔpH) with vertical lines indicating s.e. as shown in (A): numbers refer to the number of stomach preparations. In stomach preparations from fed and fasted mice omeprazole and sodium thiocyanate (NaSCN) significantly increased the pH of the lumen perfusate.

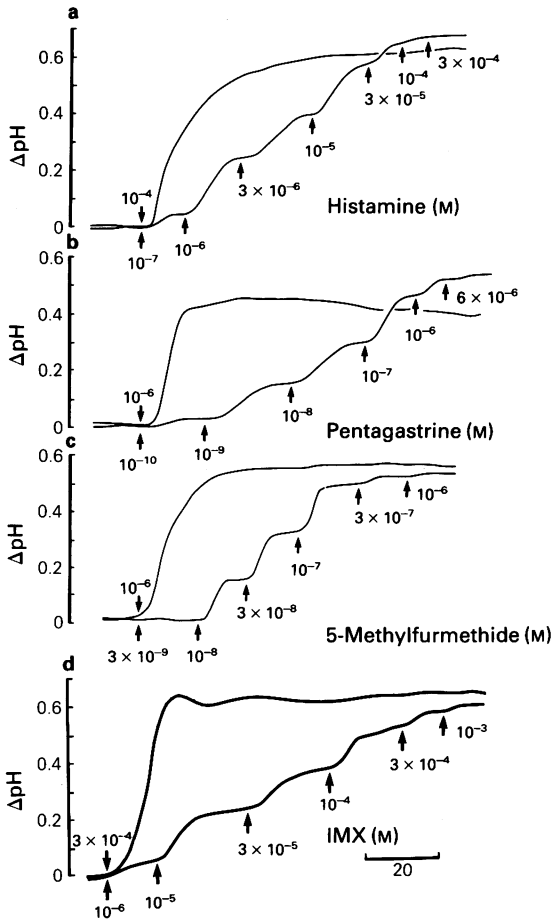

Figure 4 Experimental records of individual responses and cumulative concentration-effect curves obtained with histamine (a), 5-methylfurmethide (b), pentagastrin (c) and (d) isobutylmethylxanthine (IMX). Ordinate scales (ΔpH) refer to the changes in pH of the lumen perfusate. Agonist doses were added when responses reached a plateau to give the organ bath concentrations indicated.

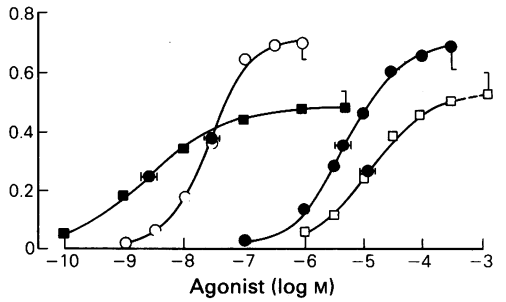

Figure 5 Histamine (●), 5-methylfurmethide (○), pentagastrin (■) and isobutylmethylxanthine (IMX; □) concentration-effect curves. The curves drawn through the mean experimental data points ($n = 5/6$) were obtained from a logistic curve fitting procedure (see methods). Error bars show standard errors. Due to the relatively poor solubility of IMX it was not possible to define experimentally the IMX maximal effect. Therefore, the computed maximal asymptote is shown.

Histamine, 5-methylfurmethide, pentagastrin and isobutylmethylxanthine (IMX) concentration-effect curves

The three agonists and IMX produced concentration-dependent, sustained increases in basal gastric acid secretion in stomach preparations with 12 cmH_2O intragastric pressure from fasted mice. The cumulative dosing method permitted the full definition of a concentration-effect curve in a single stomach preparation (Figure 4). Logistic curve fitting of individual concentration-effect curve data gave the mean parameter estimates presented in Table 2. These parameters were used to simulate the logistic curves superimposed on experimental data in Figure 5.

Discussion

This investigation finds that stomachs isolated from fasted mice and perfused with a distending pressure of

Table 2 Histamine, 5-methylfurmethide, pentagastrin and insobutylmethylxanthine (IMX) logistic curve fitting parameters

	No. of replicates	*Maximal asymptote (α)* log [A_{50}]	*(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
5-Methylfurmethide	5	− 7.58 ± 0.05	0.72 ± 0.05	1.28* ± 0.06
IMX	4	− 4.89 ± 0.12	0.51 ± 0.08	0.88 ± 0.08

The parameters are expressed as means (± s.e.) of computed estimates from individual experimental concentration-effect curve data.
* Significant difference from unity ($P < 0.05$).

12 cmH_2O provide adequate, though not necessarily optimal, conditions for a bioassay using acid secretion as the end point. Under these conditions basal secretion is low and steady for at least 2 h, sufficient time to allow full cumulative-response curves to be generated by histamine, pentagastrin and 5-methylfurmethide. A question arises, however, about the nature of this basal secretion and the possibility that its mechanism may confound the responses to these agonists.

Previous studies have implicated histamine release because the basal secretion could be reduced by histamine H_2-receptor blockade (Wan, 1977; Angus *et al.*, 1980; Szelenyi & Vergin, 1980). However, using the standardized conditions adopted in this paper, no effect of histamine antagonism has been seen. The difference between the present and previous findings seems to be the degree and duration of gastric distention. Both the distention by food and by intragastric pressures greater than 12 cmH_2O during perfusion are needed. Even so, the histamine release, and the associated rate of basal secretion, gradually decrease till no evidence of release could be seen after 1 h.

No effects of histamine antagonism were seen in any of the fasted animals at either 12 or 18 cmH_2O pressure. Nevertheless, the basal secretion was higher at 18 cmH_2O pressure (Table 1), implying a positive relation between distension and secretion which does not involve histamine release. The nature of this basal secretion and the mechanism relating secretion to distension are unknown. Grossman (1967) found that distension evoked secretion in dogs, fitted with Heidenhain pouches, could be suppressed by atropine. However, in the present experiments muscarinic receptor blockade had no effect on basal secretion confirming the findings of Szelenyi & Vergin (1980) and Angus *et al.*, (1980). No blockade was seen with tetrodotoxin either, supporting the idea that activity in submucosal nerve networks is not necessarily involved.

The possibility that these low levels of acid production by the stomach are produced by cellular activity other than oxyntic cells seems to be excluded by the marked inhibition produced by omeprazole, an effect assumed to be related to its ability to inhibit the oxyntic-cell specific enzyme, H^+/K^+-ATPase (Fellenius *et al.*, 1981). Even so, omeprazole failed to suppress acid production completely. Further reduction could always be produced by inhibition of cellular respiration with NaSCN. Acid secretion is known to be tightly coupled to cellular respiration and so we have provisionally assumed that oxyntic cells would be particularly sensitive to NaSCN poisoning. Perhaps, this omeprazole-insensitive secretion of H^+ is associated with the activity of carbonic anhydrase, an enzyme located, like the H^+/K^+-ATPase, on the apical membranes of oxyntic cells (Cross, 1970). The H^+ output by these enzymes located on the apical membranes is normally regulated by hormone-receptor interactions on the basal membranes of oxyntic cells. Receptors coupled to adenylate cyclase on the basal membranes dominate oxyntic cell regulation. Therefore, it was interesting to find that a phosphodiesterase inhibitor, IMX, produced a dose-related increase in oxyntic cell secretion to reach a maximum similar to histamine (Table 2) in spite of the fact that we could find no evidence of histamine release being involved, as shown from preliminary experiments by the absence of an effect of tiotidine (data not shown). Our provisional conclusion, then, is that basal secretion is related to unregulated basal activity of adenylate cyclase and that this activity is a function of distension. In the *in vitro* situation oxygen delivery seems to be the response-determining process. Davenport & Chaure (1950) tried to improve delivery using hyperbaric oxygen. Wan (1977) found that mouse stomachs would only secrete when distended. All the preparations which secrete well, *in vitro*, have thin walls – immature rat stomachs, mature mouse stomachs, guinea-pig mucosal sheets. We imagine that the factor relating distension to acid secretion is simply thinning of the wall leading to improved oxygenation and a higher yield of ATP.

Although this analysis of basal secretion is provisional and speculative it is, nevertheless, reassuring that the concentration-effect relations found for histamine, pentagastrin and 5-methylfurmethide are unlikely to be confouded by local factors.

The remarkable features about these concentration-effect relations are the differences in all three parameters in the logistic equation curve-fitting. Differences between the A_{50} values for histamine and pentagastrin are well-known in *in vivo* studies and have been associated with speculation about relative physiological significance. However, this has to be taken along with the inability of pentagastrin to achieve the same maximum response as histamine, a phenomenon which has also been recognised in intact animal studies (Black, 1973; Daly *et al.*, 1981) and in man (Thjodleifsson & Wormsley, 1975). The inability of pentagastrin to achieve efficacy in the system equivalent to histamine must be a part of any hypothesis about their modes of action. In some ways, the most striking difference between the agonists is in their slope functions. Histamine, with a slope parameter $\simeq 1$, that is approximating to rectangular hyperbolic, is behaving in this system as it does in other H_2-receptor mediated systems, such as heart muscle (Stanovnik & Erjavec, 1982). 5-Methylfurmethide, with a slope > 1 is displaying the same characteristic as muscarinic agonists generally do. However, pentagastrin with a slope < 1, while showing this same characteristic even in intact animals, presents a challenge to the hypothesis-maker. In a subsequent paper, we shall present indirect evidence that this 'flat'

function is determined by the secretory process of the histamine cells on which pentagastrin is imagined to act (Black *et al.*, 1985).

This view, originating with MacIntosh (1938), Code (1965) and Kahlson & Rosengren (1972), that gastrin (pentagastrin) acts indirectly to excite oxyntic cells by stimulating adjacent histamine-secreting cells is in sharp contrast to the view originating with Grossman & Konturek (1974) and subsequently developed by Soll (1977). In the Grossman-Konturek-Soll hypothesis gastrin acts directly on the oxyntic cells but the activation of gastrin receptors requires the permissive activation of the histamine receptors on the same cells. This should explain why histamine receptor blockade also blocks the effects of gastrin. However, the corollary to this hypothesis is that pentagastrin will be inactive in the absence of histamine secretion. An interesting finding, then, is that pentagastrin is fully active on the mouse-stomach preparation under the standardized conditions where there is no pharmacological evidence that histamine secretion is taking place.

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