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INCREASED HISTAMINE-OUTPUT FROM THE ISOLATED GASTRIC MUCOSA OF THE RAT IN RESPONSE TO PENTAGASTRIN AND METHACHOLINE

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1 A rat isolated gastric mucosal preparation was used to monitor histamine output and acid secretion during stimulation by different secretagogues.

2 In non-stimulated preparations, spontaneous histamine output decreased over 450 min.

3 Stimulation of secretion with 4(5)-methylhistamine or dibutyryl cyclic adenosine 3′,5′-monophosphate (db cyclic AMP) and theophylline did not influence histamine output.

4 Pentagastrin, gastrin and methacholine increased both acid secretion and histamine output. Pentagastrin and gastrin mobilized six times more histamine in relation to acid secretion than did methacholine.

5 Spontaneous histamine output and secretagogue-induced increases were unaffected by changes in external Ca^{2+} (0.0 to 7.2 mM) or Mg^{2+} (1.2 to 4.8 mM).

6 These results support the hypothesis that mucosal histamine plays a more important role in the action of gastrin than of cholinomimetics on the parietal cell.

Introduction

The discovery that H_2-receptor antagonists inhibit gastrin-stimulated gastric acid secretion (Black, Duncan, Durant, Ganellin & Parsons, 1972) provided strong support for the hypothesis that mucosal histamine has a physiological role in the control of acid secretion. The nature of such a role, whether as mediator or modulator of secretion, has not been clearly defined and the evidence implicating histamine in the secretory action of vagal stimulation and other secretagogues is less satisfactory.

The rat has been frequently used in studies on acid secretion and on the formation and metabolism of mucosal histamine (reviewed by Kahlson & Rosengren, 1971). The formation of gastric histamine *in vivo* is regulated by gastrin and by the vagus nerve which can cause the release of antral gastrin (Rosengren & Svensson, 1969; Håkanson & Liedberg, 1970; Johansson, Lundell, Rosengren & Svensson, 1972). Feeding or pentagastrin administration increases mucosal histidine-decarboxylase activity and is associated with a reduction in histamine content, indicating the concurrent mobilization of stored histamine (Johnson, Jones, Aures & Håkanson, 1969; Håkanson & Liedberg, 1972; Weidle & Sewing, 1973). Although insulin-induced vagal stimulation increases gastric histimine turnover, methacholine and carbachol have no effect on the formation of histamine (Rosengren & Svensson, 1969). These results were interpreted as showing direct stimulation of the parietal cell by stable choline esters, and an indirect action of the vagus nerve and gastrin via a histamine-forming or storing cell.

Further evidence for a requirement for mucosal histamine in the secretagogue action of gastrin in the rat has been provided by studies with the H_2-antagonist, metiamide. Although metiamide is non-selective *in vivo*, inhibiting secretion induced by histamine, pentagastrin and bethanechol (Bunce & Parsons, 1978), a number of reports suggest that it exhibits a partial selectivity of action *in vitro*. In the rat whole stomach (Bunce & Parsons, 1976; Bunce, Parsons & Rollings, 1976) and isolated gastric mucosal preparations (Main & Pearce, 1978c), metiamide is able to produce only a partial reduction of gastrin-induced secretion, while responses to cholinomimetic agents are unaffected.

The histamine mobilized within the mucosa may diffuse into the gastric juice or gastric venous blood. We have studied changes in histamine mobilization, during stimulation of acid secretion by various secretagogues, by monitoring the output of histamine from mucosal and serosal surfaces of the rat isolated gastric mucosa (Main & Pearce, 1978a). With this preparation, the lack of an external muscle layer

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facilitates the diffusion of histamine into the serosal bathing solution. Since histamine release from rat peritoneal mast cells is Ca^{2+}-dependent (Foreman & Mongar, 1972), we have also studied histamine output in the presence of altered external Ca^{2+}. Some of the results have been communicated to the British Pharmacological Society (Main & Pearce, 1977).

Methods

Fed, home-bred rats (Olac strain, male) weighing 80 to 110 g were anaesthetized with pentobarbitone (60 mg kg^{-1} s.c.). The non-glandular portion of the stomach was cut away and the muscle layer overlying the non-antral region separated from the mucosa by blistering (Main & Pearce, 1978a). Two pieces of mucosa from the stomach, each 1 cm^2 in area, were placed in organ baths containing 35 ml of a modified Krebs solution (serosal solution, Sernka & Hogben, 1969) which bathed the serosal surface and was gassed vigorously with a 95% O_2 and 5% CO_2 mixture. The mucosal surface was superfused at the rate of 0.5 ml min^{-1} with an unbuffered solution of similar ionic composition gassed with 100% O_2. Acid secretion was recorded continuously via a dual microelectrode in the mucosal solution connected to an antilog unit and a potentiometric pen recorder. The H^+-ion concentration was noted every 15 min and expressed as apparent secretion rate. The mucosal superfusate and serosal bathing solution were collected over consecutive 30 min periods and histamine estimated fluorimetrically after condensation with *ortho*-phthalaldehyde (see below).

Solutions

For control conditions, the serosal solution (SS) contained (mM): NaCl 110.0, KCl 5.0, $CaCl_2$ 3.6, $MgCl_2$ 1.2, $NaHCO_3$ 26.0 and glucose 16.7. The mucosal solution (MS) was of similar composition with 26.0 mM $NaHCO_3$ replaced with equimolar NaCl. For test conditions, both solutions contained Ca^{2+} in concentrations ranging from 0.0 to 7.2 mM and Mg^{2+} from 1.2 to 4.8 mM, keeping all other cations constant. All chemicals were of analytical reagent grade.

Experimental design

Paired preparations, from a single stomach, were randomly allocated to different treatments. Spontaneous acid and histamine outputs from non-stimulated preparations were monitored throughout the 450 min experimental period. A small priming dose of secretagogue was added to all other preparations at 30 min and washed out at 60 min. Control responses were obtained after 120 min, by which time spontaneous acid and histamine outputs had approached more stable levels. Drug solutions were added to the SS in volumes not exceeding 1 ml and left in contact for 30 min during which period, peak secretion rates were attained (Main & Pearce, 1978a). A standard washout procedure was carried out after all responses. The SS was drained and replaced with warmed solution at 0, 5, 15 and 30 min after the end of the response and at 30 min intervals thereafter. Serosal samples (35 ml, 30 min contact), collected throughout the experiment apart from washout periods, were adjusted to pH 2 by the addition of 1 M HCl (1.5 ml), and stored frozen until required for assay. Samples of the mucosal superfusate (15 ml, 30 min) were also adjusted to pH 2 (1 M HCl, as required) before freezing. In repeated dose studies, responses to the appropriate secretagogue were obtained at 120, 270 and 360 min (see Figure 1). In dose-response studies, responses to increasing doses of pentagastrin or methacholine were obtained at 90 min intervals (120, 210, 300 and 390 min). Where the external Ca^{2+} concentration was changed, mucosae were washed with test solutions for 120 min after the control response before a further two responses to the same secretagogue were obtained (120 (3.6 mM Ca^{2+}), 270 and 360 min (altered Ca^{2+})).

Histamine assay

Histamine released into the bathing solutions was estimated fluorimetrically after condensation with *ortho*-phthalaldehyde (OPT, BDH Chemicals) by a method adapted from that described by Håkanson, Rönnberg & Sjolund (1972). After overnight storage at −20°C, the samples were thawed and shaken to ensure mixing. Aliquots (2.0 ml at pH 2) were adjusted to pH 12.5 by the addition of 1 M NaOH (0.2 ml), before the addition of the OPT reagent (0.1 ml of a 0.2% w/v solution in methanol). The samples were mixed thoroughly after each addition. The condensation reaction was carried out on ice (at 0°C), and stopped after 40 min by the addition of 2 M citric acid (0.2 ml). The samples were allowed to warm to room temperature before the fluorescence was measured at 350 nm (absorption) and 440 nm (emission) in an Aminco-Bowman Spectrofluorimeter. All estimations were carried out in duplicate and values adjusted for 'blank' fluorescence. Histamine standards, containing 0, 5, 10, 20 and 50 ng ml^{-1} free base in SS, and OPT reagent solutions were prepared daily. All drugs were tested for interference in the assay; in the concentrations used, only theophylline (2×10^{-3} M) altered fluorescence (equivalent to approx. 5 ng ml^{-1} histamine). Standard samples included theophylline when appropriate.

The selective H_2-agonist, 4(5)-methylhistamine (2.5×10^{-4} M, approx. 30 μg ml^{-1}) did not alter the fluorescence intensity obtained with standard solutions of histamine (0 to 50 ng ml^{-1}). Metabolites of histamine, including acetyl and methyl derivatives, are not detected by this method (Anton & Sayre, 1969). In a few experiments, the histamine content of the samples was confirmed by bioassay on guinea-pig ileum; activity was abolished by mepyramine.

Drug solutions

Stock solutions of drugs in 0.15 M NaCl solution (saline) were diluted for immediate use or stored frozen until required. The following drugs were used: pentobarbitone (Nembutal, Abbott Laboratories), histamine acid phosphate (BDH Chemicals), 4(5)-methylhistamine (S.K. & F.), pentagastrin (Peptavlon, I.C.I.), gastrin 2–17 (heptadecapeptide amide, I.C.I.), methacholine chloride (acetyl-β-methylcholine chloride, Sigma Chemical Co), dibutyryl cyclic adenosine-3′,5′-monophosphate (db cyclic AMP, Boehringer) and theophylline (1:3-dimethyl-xanthine, BDH Chemicals).

Analysis of results

The acid secretory response is calculated as the difference in secretion rate between the peak of the response and the preceding basal rate (i.e. peak-basal). Since the basal output of histamine declines steadily (Figure 1), secretagogue-induced changes in output were calculated as the difference between the observed value and the calculated basal value obtained by interpolation between preceding and subsequent basal samples (Figure 6).

Results are expressed as the mean ± standard error of the mean (s.e.mean). Changes in responses were compared within experiments (paired *t* test) or with control groups (unpaired *t* test). A *P* value of less than 0.05 was considered to be significant.

Results

Repeated dose studies

4(5)-Methylhistamine In experiments to investigate the effect of a selective H_2-agonist on mucosal histamine output into the serosal bathing solution, one preparation from each pair was kept as a non-stimulated control and the other exposed to 4(5)-methylhistamine (2.5×10^{-4} M). In resting preparations the characteristic initial fall and subsequent increase in spontaneous acid output was observed (see Figure 1), reaching a level of 1.85 ± 0.50 μmol cm^{-2} h^{-1} at 120 min (mean ± s.e.mean, $n = 4$); this basal output was well maintained for the remainder of the experiment. Histamine output from the same preparations decreased steadily from 374 ± 61 ng, between 90 and 120 min, to 198 ± 26 ng, between 420 and 450 min. In the paired tissues, subsequently stimulated with 4(5)-methylhistamine, spontaneous acid and histamine outputs followed the patterns described but reached much higher levels in the early stages of the experiment before declining to the same basal level at 120 min (acid, 1.75 ± 0.33; histamine, 373 ± 60 ng, 90 to 120 min). Acid secretion increased during contact with 4(5)-methylhistamine, with successive responses increasing in magnitude (0.29 ± 0.12 μmol cm^{-2} h^{-1}, 1.20 ± 0.50 and 1.77 ± 0.62). Histamine output was unaltered during periods of increased acid secretion and closely matched the pattern observed for resting mucosae. These changes in acid and histamine outputs have also been expressed as a histogram (Figure 2a) to facilitate comparison between secretagogues.

Pentagastrin (1.8×10^{-8} M, $n = 20$, Figure 2b) increased both acid secretion and mucosal histamine output. In contrast to the progressive increase in responses observed with 4(5)-methylhistamine, pentagastrin-induced secretion was greatest at the first response (1.73 ± 0.40 μmol cm^{-2} h^{-1}); subse-

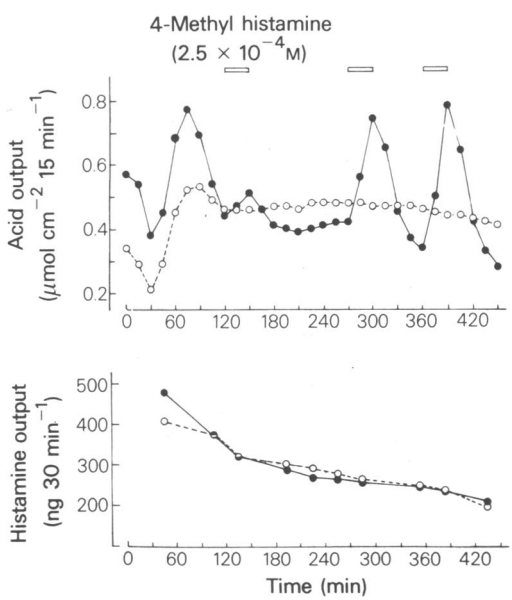

Figure 1 Acid secretion and histamine output (into SS) from resting preparations (○) and paired mucosae exposed to 4(5)-methylhistamine (2.5×10^{-4} M, ●). Each point represents the mean of 4 observations.

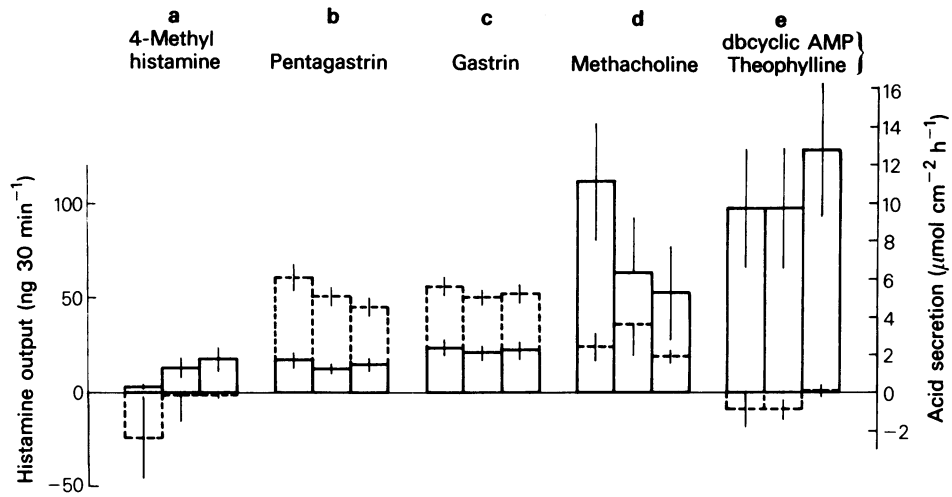

Figure 2 Increments in histamine output (into SS) and acid secretion induced by 4(5)-methylhistamine (2.5×10^{-4} M, $n = 4$), pentagastrin (1.8×10^{-8} M, $n = 20$), gastrin (2×10^{-8} M, $n = 8$), methacholine (5×10^{-7} M, $n = 6$) and db cyclic AMP (10^{-4} M) with theophylline (2×10^{-3} M, $n = 6$). Each column indicates the mean of n observations; vertical lines indicate the s.e.mean. Broken lines represent histamine output and solid lines represent acid secretion.

quent responses were 1.25 ± 0.26 and 1.48 ± 0.34. Each acid response was associated with a significant increase in the amount of histamine detected in the serosal solution. Successive increments were $+61 \pm 7$ ng, $+51 \pm 5$ and $+45 \pm 5$; each response represents an increase of approximately 30% over background histamine output.

Gastrin Similar results were obtained with gastrin (2×10^{-8} M, $n = 8$, Figure 2c). Acid secretion followed the pattern described above, (2.37 ± 0.39 μmol cm^{-2} h^{-1}, 2.11 ± 0.35 and 2.25 ± 0.41) and the increments in histamine output ($+56 \pm 5$ ng, $+50 \pm 4$, $+52 \pm 5$) represented an increase of 30% over basal levels.

Methacholine On repeated stimulation, responses to methacholine (5×10^{-7} M) decreased from 11.13 ± 3.09 μmol cm^{-2} h^{-1} ($n = 6$, Figure 2d) to 6.28 ± 2.96 and 5.25 ± 2.46. The increments in histamine output induced by methacholine were smaller and more variable than those observed with pentagastrin or gastrin. These responses ($+24 \pm 7$ ng, $+36 \pm 16$, $+19 \pm 3$) represent increases of only 10% over spontaneous output.

Dibutyryl cyclic adenosine 3',5'-monophosphate In a group of 6 experiments, one preparation from each pair was exposed to db cyclic AMP (10^{-4} M) in the presence of theophylline (2×10^{-3} M, Figure 2e). Although very large increases in acid secretion were produced (9.68 ± 3.13, 9.68 ± 3.13 and 12.75 ± 3.51 μmol cm^{-2} h^{-1}, $n = 6$), histamine output from the stimulated preparation was very similar to that from the resting tissue throughout the experiment. The calculated increments in histamine output, during contact with the secretagogues, were -9 ± 9 ng, -9 ± 5 and $+1 \pm 3$. Spontaneous acid and histamine outputs from resting mucosae followed the patterns illustrated in Figure 1.

Dose-response studies

Further experiments were carried out to investigate the relationship between acid and histamine with increasing concentrations of secretagogue. The results, illustrated in Figures 3 and 4, have been plotted to the scales used for Figure 2 to allow comparison between different treatments.

Pentagastrin Responses to increasing concentrations of pentagastrin (30 min contact) were obtained at 90 min intervals. One preparation from each pair was exposed to two fold increases in concentration (Figure 3a), the other to ten fold increases (Figure 3b, $n = 4$ for each group). The initial response to pentagastrin (1.8×10^{-8} M) was similar for both groups of mucosae for acid secretion ((a) 1.17 ± 0.34 and (b) 1.04 ± 0.15 μmol cm^{-2} h^{-1}) and for histamine output into the serosal solution ((a), 50 ± 13 and (b) 54 ± 9 ng). Mean values for the whole group were 1.11 ± 0.17 μmol cm^{-2} h^{-1} ($n = 8$) and 52 ± 8 ng respectively.

Pentagastrin-induced acid secretion did not in-

crease in parallel with an increase in concentration. Although the second response was greater than the first in both groups of mucosae (Figure 3a, b), a further increase in concentration produced a smaller response. However, the final responses to pentagastrin were increased.

These secretory responses were all associated with a significant increase in histamine output into the serosal solution. Histamine output was greater after 3.6 ± 10^{-8} M pentagastrin than in the initial response (Figure 3a); higher concentrations produced a progressive decrease in output. In the paired mucosae, output increased slightly after 1.8×10^{-7} M pentagastrin (Figure 3b) and showed a further increase with 1.8×10^{-6} M. However, when this latter dose was repeated histamine output was reduced.

In a separate series of experiments, in which to avoid tachyphylaxis only one dose was added to each preparation, acid secretion and the simultaneous release of histamine into both mucosal and serosal solutions were measured. After an initial 2 h period, one preparation from each pair was exposed to 1.8×10^{-8} M (a) and the other to 1.8×10^{-7} M (b) pentagastrin. Spontaneous acid output ((a) 2.21 ± 0.24 μmol cm^{-2} h^{-1} at 120 min and (b) 2.19 ± 0.24, $n = 12$ for each group) and histamine release into the mucosal ((a) 140 ± 20 ng, 90 to 120 min, and (b) 160 ± 26, $n = 8$) and serosal solutions ((a) 376 ± 64 ng, 90 to 120 min, and (b) 341 ± 71, $n = 8$) were similar for both groups of preparations. The histamine content of the mucosal superfusate represented approximately 25% of the total output.

In these experiments, acid secretion induced by pentagastrin showed a positive dose-response relationship, with 1.8×10^{-8} M (1.66 ± 0.22 μmol cm^{-2} h^{-1}, $n = 12$) producing a significantly smaller response than 1.8×10^{-7} M (2.94 ± 0.26, $P < 0.05$). A similar relationship was observed in the pattern of histamine release. The increase in output induced by 1.8×10^{-8} M (MS, $+21 \pm 2$ ng; SS, $+31 \pm 9$, $n = 8$) was less than after 1.8×10^{-7} M pentagastrin (MS, $+32 \pm 8$; SS, $+37 \pm 7$) although the differences were not significant. These increments, expressed as a percentage of the extrapolated basal output, reflect a relatively greater increase in output from the mucosal ((a) $+18 \pm 5\%$ and (b) $+24 \pm 6\%$) than from the serosal surface ((a) $+8 \pm 2\%$ and (b) $+18 \pm 6\%$).

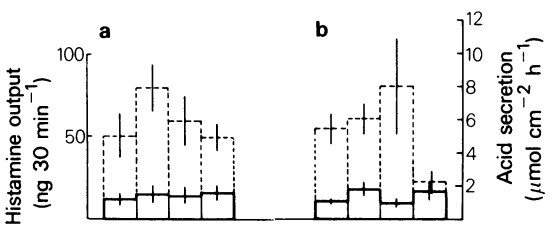

Figure 3 Histamine output (into SS) and acid secretion induced by pentagastrin. Each column indicates the mean and vertical lines the s.e.mean of 4 observations. Successive concentrations of pentagastrin used were: (a) 1.8×10^{-8} M, 3.6×10^{-8} M, 7.2×10^{-8} M and 14.4×10^{-8} M; (b) 1.8×10^{-8} M, 1.8×10^{-7} M, 1.8×10^{-6} M and 1.8×10^{-6} M. Broken lines represent histamine output and solid lines represent acid secretion.

Methacholine Responses to increasing concentrations of methacholine were obtained using the experimental design and dosage schedule described for pentagastrin. The initial responses to methacholine (2.5×10^{-7} M, Figure 4) were similar for both groups for acid secretion ((a) 1.77 ± 0.44 and (b) 3.13 ± 1.26 μmol cm^{-2} h^{-1}, $n = 4$) and histamine output into the serosal solution ((a) 16 ± 7 and (b) 19 ± 6 ng). Mean values for the whole group were 2.45 ± 0.67 μmol cm^{-2} h^{-1} ($n = 8$) and 17 ± 4 ng. The ratio of these increases in histamine and acid with methacholine was only 15% of that with pentagastrin.

A two or ten fold increase in the concentration of methacholine, from 2.5×10^{-7} M, produced a large increase in the secretory response. However, subsequent increases in concentration resulted in only a slight further increase in secretion (Figure 4a) or, with the highest concentration, a progressive reduction in responses (Figure 4b).

The increments in histamine output induced by methacholine were smaller than those observed with pentagastrin and did not follow the pattern of acid secretory responses. Both acid and histamine responses to methacholine (2×10^{-6} M) were abolished by pretreatment with atropine (3×10^{-6} M, 30 min). Pentagastrin (1.8×10^{-6} M) increased histamine output but did not stimulate acid secretion from mucosae made insensitive to methacholine.

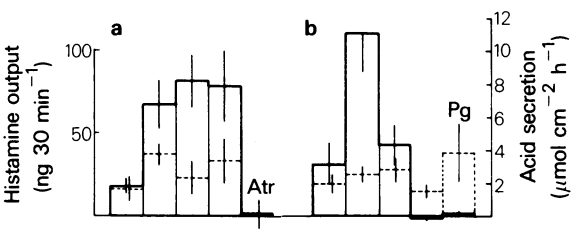

Figure 4 Histamine output (into SS) and acid secretion induced by methacholine. Each column represents the mean and vertical lines the s.e.mean of 4 observations. Successive concentrations of methacholine used were: (a) 2.5×10^{-7} M, 5×10^{-7} M, 10^{-6} M, 2×10^{-6} M and 2×10^{-6} M repeated in the presence of atropine (Atr, 3×10^{-6} M); (b) 2.5×10^{-7} M, 2.5×10^{-6} M, 2.5×10^{-5} M and 2.5×10^{-5} M followed by pentagastrin (Pg, 1.8×10^{-6} M). Broken lines represent histamine output and solid lines represent acid secretion.

Altered external Ca^{2+}

Non-stimulated mucosae In 24 randomized experiments, one preparation from each pair was kept as a non-stimulated control (Figure 5) and the other exposed to perntagastrin (Figure 6). All tissues were bathed in control solutions (3.6 mM Ca^{2+}, 1.2 mM Mg^{2+}) for the first 150 min of the experiment.

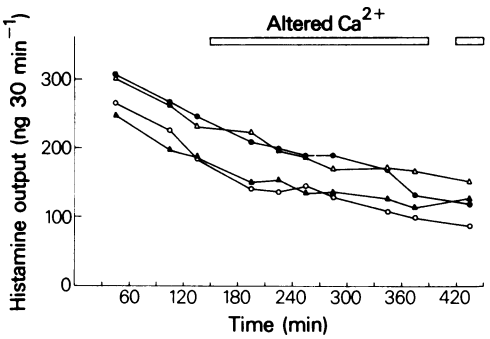

Figure 5 Histamine output (into SS) from non-stimulated preparations. Solutions were changed from 3.6 mM Ca^{2+} (control) at 150 min; each point represents the mean of 6 observations. Groups are described by the following symbols: 7.2 mM (○), 3.6 mM (●), 0.9 mM (▲), Ca^{2+}-free (△).

In resting mucosae, spontaneous acid output was 0.40 ± 0.09 μmol cm^{-2} h^{-1} (120 min, $n = 24$) and serosal histamine output was 239 ± 18 ng (90–120 min). These mucosae exhibited a steady decrease in histamine output (Figure 5), falling from 267 ± 23 ng ($n = 6$) at 90–120 min to 119 ± 20 ng at 420–450 min in the control group. Alteration of the external Ca^{2+}-concentration to 7.2 mM, 0.9 mM or Ca^{2+}-free (no added calcium) had no effect on histamine output. In these mucosae, raising Ca^{2+} to 7.2 mM decreased acid output while a reduction in concentration to 0.9 mM or to Ca^{2+}-free produced a large increase (Main & Pearce, 1978b).

Pentagastrin and methacholine The effects of altered Ca^{2+} on secretagogue-induced histamine output were studied in a group of experiments with pentagastrin (1.8×10^{-8} M) and methacholine (5×10^{-7} M). After the control response, mucosae were exposed to test solutions for 120 min before a further two responses were obtained (Main & Pearce, 1978b). Both secretagogues produced significant increases in histamine output, over the extrapolated basal level (Figure 6). During the initial response pentagastrin produced mean increases of 48 ± 5 ng ($P < 0.001$, $n = 25$) in histamine output and 1.18 ± 0.24 μmol cm^{-2} h^{-1} ($P < 0.001$) in acid secretion. Methacholine produced increases of 28 ± 4 ng

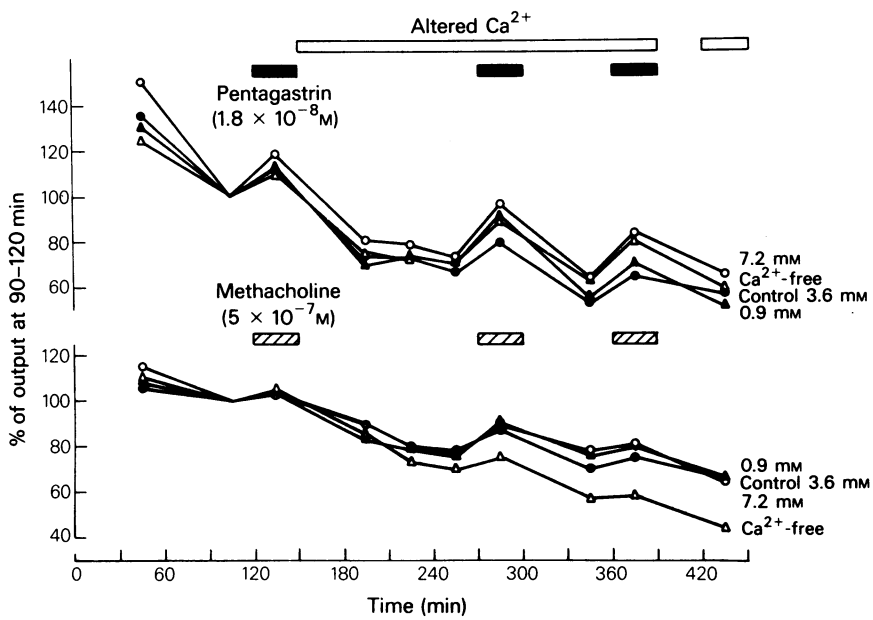

Figure 6 Effects of calcium on histamine output (into SS) from preparations stimulated by pentagastrin (1.8×10^{-8} M) or methacholine (5×10^{-7} M). Secretagogues were present at the times indicated and solutions changed from 3.6 mM Ca^{2+} at 150 min from the start of the experiment. Each point represents the mean of 6 observations with 7 for Ca^{2+}-free, pentagastrin and 4 for 7.2 nM Ca^{2+}, methacholine.

($P<0.001$, $n=22$) in histamine and $8.14 \pm 1.26\,\mu mol\,cm^{-2}\,h^{-1}$ ($P<0.001$) in acid outputs. Neither spontaneous histamine output nor the increments induced by the secretagogues were affected by changes in external Ca^{2+} between 0.0 and 7.2 mM (Figure 6). However, this treatment did cause significant changes in acid secretory responses to both pentagastrin and methacholine. The decrease in response observed under control conditions was greater in 7.2 mM Ca^{2+} and was converted to an increase by a reduction in concentration to 0.9 mM or to Ca^{2+}-free (Main & Pearce, 1978b). Raising the external Mg^{2+} concentration (1.2 to 4.8 mM) also failed to influence spontaneous or pentagastrin-induced histamine output, and had little effect on acid secretory responses (Main & Pearce, 1978b). There was a significant correlation between the increments in acid and histamine output (all three responses for the six mucosae in each control group, 3.6 mM Ca^{2+}, 1.2 mM Mg^{2+}) for pentagastrin, $r=0.65$, $n=36$, $P<0.01$, but not for methacholine, $r=0.03$, $n=18$.

Discussion

The rat isolated gastric mucosa (Main & Pearce, 1978a) has been used to study the simultaneous effects of drugs on acid secretion and endogenous histamine output. Both parameters were monitored continuously before and during responses to a range of secretagogues.

Under control conditions (3.6 mM Ca^{2+}, 1.2 mM Mg^{2+}), spontaneous histamine output decreased steadily throughout the experimental period while acid output declined slightly after reaching an initial peak but was then well maintained. Pentagastrin, gastrin and methacholine increased histamine output and acid secretion. At equisecretory concentrations, pentagastrin and gastrin mobilized six times more histamine relative to the increase in acid secretion than did methacholine. With higher concentrations of these secretagogues, the difference in the ratio of histamine to acid increased. 4(5)-Methylhistamine and db cyclic AMP with theophylline increased acid secretion but had no effect on histamine output. The ratio of the quantity of histamine appearing in the mucosal superfusate to that in the serosal solution was 1:3. The question of the presence of metabolites of histamine and their relative distribution in the bathing solutions has not been investigated.

The high level of spontaneous histamine output may reflect new synthesis or diffusion from enterochromaffin-like storage cells (Håkanson, Lindstrand, Nordgren & Owman, 1971) situated at the base of the gastric glands (Thunberg, 1967). Neither this spontaneous output nor the increments induced by pentagastrin and methacholine were affected by changes in the external concentration of Ca^{2+} (0.0 to 7.2 mM) or Mg^{2+} (1.2 to 4.8 mM), indicating that the release of histamine or change in histamine metabolism is independent of extracellular calcium.

The absence of changes in mucosal histamine output during stimulation of secretion with db cyclic AMP and theophylline, secretagogues which act within the parietal cell, or with 4(5)-methylhistamine, which has a direct action via H_2-receptors, indicates that the mobilization of histamine is not a result of increased secretory activity, and that spontaneous histamine output is not regulated by an H_2-receptor-mediated auto-feedback (Håkanson, Larsson, Liedberg & Sundler, 1978).

Stimulation with pentagastrin produced an increase in histamine output which coincided with the acid secretory response. These increments showed a significant correlation, compatible with the hypothesis that the secretagogue action of gastrin may be due, at least in part, to the mobilization of endogenous histamine. Methacholine induced a much smaller output of histamine relative to acid secretion than that produced by pentagastrin. Our results may be compared with the lack of effect of carbachol and methacholine on rat mucosal histamine-forming capacity reported by Rosengren & Svensson (1969), whose results do not entirely exclude the possibility of histamine release by these choline esters. A recent investigation using isolated gastric glands from the rabbit has shown that, in that species, carbachol (10^{-6} to 10^{-3} M) and pentagastrin (10^{-10} to 10^{-8} M) both liberate histamine in a dose-related manner and exhibit a similar maximum effect (Bergqvist, Waller, Hammer & Öbrink, 1980) although they produce only a transient increase or no change in O_2-consumption and [^{14}C]-aminopyrine accumulation by the glands (Berglindh, Helander, & Öbrink, 1976).

The dual action of pentagastrin on acid secretion and histamine output from the rat isolated mucosa may reflect two parallel, unrelated effects or a sequential increase in histamine and acid which might be causally related. Repeated exposure to pentagastrin resulted in a decrease in histamine output, but an increased acid response. Furthermore, pentagastrin was able to increase histamine output but not acid secretion from mucosae unable to respond to very high concentrations of methacholine. This evidence indicates the existence of separate effects of pentagastrin and methacholine on acid secretion and histamine output but does not exclude a subsequent interaction at the parietal cell between mobilized histamine and the exogenous secretagogue.

In the bullfrog isolated mucosa, equisecretory con-

centrations of pentagastrin and acetylcholine produce similar increases in histamine output (Rangachari, 1975), whereas preparations refractory to stimulation with these secretagogues still respond to histamine (Kasbekar, 1972). This evidence, together with the observation that the acid secretory response to pentagastrin is Ca^{2+}-dependent (Kasbekar, 1974), is consistent with the hypothesis that pentagastrin and acetylcholine act via the release of mucosal histamine, at least in amphibian preparations.

In the mouse isolated whole stomach preparation (Black & Welch, 1977) acid secretory responses to pentagastrin are inhibited in Ca^{2+}-free solutions without changes in histamine-induced secretion being observed. This result, and the observed inhibition by metiamide of acid secretion induced by the Ca^{2+}-ionophore A 23187 (Black, Jenkinson & Welch, 1978), is also consistent with the hypothesis that pentagastrin stimulates the parietal cell indirectly via release of histamine by a Ca^{2+}-dependent process. However, a mediator role for histamine is not supported by similar studies in other *in vitro* mammalian systems. Stimulation by pentagastrin or gastrin in the rat whole stomach (Bunce, Honey & Parsons, 1979) or gastric mucosa (Main & Pearce, 1978b), or in canine parietal cell suspensions (Soll, 1981), is not affected by Ca^{2+}-free solutions which completely but reversibly inhibit secretory responses to electrical field stimulation (Baird & Main, 1978).

In experiments with H_2-antagonists, pentagastrin-induced secretion from the kitten isolated mucosa was abolished by metiamide (Tepperman, Schofield & Tepperman, 1975) although evidence from other *in vitro* preparations favours a modulator rather than a mediator role for histamine. In the rat isolated gastric mucosa, metiamide (10^{-5} M) abolished histamine-induced secretion and caused a significant but only partial decrease ($-32 \pm 11\%$, $P < 0.05$, $n = 8$; Main & Pearce, 1978c) in the response to gastrin; only slightly greater inhibition was produced by 10^{-3} M ($-44 \pm 8\%$, $P < 0.005$, $n = 8$; unpublished observations). Similar results were obtained in the rat whole stomach preparation (Bunce & Parsons, 1976; Bunce *et al.*, 1976) and the mouse whole stomach (Wan, 1977). In none of these preparations did metiamide inhibit secretion induced by a cholinomimetic agent. In canine parietal cell suspensions (Soll, 1978) responses to histamine are inhibited only by metiamide and those to carbachol only by atropine, while gastrin is inhibited by neither. Furthermore, metiamide only inhibits the histamine component of the potentiated response to a combination of secretagogues. This evidence suggests that parietal cells possess separate receptors for histamine, acetylcholine and gastrin.

Secretory responses to cholinomimetics and db cyclic AMP, which in the rat isolated mucosa mobilize little or no histamine, are not inhibited by metiamide. This suggests that spontaneously released histamine does not provide a background facilitating influence for the parietal cell but may reflect an involvement in tissue damage or repair. In contrast, the increase in histamine output induced by gastrin and the partial reduction by metiamide of the acid secretory response suggests that gastrin acts directly on the parietal cell but also augments its own effect by releasing histamine locally.

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