Pentagastrin gastroprotection against acid is related to H₂ receptor activation but not acid secretion

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Abstract

Background—Pentagastrin enhances gastric mucosal defence mechanisms against acid and protects the gastric mucosa from experimental injury.

Aims—To investigate whether this gastroprotection is mediated by histamine receptors or occurs as a secondary effect of acid secretion stimulation.

Methods—The effects of omeprazole (100 µmol/kg), ranitidine (20 mg/kg), and pyrilamine (10 mg/kg) on pentagastrin (80 µg/kg/h) induced gastroprotection against acidified aspirin injury were examined in a luminal pH controlled model. The effects of these compounds on pentagastrin enhanced gastroprotective mechanisms were investigated using intravital microscopy, in which intracellular pH of gastric surface cells (pH_i), mucus gel thickness, gastric mucosal blood flow, and acid output were measured simultaneously.

Results—Pentagastrin protected rat gastric mucosa from acidified aspirin injury. This gastroprotection was abolished by ranitidine, but not omeprazole or pyrilamine. Pentagastrin induced a hyperaemic response to luminal acid challenge, increased mucus gel thickness, and elevated pH_i during acid challenge. Ranitidine reversed these enhanced defence mechanisms, whereas omeprazole and pyrilamine preserved these effects.

Conclusions—These data indicate that pentagastrin associated gastroprotection and enhanced defence mechanisms against acid result mainly from activation of histamine H₂ receptors, and not as an effect of the stimulation of acid secretion.

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lated mucosal blood flow and mucus gel thickness. The net effect of this coregulation of acid secretion and host defences is the preservation of the balance between the two opposing factors, which prevents mucosal injury during maximal acid secretion, and conserves energy when luminal pH is elevated.

Gastrin analogues protect the gastric mucosa from injury caused by ethanol,^{4-9,10} 0.6 N HCl,^{7,8} and stress.^{3,11} The mechanisms underlying this protective effect have thus far been poorly understood. Study of the gastroprotective mechanisms of acid secretagogues is hampered by the confounding effect of inconstant gastric luminal pH, especially when acid secretion is either maximally stimulated or inhibited, making wide pH variations likely. As luminal acid is the major aggressive factor, experiments performed in which luminal pH varies fail to control for this variable. None of the studies that have addressed the gastroprotective effects of prosecretory compounds or stimuli in an injury model have included control of luminal pH in the experimental design. We hence controlled luminal pH in our studies by a rapid perfusion technique, in order to minimise pH fluctuations, and therefore control aggressive factors.

As many of the effects of gastrin on acid secretion can be attributed to secondary effects of histamine,¹² and as histamine and H₂ receptor agonists can increase mucosal blood flow and prevent experimental ulcers,^{7,13-15} we hypothesised that histamine mediates the gastroprotective effects of pentagastrin via activation of H₂ receptors. To test this hypothesis, we applied our unique microscopic technique in which gastric mucosal intracellular pH (pH_i), mucus gel thickness, mucosal blood flow, and acid secretion are simultaneously measured in vivo in anaesthetised rats. One advantage of this technique over conventional means of measuring gastric host defences is that superfusate pH is held to within 0.05 pH units, even during maximal acid secretion or inhibition. This pH "clamp" is achieved by rapid superfusion of a mildly buffered solution. We thus stimulate acid secretion while maintaining superfusate pH at 7, and then acidify the superfusate to pH 1 in order to simulate the presence of luminal acid. Dissociation of luminal acid and acid secretion in this manner has enabled us to study the effects of acid secretion stimulation and luminally applied acid as independent variables. Other advantages are that discrete host defences such as mucosal blood flow and mucus gel thickness can be measured simultaneously with epithelial cell pH_i. The importance of the latter measurement is that

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The gastric mucosal barrier to acid is a laminar structure, consisting of a pre-epithelial mucus-bicarbonate layer, an epithelial layer, and a postepithelial layer consisting of blood vessels, non-epithelial cells, and enteric nerves. We, and others, have previously shown that gastric defence mechanisms, which prevent mucosal injury, are enhanced by the same factors that increase acid secretion, such as central vagal stimulation and administration of acid secretagogues.¹⁻⁸ In this fashion, the same factors (for example, gastrin) that increase acid secretion, which is the primary gastric aggressor, also enhance host defensive factors such as stimu-

failure to maintain pH_i during an acid challenge correlates with enhanced injury susceptibility when similar conditions are applied to a rat injury model.^{16–19} The fall in pH_i during acid challenge is hence an early indicator of impending damage which can be measured in the absence of barrier disrupting compounds.

Using this technique, we previously showed that pentagastrin increased mucus gel thickness, and produced a hyperaemic response to luminally superfused acid, with the net effect of improving the ability of the surface cell to preserve pH_i during a luminal acid challenge.² In that study, the H_2 receptor antagonist cimetidine reversed the effects of pentagastrin on mucosal blood flow, but had puzzling effects on mucus gel thickness. Further concerns regarding the effects of acid secretion inhibition per se, as opposed to specific effects of histamine receptor antagonism on defensive factors prompted this study. In the present study, H_1 and H_2 receptors were selectively antagonised with pyrilamine and ranitidine, respectively, and the gastric H^+/K^+ ATPase was selectively inhibited with omeprazole to control for non-specific histamine receptor antagonism and for the effects of acid secretion, respectively. Parallel experiments were performed using a model in which gastric injury was produced with a constant, rapid superfusion of acidified aspirin. As our *in vivo* system is predicated on the assumption that luminal acid is essential for mucosal damage, we chose a model in which injury is acid dependent, wherein luminal acid is requisite for mucosal injury, and antiseecretory compounds such as omeprazole prevent injury. Moreover, the pathogenesis of acidified aspirin (A-ASA) injury is relatively well understood.^{20–23} Other injury models, such as those using luminal ethanol, produce acid independent injury, which is due to the direct damaging effects of the added compound, and is not prevented by antiseecretory agents. This model was also designed to minimise fluctuations in luminal pH while causing acid induced injury.

Methods

ANIMALS

Male Sprague-Dawley rats weighing approximately 200 g (Harlan Laboratories, San Diego, California) were fasted overnight, but had free access to water. All studies were approved by the Animal Use Committee of the West Los Angeles Veterans Administration Medical Center.

SURGERY

Rats were anaesthetised by urethane (1.25 g/kg intraperitoneally). Body temperature was maintained at 36–37°C by a heating pad and a heat lamp. A tracheotomy was performed and the trachea was cannulated to ensure a patent airway. Rats were infused with saline via the femoral vein at a rate of 1.56 ml/min to prevent dehydration. The gastric lumen was continuously perfused by a technique described previously.²⁴ Briefly, a polyethylene tube (PE 50) was inserted into the stomach through an incision in the cervical oesophagus and held in place by a ligature, and saline was perfused

through the gastric lumen at a rate of 0.75 ml/min using a Harvard infusion pump. Gastric outflow was collected by a cannula inserted into the stomach via an incision in the duodenum. After completion of surgery an equilibration period of one hour was allowed until the experiments were started.

ASSESSMENT OF GASTRIC MUCOSAL LESIONS

After a 30 minute gastric perfusion with either A-ASA or saline, the stomachs were removed, opened along the greater curvature, rinsed with saline, and pinned flat on a corkboard. Gross lesions were assessed according to previously described methods.²⁴ The lesions were measured along their greatest length; lesions measuring less than 1 mm were assigned a rating of 1, lesions measuring 1–2 mm were assigned a rating of 2, and lesions measuring more than 2 mm were given a rating according to their length in millimetres. The overall total was designated the “macroscopic lesion score”.

Histological damage was assessed as described previously.¹⁷ Three strips of tissue were taken from across the entire posterior wall (parallel to the limiting ridge): just below the limiting ridge; the mid corpus; and the distal corpus. The severity of gastric mucosal injury was evaluated on the sections stained with haematoxylin and eosin using a modification of established criteria.²⁵ Damage was graded as follows: surface mucous cell damage (surface damage); erosions down to, but not deeper than, the mucous neck cell area (superficial erosions); and erosions extending down into the parietal cell area (deep erosions). At least 100 glands were assessed in each specimen. The lesion score was calculated by multiplying the percentage of glands with no injury by 0, the percentage with surface damage by 1, the percentage with superficial erosions by 2, and the percentage with deep lesions by 3. The scores were then added and averaged among the three specimens.

IN VIVO MICROSCOPIC PREPARATION

An *in vivo* microfluorometric technique, described in detail elsewhere,^{16 26–28} was used to measure intracellular pH (pH_i). After urethane (1.25 g/kg intraperitoneally) anaesthesia, the rat was placed supine on a plastic stage. Body temperature was maintained at 36–37°C by a heating pad and a heat lamp. The abdomen was opened via a 3 cm midline incision and the stomach exposed. The anterior wall of the forestomach was incised just proximal and parallel to the limiting ridge, and a portion of the posterior wall of the corpus was everted through the incision. A stainless steel disc (2.5 cm in diameter and 5 mm deep) with a 5 mm central aperture was fixed watertight on the mucosal surface with a silicone plastic adherent (Silly Putty, Binney & Smith Co., Easton, Pennsylvania). A thin plastic coverslip was fixed to the disc with the silicone adherent to permit closed superfusion with solutions at a rate of 0.39 ml/min using a Harvard infusion pump. The exposed mucosa was continuously superfused with Krebs solution (pH 7.4) containing 100 μM 5,6-carboxyfluorescein

diacetate (CF; Molecular Probes Inc., Eugene, Oregon) for 15 minutes to load the surface mucous cells before starting the experiment. CF was omitted from acidic solutions due to solubility problems. It was found that, although total fluorescence diminished during superfusion with CF free superfusate, fluorescence was maintained above background at a level sufficient to obtain reproducible fluorescence ratios for 20 minutes. The CF labelled surface cells were visualised using a microscope connected to a closed circuit video system. All experiments were videotaped for later analysis. On playback of the videotapes, an image analyser was used to measure the intensity of fluorescence from a small area.

IMAGE ANALYSIS

Fluorescence of the microscopically observed chambered segment of gastric mucosa at 515 nm emission was recorded on VHS videotape. The intensity of emitted fluorescence at 495 nm stimulation is pH dependent, whereas that at 450 nm is not. Therefore 450 nm and 495 nm narrow bandpass interference filters (Chroma Inc., Brattleboro, Vermont) were exchanged every 2 minutes. Readings were taken at 10 seconds before and after each time point. The paired readings needed to calculate a fluorescence ratio were thus taken at a maximum of 20 seconds apart. Image analysis was performed on the recorded images as follows. Initially a small area of a gastric gland corresponding to three surface cells was selected at random and then followed throughout the experiment. Fluorescence intensity of the selected area was measured by first capturing the image using an Intel Pentium based IBM compatible microcomputer equipped with a FlashPoint framegrabbing videographic card (Integral Technologies Inc., Silver Spring, Maryland), and digitised, with area of interest defined and intensity measured using an image analyser (Image-Pro Plus v. 1.3, Media Cybernetics, Silver Spring, Maryland). Calibration and background compensation were done as described previously.²⁶ The intensity at 495 nm was divided by that at 450 nm and the resulting ratio was converted to pH_i using an *in vivo* calibration curve as described previously.²⁶

MEASUREMENT OF ACID SECRETION

The effluent of each superfusion was back titrated with 0.1 N NaOH in order to measure the amount of acid secreted during the superfusion according to the method of Garner and Heylings.²⁹

GASTRIC MUCOSAL BLOOD FLOW MEASUREMENT

For the simultaneous measurement of mucosal blood flow and pH_i , a soft tip pencil probe (Model P435, Vasomedics Inc., St Paul, Minnesota) was placed through the central aperture at a 45° angle so as to rest gently on the surface of the everted mucosal surface. Blood flow was measured as the voltage output of the laser Doppler instrument (LaserFlo BPM803A, Vasomedics Inc., St Paul, Minnesota) and was expressed relative to four or five measurements made during the last 4–5 minutes of the dye loading period, as described previously.²⁸

MEASUREMENT OF MUCUS GEL THICKNESS

Following CF loading, graphite particles were placed over the mucosa to delineate the luminal surface of the gel layer. The microscope was alternately focused from the fluorescent apical cell surface to the graphite layer. The distance of vertical travel of the microscope objective was measured by using a digital z axis measuring device (Quick-Check, Metronics, Bedford, New Hampshire) connected to the microscope, providing a measure of gel thickness. We have shown previously, in a systematic study of gel thickness,¹⁶ that measurement of gel thickness by our technique (118 (4) μm , range 52–247 μm ; $n=89$) compared favourably with gel thickness measured by two other techniques: staining of thick sections of unfixed mucosa^{30, 31} (73 (5) and 145 (22) μm), and slit lamp pachymetry³² (166 (10) μm).

CALCULATION OF THE INITIAL ACIDIFICATION RATE

Initial acidification rates were calculated by subtracting pH_i measured at 0 minutes from pH_i measured two minutes after the start of superfusion with acidic buffer. Intrinsic buffer capacity (intracellular buffering power exclusive of bicarbonate; β_i) of surface cells was estimated according to published measurements^{33, 34} as 15 mM/pH at pH 7.1. Total buffer capacity (β_t) was calculated according to the equation³⁴: $\beta_t = \beta_i + \beta_{\text{CO}_2} = \beta_i + 2.3 \cdot [\text{HCO}_3^-]$ as 44 mM/pH

EXPERIMENTAL DESIGN

Injury studies

Rats were pretreated with either omeprazole (100 $\mu\text{mol/kg}$ intraperitoneally), ranitidine (20 mg/kg intraperitoneally), pyrilamine (10 mg/kg intraperitoneally), or vehicle (0.56 mg/ml NaHCO_3 solution intraperitoneally) 60 minutes before continuous gastric perfusion with A-ASA (25 mM aspirin in pH 1.0 buffer). Pentagastrin (80 $\mu\text{g/kg/h}$ intravenously) was started 20 minutes before A-ASA perfusion. After 30 minutes perfusion of the gastric lumen with either A-ASA or saline, rats were killed and gastric injury was assessed.

In vivo microscopy studies

After urethane anaesthesia, the mucosa was prepared and the cells were loaded with CF as described in the preceding section. The end of this preloading period was designated time 0, at which time an intravenous infusion of pentagastrin (80 $\mu\text{g/kg/h}$) or saline was begun. Rats were divided into eight groups, four of which were saline infused: saline control (vehicle intraperitoneally, saline intravenously), OMP (omeprazole intraperitoneally, saline intravenously), Ranit (ranitidine intraperitoneally, saline intravenously), and Pyl (pyrilamine intraperitoneally, saline intravenously); and four of which were pentagastrin infused: Gast control (vehicle intraperitoneally, pentagastrin intravenously), OMP+Gast (omeprazole intraperitoneally, pentagastrin intravenously), Ranit+Gast (ranitidine intraperitoneally, pentagastrin intravenously), and Pyl+Gast (pyrilamine intraperitoneally, pentagastrin intravenously). Omeprazole (100 $\mu\text{mol/kg}$ intraperitoneally), ranitidine

(20 mg/kg intraperitoneally), pyrilamine (10 mg/kg intraperitoneally), or vehicle (0.56 mg/ml NaHCO₃ solution intraperitoneally) was injected at -60 minutes. The concentrations of these compounds were chosen so as to produce maximal suppression (with the exception of pyrilamine) while maintaining optimal receptor selectivity.³⁵ The chamber was superfused with pH 7.4 Krebs solution for the first 30 minutes (0–30 minutes), then the superfusate was changed to pH 1.0 solution for 20 minutes (30–50 minutes).

CHEMICALS AND SOLUTIONS

5,6-Carboxyfluorescein diacetate (Molecular Probes Inc., Eugene, Oregon) was first dissolved in dimethylsulphoxide and diluted with Krebs solution. The final concentration of the solvent was less than 0.5%. Krebs solution contains (in mM): 136 NaCl, 2.6 KCl, 1.8 CaCl₂, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) (pH 7.4). For acid superfusion, Krebs solution was (in mM): 36 NaCl, 2.6 KCl, 1.8 CaCl₂, 10 HEPES, and titrated to pH 1.0 with 5 N HCl. Ranitidine, pyrilamine (Sigma Chemicals, St Louis, Missouri), and omeprazole (Astra Hässle AB, Sweden) were dissolved in NaHCO₃ (0.56 mg/ml) solution, and administered in a 1 ml/kg intraperitoneal injection. Pentagastrin (Peptavlon, Ayerst Laboratories, New York, NY) was diluted with saline and injected intravenously at a rate of 1.56 ml/kg/h. Aspirin (Sigma Chemicals, St Louis, Missouri) was dissolved in pH 1.0 Krebs solution containing 3% Tween 80, which was freshly prepared for each experiment.

STATISTICAL ANALYSIS

Results are expressed as means (SEM). Multiple group comparisons in all cases were performed by analysis of variance (ANOVA, factorial) followed by Fisher's contrast. A probability level of less than 0.05 was considered significant. In the in vivo microscopic study, the eight groups were divided into two sets of four: those that were infused with pentagastrin and those that were saline infused, so as to vary only one intervention within each set, and ANOVA contrasts (factorial or repeated) were used for comparison in each set.

Results

INJURY STUDY

Gastric perfusion with A-ASA produced significant gross lesions in vehicle pretreated,

saline infused rats compared with saline perfusion (33.8 (3.5), n=8 versus 0.5 (0.5), n=4). Pentagastrin pretreatment significantly reduced gross injury produced by A-ASA perfusion by 78%. Pretreatment with omeprazole or pyrilamine had no effect on the protective effect of pentagastrin against A-ASA, whereas ranitidine completely reversed the protective effect of pentagastrin. Pentagastrin given alone did not increase gross lesion scores compared with saline infused rats (table 1).

The histological lesion score in rats treated with A-ASA alone was 156.5 (12.0) (n=8). Infusion with pentagastrin significantly reduced the lesion scores by 64% (to 56.5 (9.1), n=6). Pretreatment with omeprazole or pyrilamine had no effect on the protective effect of pentagastrin against A-ASA, but ranitidine completely reversed the lesion score to 152.8 (16.8) (n=6). Deep lesions, defined as injury extending into the glandular portion of the gastric pits, were significantly prevented by pentagastrin (by 85%). Pretreatment with omeprazole did not affect the protective effect of pentagastrin, but ranitidine and pyrilamine reversed the protection (table 1).

IN VIVO MICROSCOPY STUDY

Acid output

Acid output was 0.20 (0.03) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the saline control group (n=6), 0.05 (0.01) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the OMP group (n=7), 0.05 (0.01) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the Ranit group (n=7), 0.19 (0.02) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the Pyl group (n=7), 0.45 (0.07) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the Gast control group (n=8), 0.05 (0.01) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the OMP+Gast group (n=8), 0.06 (0.01) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the Ranit+Gast group (n=8), and 0.44 (0.07) $\mu\text{mol}/\text{min}/\text{cm}^2$ in the Pyl+Gast group (n=7) (fig 1). Omeprazole or ranitidine significantly suppressed, as expected, whereas pyrilamine had no effect on basal and pentagastrin stimulated acid secretion.

Mucosal blood flow

Figure 2 depicts relative gastric mucosal blood flow. There was no statistical difference in relative mucosal blood flow during superfusion with pH 7.4 solution among all treatment groups. After acid challenge, relative blood flow significantly increased in rats infused with pentagastrin (Gast control group) compared with saline control animals (p<0.05). OMP, Ranit, or Pyl did not affect relative gastric mucosal blood flow compared with saline controls (fig

Table 1 Effect of omeprazole, ranitidine, and pyrilamine on pentagastrin induced gastroprotection against acidified aspirin injury

Pretreatment Intravenous treatment A-ASA	Vehicle Saline -	Vehicle Pentagastrin -	Vehicle Saline +	Vehicle Pentagastrin +	OMP Pentagastrin +	Ranitidine Pentagastrin +	Pyrilamine Pentagastrin +
Macroscopic injury score [n]	0.5 (0.5) [4]	1.3 (0.7) [3]	33.8 (6.3) [8]	7.3 (3.5)* [6]	17.8 (3.9)* [6]	45.7 (8.6)†‡§ [6]	6.8 (2.9)* [5]
Histological injury score [n]		21.6 (12.0) [3]	156.5 (12.0) [8]	56.5 (9.1)* [6]	88.3 (20.8)* [6]	152.8 (16.8)†‡§ [6]	90.5 (16.6)* [5]
% Surface injury		9.1 (5.0)	10.2 (2.3)	12.1 (1.4)	17.6 (4.9)	10.0 (2.5)‡	11.6 (3.1)
% Shallow injury		6.3 (3.5)	42.7 (4.8)	19.8 (4.5)*	12.3 (7.0)*	26.3 (5.3)*	14.8 (4.7)*
% Deep injury		0.0 (0.0)	21.3 (5.5)	3.2 (1.9)*	8.0 (2.7)	29.8 (7.3)†‡	16.4 (4.3)†

Values expressed as mean (SEM) obtained from the number of rats indicated in square brackets.

* p<0.05 v vehicle + saline + A-ASA, † p<0.05 v vehicle + pentagastrin + A-ASA, ‡ p<0.05 v OMP + pentagastrin + A-ASA, § p<0.05 v pyrilamine + pentagastrin + A-ASA among five groups treated with A-ASA.

OMP, omeprazole.

Figure 1 Effect of omeprazole (OMP), ranitidine, and pyrilamine on pentagastrin stimulated gastric acid output. Values are the means (SEM) of measurements obtained from the number of rats indicated in parentheses during the first 30 minutes. * $p < 0.05$ versus saline control among the four saline infused groups and pentagastrin control group; † $p < 0.05$ versus pentagastrin control among the four pentagastrin infused groups.

2A). The pentagastrin induced hyperaemic response during acid challenge was not decreased by OMP or pyrilamine, but was reversed by ranitidine in the 45–50 minute time period ($p < 0.05$ versus Gast controls) (fig 2B).

Mucus gel thickness

Figure 3 depicts gastric mucus gel thickness, as measured by the in vivo microscopy fluorometric technique. Gel thickness at time 0 minutes (before pentagastrin infusion) was 122.8 (13.1) μm ($n=6$), 126.0 (12.8) μm ($n=7$), 111.6 (12.7) μm ($n=7$), 121.6 (13.0) μm ($n=7$), 111.0 (8.3) μm ($n=8$), 105.4 (8.9) μm ($n=8$), 128.1 (12.4) μm ($n=8$), and 105.2 (9.4) μm ($n=7$) in saline control, OMP, Ranit, Pyril, Gast control, OMP+Gast, Ranit+Gast, and Pyril+Gast groups, respectively. Baseline gel thickness was not significantly different among

the eight groups. Gel thickness significantly increased in the Gast control compared with the saline control, OMP, Ranit, and Pyril groups ($p < 0.05$; fig 3A). In the pentagastrin infused groups, ranitidine decreased gel thickness at 20, 40, and 50 minutes compared with the Gast control and Pyril+Gast groups. Omeprazole decreased gel thickness at 40 and 50 minutes compared with Gast control. Pyrilamine did not affect gel thickness during both neutral and acid superfusions as compared with the Gast control group (fig 3B).

Intracellular pH and initial acidification rate

Intracellular pH was significantly higher in the Gast control group than in the saline control, OMP, Ranit, and Pyril groups ($p < 0.05$), consistent with our previous study.² OMP, Ranit, or Pyril did not affect pH_i as compared with the saline control group (fig 4A). pH_i during acid challenge was not affected by omeprazole or pyrilamine. Ranitidine decreased pH_i at all time points during acid challenge compared with the Gast control group, and at some time points compared with the other two groups (fig 4B).

Initial acidification rate calculated from the initial drop of pH_i after the superfusion of pH 1.0 buffer was 8.40 (0.87) mM ($n=12$), 8.26 (0.73) mM ($n=14$), 7.94 (0.43) mM ($n=14$), 8.68 (0.69) mM ($n=14$), 5.38 (0.38) mM ($n=16$), 7.22 (0.37) mM ($n=16$), 7.69 (0.48) mM ($n=16$), and 6.64 (0.49) mM ($n=14$) in saline control, OMP, Ranit, Pyril, Gast control, OMP+Gast, Ranit+Gast, and Pyril+Gast groups, respectively. The acidification rate was significantly slower in the Gast control group than in the saline control group ($p < 0.05$). OMP and ranitidine, but not pyrilamine, reversed the effect of pentagastrin on acidification. In the four saline infused groups, OMP, Ranit, or Pyril by themselves did not affect the acidification rates as compared with the saline control group.

Figure 2 Effect of omeprazole (OMP), ranitidine, and pyrilamine on relative gastric mucosal blood flow. (A) Effect on gastric mucosal blood flow in saline infused groups during superfusion with neutral and acid solutions. (B) Effect on gastric mucosal blood flow in pentagastrin infused groups during superfusion with neutral and acid solutions. Each point and error bar represents the mean (SEM) of the number indicated in parentheses. * $p < 0.05$ versus saline control; † $p < 0.05$ versus pentagastrin control; ‡ $p < 0.05$ versus OMP plus pentagastrin; § $p < 0.05$ versus pyrilamine plus pentagastrin.

Figure 3 Effect of omeprazole (OMP), ranitidine, and pyrilamine on mucus gel thickness. (A) Effect on gel thickness in saline infused groups during superfusion with neutral and acid solutions. (B) Effect on gel thickness in pentagastrin infused groups during superfusion with neutral and acid solutions. Each point represents the mean (SEM) of the number indicated in parenthesis. * $p < 0.05$ versus saline control; † $p < 0.05$ versus pentagastrin control; ‡ $p < 0.05$ versus OMP plus pentagastrin; § $p < 0.05$ versus pyrilamine plus pentagastrin.

Figure 4 Effect of omeprazole, ranitidine, and pyrilamine on intracellular pH of gastric surface cells. (A) Effect on pH_i in saline infused groups during superfusion with neutral and acid solutions. (B) Effect on pH_i in pentagastrin infused groups during superfusion with neutral and acid solutions. Each point represents the mean (SEM) of the number indicated in parenthesis. * $p < 0.05$ versus saline control; † $p < 0.05$ versus pentagastrin control; ‡ $p < 0.05$ versus OMP plus pentagastrin; § $p < 0.05$ versus pyrilamine plus pentagastrin.

Discussion

Pentagastrin significantly reduced acidified aspirin (A-ASA) induced gastric injury. This gastroprotection was completely abolished by ranitidine, whereas omeprazole and pyrilamine had no effect on the protective effects of pentagastrin. In the *in vivo* microscopy studies, ranitidine, but not omeprazole and pyrilamine, partially reversed the pentagastrin induced hyperaemic response to luminal acid challenge. Ranitidine and omeprazole inhibited the penta-

gastrin induced increase in mucus gel thickness. Pentagastrin improved the ability of surface cells to preserve pH_i during luminal acid challenge. This improvement was abolished by ranitidine, but not by omeprazole or pyrilamine.

Gastrin protects the gastric mucosa from injury caused by ethanol,^{4 5 9 10} 0.6 N HCl,⁷ and stress,^{3 11} although Konturek *et al* showed that acute gastric lesions induced by acidified aspirin were not prevented by gastrin 17.⁹ Interpretation of this latter study, however, was potentially

impaired by a fall in gastric luminal pH following injection of gastrin 17, which increased the difficulty of separating the aggressive (for example, stimulation of acid secretion) from the defensive (for example, enhancing mucosal defence mechanisms) roles of gastrin.

There are only a few reports available concerning the effect of acid inhibition on the gastroprotective effect of acid secretagogues. Several investigators have shown that H_2 receptor antagonists and omeprazole can reverse the gastroprotective effects of stimulated acid secretion.^{5 8 36} These studies, however, differed from the present study in that luminal perfusion was not used to control luminal pH, which is an important factor in studies in which acid secretion is either maximally suppressed or induced. Furthermore, studies of acid independent gastric injury (induced by ethanol, for example) are difficult to compare with acid dependent injury due to aspirin. The present study shows that stimulation of H_2 receptors plays a major role in mediating the gastroprotective effects of pentagastrin.

As was seen in our previous studies,^{2 37} pentagastrin infusion was associated with a hyperaemic response to superfused luminal acid. Generally, stimulated acid secretion is associated with increased gastric mucosal blood flow. In most studies, however, the effects of luminal acid and of acid secretion are not dissociated, so it is difficult to ascertain whether the increased blood flow due to pentagastrin results from increased metabolic needs of the secreting mucosa or stimulation of mucosal afferent nerves by back diffusing luminal or superfused acid. The present study supports the latter possibility, as increased blood flow by pentagastrin was exclusively associated with the presence of superfused acid, and acid inhibition with omeprazole did not abolish the pentagastrin associated hyperaemic response to luminal acid. Several investigators have shown that pentagastrin associated hyperaemia is inhibited by inhibition of nitric oxide (NO) synthesis.^{38 39} Pentagastrin may thus increase the sensitivity of afferent nerves to luminal acid, activate the calcitonin gene related peptide-NO pathway, and increase mucosal blood flow. It is not apparent whether this potentiation is at the level of sensory nerves or in the vascular smooth muscle.

The role of acid secretion versus luminal acid on gastrin induced gastric hyperaemia is controversial. Piqué *et al* showed, using hydrogen gas clearance, that omeprazole and ranitidine reduced pentagastrin induced increases in acid output and corpus mucosal blood flow to basal levels.⁴⁰ In contrast, Holm and Jägaré showed that ranitidine injection did not significantly inhibit pentagastrin induced increases in mucosal blood flow measured by laser Doppler flowmetry in spite of significant acid inhibition.⁴¹ Recently, Kato *et al* showed that neutralisation of luminal acid by glycine abolished increased mucosal blood flow by pentagastrin, whereas increased blood flow was noted when the buffer was omitted,¹⁵ indicating that an acid secretagogue plus luminal acid were required to produce an acid dependent hyperaemic response. This is similar to the findings in this and

other recent studies from our laboratory.³⁷ The present study clearly shows that the luminal acid dependent increase in mucosal blood flow requires H_2 receptor activation, but does not result from acid secretion in and of itself.

Ranitidine decreased pentagastrin induced gel thickening, suggesting that control of mucus gel thickness is under histaminergic control, as is acid secretion and the hyperaemic response to luminal acid in the presence of acid secretion. Although our results appear to be clear cut, control of gastric mucus secretion and gel thickness is another area of controversy. Studies of mucus secretion from cultured gastric epithelial cells suggest that gastrin analogues and histaminergic and cholinergic agonists can increase mucus secretion at the cellular level.^{4 42 43} Our own previous data, however, are confusing as cimetidine not only had no effect on pentagastrin induced mucus gel thickening, but also increased thickness in its own right.² This discrepancy may have resulted from non-specific effects of cimetidine, which appear to have been solved with the use of ranitidine, or from the different anaesthetics used in the two studies. In the previous study, pentobarbital was used, in contrast to urethane in the present study. Unlike pentobarbital, urethane releases somatostatin,⁴⁴ which, however has little effect on non-steroidal anti-inflammatory drug (NSAID) induced injury or gastric blood flow,⁴⁵ suggesting that this is not the explanation for the discrepant findings. Although some groups have also shown mucus gel thickening in secretagogue treated animals, we are unaware of like studies in which this effect was abolished with H_2 receptor antagonists in an *in vivo* model.

Consistent with our previous studies,^{2 37} pentagastrin increased pH_i during acid superfusion, probably due to the net effects of increased blood flow, increased mucus gel thickness, and basolateral (alkaline tide) bicarbonate release from parietal cells due to increased acid secretion. The major reason why ranitidine abolished the pentagastrin induced increase in pH_i during luminal acid challenge may be explained by the abolition of pentagastrin increases in mucosal blood flow in response to luminal acid coupled with impaired mucus gel thickening. It is unlikely that inhibition of acid secretion *per se* contributes to the effect of ranitidine on pH_i as omeprazole preserved the pentagastrin induced increase in pH_i despite potently suppressing induced acid secretion. Ranitidine, but not omeprazole or pyrilamine, reversed the decrease in initial acidification rate (IAR) by pentagastrin, probably due to its inhibition of pentagastrin associated mucus gel thickening. This study shows the importance of individual defence mechanisms on the overall resistance of the gastric mucosa to acid. The thickened mucus gel slows proton permeation, which presumably decreases trans gel proton flux,⁴⁶ enabling the more efficient function of other mucosal compensatory mechanisms. The hyperaemic response to acid is convincingly linked to a reduction in injury susceptibility in this and prior studies,⁴⁷ presumably by increasing mucosal bicarbonate delivery and by enhancing tissue proton removal.

In conclusion, the H_2 antagonist ranitidine, but not omeprazole or pyrilamine, abolished pentagastrin associated gross and histological gastroprotection in a luminal pH controlled gastric injury model, and abolished the important gastric defensive mechanisms associated with systemic gastrin analogue infusion: the hyperaemic response to acid, the increase in gel thickness, and the decrease in IAR. These findings indicate that pentagastrin induced gastroprotective effects against acid result from activation of H_2 receptors rather than from activation of H_1 receptors or from induction of acid secretion alone. Clinically, the antiaggressive effects of H_2 receptor antagonists generally appear to outweigh their antidefensive properties. The antidefensive properties of these compounds, however, might be consequential in situations in which defensive impairment may be relatively more important, such as in the prevention of gastric ulcers due to non-steroidal anti-inflammatory drugs (NSAIDs) or stress. In most studies, H_2 receptor antagonists have been proved to be ineffective for the prevention of NSAID related gastric injury.^{48 49}

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