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Histamine Dependence of Pentagastrin-Stimulated Gastric Acid Secretion in Rats

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Does gastrin stimulate gastric acid secretion by direct action on oxyntic cells, by releasing histamine, or by being potentiated by histamine? Previous studies in the mouse pointed to gastrin-regulated histamine release. Guinea pig and rat are well known to vary in their sensitivity to histamine. Therefore, the effects of histamine and pentagastrin were compared quantitatively on isolated, lumen-perfused, stomach preparations from these species in the absence and presence of histamine H_2-receptor blockade. The loss of potency of histamine in the rat was mirrored by a loss of potency of pentagastrin consistent with the idea that pentagastrin acts by releasing histamine. In the rat, a well-defined pentagastrin curve was obtained in the presence of histamine H_2-receptor block as though pentagastrin acts both directly on the oxyntic cell and indirectly by releasing histamine. It was not necessary to invoke a potentiating interaction between histamine and pentagastrin at the oxyntic cell; the two effects appeared simply to add. Potentiation was observed, however, between other combinations of stimuli, for example, between vagal nerve and pentagastrin stimulation. The physiological consequences of these results are discussed.

INTRODUCTION

There are currently two hypotheses which describe the regulation of gastric acid secretion by the three primary secretagogues: gastrin, histamine, and acetylcholine (Fig. 1). The first hypothesis was formulated by Grossman and Konturek [1] and subsequently developed by Soll [2]. In this hypothesis, the three secretogogues are imagined to act directly on the oxyntic cell to stimulate acid secretion. The finding that histamine H_2-receptor antagonists can, under certain experimental conditions, block all forms of stimulated secretion is accounted for by the existence of potentiating interactions beyond the oxyntic cell receptors. Previously, we have referred to this model as the "permission hypothesis" because gastrin and acetylcholine effectively work by permission of histamine [3]. In the alternative hypothesis, initiated by MacIntosh [4], revived by Code [5], and further developed by Kahlson and Rosengren [6], gastrin and acetylcholine act indirectly by releasing histamine from cells located adjacent to the oxyntic cells. In this "transmission hypothesis" [3], histamine transmits the actions of gastrin and acetylcholine. Although the permission model recognizes the *importance* of histamine, only the transmission hypothesis provides a description of the *control* of histamine.

We have previously investigated the regulation of gastric acid secretion using isolated, lumen-perfused, whole-stomach preparations from young adult mice [7].

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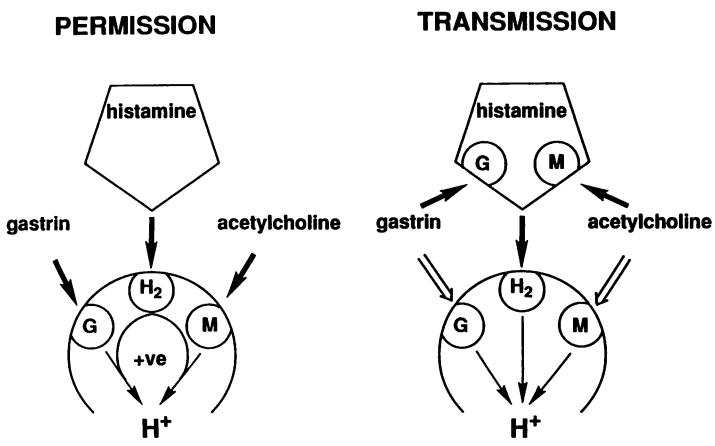

FIG. 1. The two alternative hypotheses for the regulation of gastric acid secretion by histamine, gastrin, and acetylcholine (see text for details).

These assays have the advantage of retaining the gastric mucosal architecture, and the three primary stimulants act in a way which is compatible with their behavior in intact animals. Pharmacological models of agonist and antagonist action can be reliably applied because fixed and known concentrations of pharmacological reagents can be used in these *in vitro* assays.

The isolated-stomach preparations can be made to secrete an unregulated basal level of acid by, for example, prior fasting and adjusting of the intragastric pressure [7]. This fact is important for the interpretation of experimental data. The fact that histamine H_2-receptor antagonists, like tiotidine (0.1 μM, 1,000-fold above its K_B at histamine H_2-receptors [8]), do not inhibit basal secretion means that free, endogenous histamine can only be present in these assays at sub-threshold levels. For example, the mouse stomach assay concentration-effect curves to pentagastrin, used as an experimentally amenable surrogate for native gastrin, were progressively shifted to the right and the upper asymptote depressed in the presence of increasing concentrations of the histamine H_2-receptor antagonist, tiotidine. These data were quantitatively consistent with a model describing the competitive antagonism of endogenous histamine released by pentagastrin [9]. According to the model, the depression of the upper asymptotes of the pentagastrin concentration-effect curves by the competitive histamine H_2-receptor antagonist is due to the inability of pentagastrin to release supramaximal quantities of histamine, which would be required to surmount the receptor blockade. In the absence of suprathreshold background endogenous histamine, these data were consistent with the transmission model for the regulation of gastric acid secretion. Pentagastrin was apparently acting by releasing endogenous histamine, and, under these particular experimental conditions, there was no evidence of a significant direct action of pentagastrin on the oxyntic cell in the mouse.

We now report the extension of these studies on isolated, lumen-perfused, stomach assays prepared from immature rats and guinea pigs.

METHODS

Immature Rat and Mouse Isolated, Lumen-Perfused, Stomach Assays

Isolated, lumen-perfused, stomach assays from mice, guinea pigs, and immature rats were prepared as described previously [7,10]. Briefly, whole stomachs from mice

(22–30 g) and rats (32–37 g) were emptied and placed in a 40 ml organ bath containing buffered serosal solution and perfused with unbuffered mucosal solution at 1 ml min^{-1}. This solution was passed over a pH electrode system set at 12 cm above the preparation to distend the stomach wall.

Guinea Pig Stomach Preparation

Stomachs were removed from guinea pigs (180–220 g). The forestomach was removed, and the remaining section resealed around the perfusion cannula. In all other respects, the assay was identical to the immature rat and mouse stomach assays.

Field Stimulation

Electrical field stimulation of the vagus was achieved by placing a pair of platinum ring electrodes (ring diameter, 2 mm; wire diameter, 0.5 mm) on either side of the stomach preparations to stimulate the region of the fundic glands [13]. Square-wave pulses of 0.5 ms duration and 10 V measured intensity were applied over the frequency range 1–7 Hz.

ANALYSIS

Six preparations were used simultaneously and, after an initial 60-minute stabilization period, those not producing a stable basal acid secretion (approximately 5 percent) were discarded. All drugs were added directly to the organ bath (serosal side) and, where appropriate, following a further 60-minute period, a single cumulative agonist concentration effect was obtained to histamine or to pentagastrin.

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.

Acid secretion was expressed as 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. Agonist concentration-effect data were fitted to a logistic function of the form:

$$E = \frac{\alpha [A]^p}{[A_{50}]^p + [A]^p}$$

where E = effect (ΔpH), $[A]$ = agonist concentration, and α, $[A_{50}]$, and p are the upper asymptote, midpoint location, and slope parameters, respectively.

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 and diluted in distilled water with the exception of tiotidine (a gift from ICI), which was initially dissolved in 1 N HCl. Molar stock solutions of histamine dihydrogen chloride (Sigma) were neutralized by the addition of sodium hydroxide [11]. Pentagastrin was purchased in Peptavlon™ ampoules from ICI. The total volume added to the 40 ml organ bath did not exceed 400 μl.

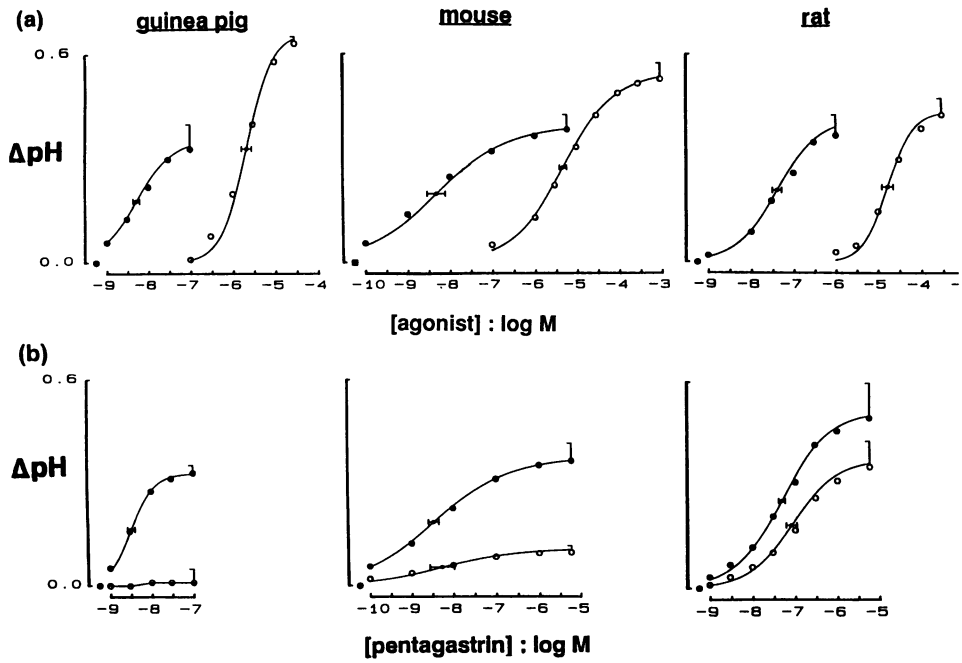

FIG. 2. (a). *Upper panel:* Pentagastrin (●) and histamine (○) concentration-effect curves obtained on isolated, lumen-perfused stomach preparations prepared from immature guinea pig, mouse, and immature rat. The curves drawn through the mean experimental data points (n = 5/7) were obtained by logistic curve-fitting. Error bars show SE mean. (b). *Lower panel:* Pentagastrin concentration-effect curves (n = 5/7) obtained on isolated, lumen-perfused stomach preparations prepared from immature guinea pig, mouse, and immature rat in the absence (●) and presence (○) of histamine H_2-receptor blockade (100 μM tiotidine or 30 μM famotidine, pre-incubated for 60 minutes).

RESULTS

Control histamine and pentagastrin concentration-effect curves were obtained on each of the three assays (Figure 2a and Table 1). Histamine was equipotent in the mouse and guinea pig but tenfold less potent in the rat. These potencies were quantitatively paralleled by the potencies of pentagastrin in the three species. In the guinea pig and mouse, pentagastrin behaved as a partial agonist with respect to histamine, only producing about 50 and 70 percent, respectively, of the maximum

TABLE 1
Logistic Function Curve-Fitting Parameters (n = 6/8 ± SEM) Derived from Histamine and Pentagastrin Control Concentration-Effect Curves Obtained on Isolated, Lumen-Perfused, Stomach Assays (standard errors shown in parentheses)

	Guinea Pig		Mouse		Rat	
	Histamine	Pentagastrin	Histamine	Pentagastrin	Histamine	Pentagastrin
$p[A_{50}]$	5.67 (0.12)	8.29 (0.08)	5.36 (0.10)	8.59 (0.10)	4.78 (0.13)	7.39 (0.11)
Maximum (ΔpH)	0.66 (0.02)	0.36 (0.07)	0.69 (0.08)	0.49 (0.06)	0.44 (0.05)	0.42 (0.06)
Slope	1.40 (0.19)	0.96 (0.11)	1.02 (0.03)	0.67 (0.07)	1.48 (0.18)	0.89 (0.10)

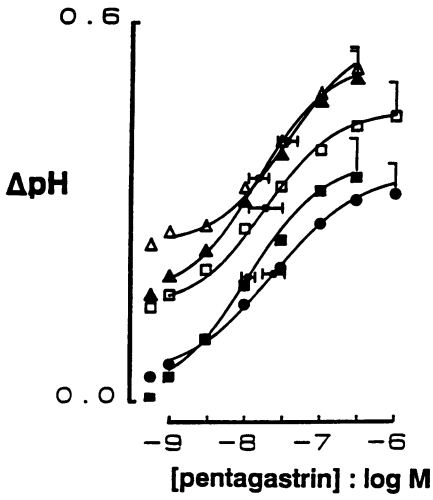

FIG. 3. Pentagastrin concentration-effect curves obtained on the immature rat stomach assay following pre-incubation (30 minutes) with increasing concentrations of histamine (●, 0; ■, 3; □, 10; ▲, 30; △, 100 μM).

response obtained with histamine. In the rat, however, pentagastrin and histamine produced the same maximal acid secretion.

We have previously shown that the histamine H_2-receptor antagonists, tiotidine and famotidine, produce a parallel rightward shift of histamine concentration-effect curves in these assays [12], consistent with a selective, competitive mechanism of action. In the presence of concentrations of these antagonists which are sufficient to produce a 3 log unit rightward shift of histamine concentration-effect curves, the pentagastrin response was abolished in both the guinea pig and the mouse, but, in the rat, pentagastrin still produced a significant, fully definable, concentration-effect curve (Fig. 2b).

Pentagastrin concentration-effect curves in the rat were obtained following pre-incubation (30 minutes) with increasing concentrations of histamine (Fig. 3). As judged by the values of the upper asymptotes, there was no increase in the amplitude of the curves, nor any sign of potentiation as judged by the midpoint location parameters. Similar experiments using other combinations of secretagogues, however, produced evidence for both potentiation and amplification (not all data shown). The example given (Fig. 4) is the interaction between pentagastrin and electrical field stimulation of the vagus nerve in the rat assay. In the presence of background vagal stimulation, the pentagastrin curves were progressively shifted to the left and amplified.

DISCUSSION

The fact that the potency of histamine was quantitatively paralleled by the potency of pentagastrin in the three species, guinea pig, mouse, and rat, might be intuitively expected if pentagastrin acts by releasing histamine. Comparison of the upper asymptotes of the pentagastrin concentration-effect curves between and within species suggested, however, a discontinuity in pentagastrin's mechanism of action between the rat and the other two species. It was as though pentagastrin was acting solely by the release of histamine in the mouse and guinea pig but was unable to release sufficient endogenous histamine to produce the same magnitude of effect as could be achieved by the addition of exogenous histamine. This finding agreed with

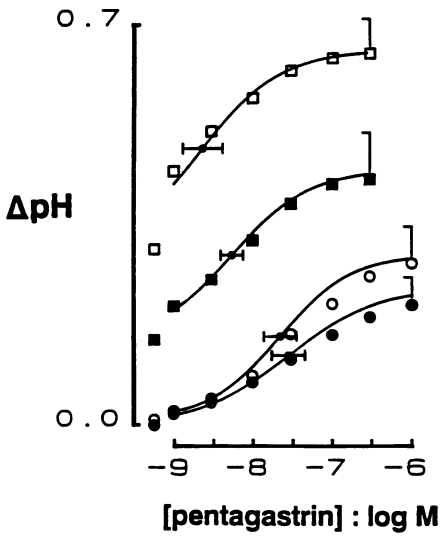

FIG. 4. Pentagastrin concentration-effect curves (n = 5/7) obtained on the immature rat stomach assay in the presence of electrical stimulation of the vagus nerve (●, 0; ○, 1; ■, 3; □, 7 Hz; 0.5 ms pulse width, 10 V).

the previous model deduction made from the observed non-surmountable antagonism of pentagastrin by tiotidine in the mouse [10]. If pentagastrin was acting in the rat as it does in the guinea pig and mouse, then we might have expected that it would only produce 50–70 percent of the histamine maximum as well; however, pentagastrin produced the same maximum as histamine in the rat. Pentagastrin was either more efficient at releasing histamine, even though it was less potent in this species (Table 1), or was acting by an additional or different mechanism. The latter conclusion was supported by the finding that, in the rat, pentagastrin still produced a significant, fully definable concentration-effect curve in the presence of histamine H_2-receptor blockade, presumably due to a direct action of pentagastrin on the oxyntic cell (Fig. 2b), which would be independent of histamine release.

These results indicate that in the rat stomach assay the action of pentagastrin, under control conditions, is due to both an indirect, histamine-mediated action and a direct action on the oxyntic cell, as though both the transmission and permission models (Fig. 1) were in play. There is only one effector in the experimental system, namely, oxyntic cell acid secretion; therefore the gastrin and histamine receptor-transduction pathways must ultimately converge prior to, or at the level of, activation of the H^+/K^+-ATPase, the enzyme ultimately responsible for acid secretion [14]. We tried to characterize the behavior of the interaction between histamine and pentagastrin at the oxyntic cell in the immature rat isolated whole-stomach assay, looking for the potentiating interactions described in the permission model. The effect of the background histamine was simply to frameshift the pentagastrin curves upward until an apparent maximum effect was achieved, as though the effects of the two pathways simply added (Fig. 3).

As exemplified by the interaction between pentagastrin and electrical field stimulation of the vagus nerve in the rat assay (Fig. 4), however, similar experiments, using other combinations of secretagogues, produced evidence for both potentiation and amplification. In the presence of background vagal stimulation, the pentagastrin curves were progressively shifted to the left and amplified. This potentiation may be indicative of the enhancement of the informational content of hormones which can

be achieved by convergent action. In this case, for example, the primary regulator of the cephalic phase of secretion, the vagus, has the effect of pepping up the response of the system to the regulator of the gastric phase of secretion, gastrin. Thus, in this particular experiment, low concentrations of pentagastrin (sub-nanomolar) only produced a significant secretory response in the presence of background vagal tone.

The apparent absence of potentiation with histamine and pentagastrin in the rat may indicate that histamine's main role is not for the local regulation of oxyntic cell activity but rather to produce synchronized vasodilation to satisfy the increased oxygen demand of the stimulated oxyntic cell.

In conclusion, the histamine dependence of the pentagastrin response is variable across species. We have evidence that this variation can also be true between stomach preparations of the same species, as might be expected from a system in which the hormone receptor density, a recognized biological variable, can be regulated by the gastrointestinal hormones themselves. In the rat, direct and indirect, histamine-mediated responses to gastrin have been exposed consistent with both the permission and transmission hypotheses. It is as though both methods of acid secretory regulation are operating. Histamine release by vagus and gastrin integrates circulatory and secretory processes; potentiating interactions between vagus and gastrin integrate the physiological control of oxyntic cell activity.

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