Evidence for the secretion of gastrin into human gastric juice

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SUMMARY Provided the intragastric pH is greater than 6 and the collected gastric juice is boiled and neutralised immediately, considerable amounts of gastrin-like immunoreactivity can be found in human gastric juice. Characterisation according to size and charge reveals that nearly all the gastrin-like immunoreactivity is similar to gastrin 1-17. Direct stimulation of the G-cells with protein solution is followed by a significant and simultaneous increase of acid secretion, serum gastrin, and gastrin output into the lumen of the stomach in control subjects and patients with duodenal ulcer.

The occurrence of gastrin in gastric juice has been a matter of dispute. Sircus¹ suggested the presence of a secretagogue in the gastric juice of Zollinger-Ellison patients. Jordan and Yip² reported that gastrin-like immunoreactivity was detectable in gastric juice of patients without gastrointestinal disease in the fasting state and after stimulation of secretion. Intraluminal gastrin has been thought to be related to peptic ulcer disease.³ Other authors have questioned the assay conditions used in the measurement of gastrin-like activity in gastric juice,^{4,5} pointing out possible artefacts in the gastrin radioimmunoassay.⁶

The present study was performed to determine whether gastrin protected from acid proteolysis and enzymatic degradation is really present in gastric juice or its apparent presence there is an artefact in the radioimmunoassay system.

Methods

COLLECTION OF GASTRIC JUICE AND BLOOD SAMPLES

Fifteen healthy volunteers and 14 duodenal ulcer patients were studied. Informed written consent was obtained from each subject. After an overnight fasting period of 12 hours, gastric tubes were positioned in the gastric fundus and antrum under fluoroscopic control. A polyethylene cannula was placed in the antecubital vein. After a rest period of 30 minutes, residual gastric contents were removed and submitted to gastrin assay. Basal acid output was

determined over 30 minutes, and two basal samples of venous blood for gastrin assay were obtained. Then gastric perfusion was performed for two periods with 50 ml 0.15 M sodium phosphate buffer pH 7.0 (299 mosm/kg) per 15 minutes and for 10 consecutive 15 minute periods with 50 ml 10% peptone pH 7.0 (540 mosm/kg, Difco Laboratories, Detroit, Michigan, USA). The perfusate entered the stomach *via* the tube positioned in the gastric fundus and was collected *via* the antral catheter in a glass cylinder standing in a boiling water bath. The gastric juice was immediately neutralised by addition of 0.1 N sodium hydroxide. Before neutralisation, the pH of the gastric juice was recorded. Acid secretion per 15 minute sample was determined. Aliquots of venous blood were taken at the end of each collection period. Serum and gastric juice samples were frozen after collection and treatment and stored at -20°C.

In five healthy volunteers and five patients with duodenal ulcer the stomach was perfused in the same way with 0.42 M sodium bicarbonate adjusted to pH 5.5 with 1 N hydrochloric acid (825 mosm/kg) and 20% peptone pH 5.5 (1080 mosm/kg). The recovered perfusates were treated as described above.

To exclude G-cell damage caused by phosphate buffer, the stomach of four control persons and four duodenal ulcer patients was alternately perfused for six consecutive 15 minute periods with 0.6 M sodium bicarbonate adjusted to pH 7.0 with 1 N hydrochloric acid and with 0.15 M sodium phosphate buffer pH 7.0 (flow rate 50 ml/15 min). The perfusates were boiled, neutralised, frozen, and stored at -20°C.

RADIOIMMUNOASSAY OF GASTRIN

To exclude artefacts in the radioimmunoassay systems caused by interaction of unknown factors in the gastric juice with the antigen-antibody binding reaction, serum and gastric juice were assayed for gastrin with two RIA systems (system A and B) using different antisera (system A: 662, system B: TB). Synthetic human gastrin 1-17 (SHG), (ICI Limited, Macclesfield, England), was used as standard and purified ^{125}I -labelled SHG as tracer in both assay systems. For separation of antibody-bound and free-labelled antigen Amberlite CG-4 I (Behringwerke AG, Marburg, Germany) was used in system A, whereas a 10 g% charcoal suspension (Norit A, Sigma Chemical Company, St Louis, USA) was used in system B. In system A (B) the standard diluent consisted of 0.02 M barbitol buffer (pH 8.4) with 0.2% (0.1%) bovine serum albumin (BSA). In both RIA systems 100 μl of serum and gastric juice were assayed for gastrin in a final incubation volume of 2.5 ml using the antisera at a dilution of 1:100 000 (662) and 1:40 000 (TB) with a sensitivity of 0.8 pg/ml (662) and 0.2 pg/ml (TB). The antiserum TB measured gastrin 1-17 and gastrin 1-34 with equimolar potency. In the RIA system using antiserum 662 gastrin 1-34 reacted with a potency about 80% that of gastrin 1-17 on a molar basis. The antisera 662 and TB showed little cross-reaction with fragments of the gastrin molecule. The ratio between ID_{50} —that is, the molar concentration of antigen able to inhibit the antibody binding of the labelled antigen to 50% of the initial value—and ID_{50} for different antigens was: gastrin 11-17 0.173 (662) and 0.016 (TB), gastrin 14-17 0.049 (662) and 0.002 (TB). Gastric juice from which gastrin had been removed did not crossreact with either antiserum.

RECOVERY STUDIES

One hundred picograms SHG were incubated with 1 ml of untreated gastric juice for 10 minutes at different pH values. After incubation, samples were immediately neutralised and boiled. Gastrin concentration was measured, and recovery was determined.

GEL FILTRATION

Two millilitre samples of gastric juice were applied to 16 mm $\times$ 1000 mm Sephadex G50 superfine columns with a constant flow-rate of 4.2 ml/h at 4°C and eluted with 0.02 M barbitol buffer (pH 8.4) containing 2 mg BSA per ml. The columns were calibrated with ^{131}I -albumin, ^{131}I , ^{125}I -SHG, and serum from a patient with the Zollinger-Ellison syndrome. Fractions of 1.3 ml were counted for radioactivity and assayed for gastrin.

STARCH GEL ELECTROPHORESIS

Eighty microlitre samples of boiled and neutralised gastric juice were run on starch gel electrophoresis according to the method of Smithies,⁷ together with SHG added to 80 μl of boiled and neutralised gastric juice from which endogenous gastrin had been removed (by repeated treatment with Amberlite anion exchange resin) and with serum from Zollinger-Ellison patients. After electrophoresis, gel segments were cut from the slab and frozen for at least four hours. After thawing, gastrin was eluted by maceration in 0.02 M barbitol buffer (pH 8.4) containing 0.2% BSA. Gel particles were removed by centrifugation and the supernatant solutions were assayed for gastrin. Bromphenol blue was added to the serum before electrophoresis to stain serum albumin.

COMPETITIVE INHIBITION CURVES

Inhibition of binding of ^{125}I -SHG to anti-SHG antibodies by gastric juice gastrin-like immunoreactivity was compared with the inhibition produced by unlabelled SHG.

STATISTICAL EVALUATION

The statistical significance of the difference of the mean values was evaluated by Wilcoxon's test⁸ for paired comparison, P values less than 0.05 being considered significant.

Results

CONDITIONS FOR DETECTION OF GASTRIN IN GASTRIC JUICE

No gastrin could be detected in untreated gastric juice. In some of the perfusates carried out with

Fig. 1 Recovery of 100 pg synthetic human gastrin 1-17, expressed in per cent, after a 10 minute incubation with 1 ml untreated gastric juice at different pH values.

Fig. 2 Sephadex G50 superfine elution profile of ^{125}I -gastrin 1-17 and immunoreactive gastrin components in neutralised gastric juice and in serum of a patient with the Zollinger-Ellison syndrome.

sodium bicarbonate pH 5.5 and 20% peptone solution pH 5.5 trace amounts of gastrin-like immunoreactivity were found. The lowest pH of the recovered perfusates varied between 3.1 (bicarbonate) and 4.0 (peptone). Immunoreactive gastrin was detected in all samples of gastric juice recovered during perfusion of the stomach with sodium phosphate pH 7 and 10% peptone pH 7. Minimum pH of the recovered perfusates was greater than 6 in all experiments. As regards the release of gastrin into gastric juice per collection period, the perfusions of the stomach with sodium bicarbonate pH 7.0 and with sodium phosphate buffer pH 7.0 produced identical results. Samples of neutralised and boiled gastric juice diluted 1 : 25 in the incubation medium for gastrin assay did not cause a significant reduction in pH. Taking the somewhat different specificity of the two antisera into account, the RIA systems used in this study yielded consistent results.

GASTRIN RECOVERY FROM GASTRIC JUICE

SHG-recovery after a 10 minute incubation with unboiled gastric juice was pH-dependent, showing a steep increase between pH 5 and 6 (Fig. 1).

MOLECULAR FORMS OF GASTRIN IN GASTRIC JUICE

When subjected to gel filtration on Sephadex G50 superfine columns nearly all the immunoreactivity

in gastric juice eluted similarly to labelled gastrin 1-17 (Fig. 2). A small amount corresponded to gastrin 1-34. Starch gel electrophoresis also showed that the predominant amount of immunoreactive material was similar to gastrin 1-17 (Fig. 3); small amounts migrated like gastrin 1-34. The competitive inhibition curve of gastrin-like immunoreactivity in gastric juice was superimposable on that of SHG (Fig. 4).

CONTENT OF GASTRIC JUICE GASTRIN AT REST AND DURING SECRETORY STIMULATION
Perfusion of the stomach with sodium phosphate pH 7 led to an insignificant increase of acid secretion in the control group (Fig. 5) and did not change serum gastrin levels. Gastrin was detected in the

Fig. 3 Distribution of immunoreactive gastrin components on starch gel electrophoresis in boiled and neutralised gastrin free gastric juice to which had been added synthetic human gastrin 1-17 (top), in neutralised and boiled gastric juice (middle), and in serum of a patient with the Zollinger-Ellison syndrome (bottom).

Fig. 4 Ratio (B/F) of antibody-bound (B) to free (F) ^{125}I -labelled synthetic human gastrin 1-17 as a function of concentration of unlabelled synthetic human gastrin 1-17 (●—●). The gastrin concentration in gastric juice (▲) was determined by reference to the standard curve of synthetic human gastrin 1-17. The concentration of each dilution was then calculated from the dilution factors employed. Antiserum: TB, final dilution 1:40 000.

gastric perfusate. Stimulation with 10% peptone solution pH 7 induced a significant ($P < 0.001$) and simultaneous increase of acid secretion, serum gastrin levels, and the amounts of gastrin released into the lumen of the stomach. There occurred a plateau in respect of all parameters for the entire period of perfusion. Duodenal ulcer patients showed results similar to the control group (Fig. 6).

The amounts of gastrin released into gastric juice ranged from 1 to 55 ng SHG equivalent per 15 min sample; usually the concentration did not exceed 300 pg/ml (range 20–700 pg/ml). There was a considerable variation between individuals for gastrin output into the gastric juice in comparable collection periods. During perfusion with sodium phosphate and after the stimulation plateau had been reached, the output of gastrin into gastric juice was rather constant for a given subject.

Discussion

Dissociation of antigen-antibody complexes at low pH and proteolytic damage of labelled antigen and antibody are the source of erroneously high concentrations in the radioimmunoassay of peptide hormones in gastric or duodenal secretions.^{6,9} On the other hand, falsely low or zero values can be due to acid hydrolysis and enzymatic degradation of gastrin before assay. In our experiments incubation damage was avoided by neutralising and boiling the collected samples of gastric juice immediately. As the dilution of gastric juice was 1:25, the pH of the incubation

volume remained unchanged. The investigations of Stadil *et al.*⁵ demonstrated a great loss of gastrin 1-17 immunoreactivity after as little as 30 seconds'

Fig. 5 Time-course of acid secretion, serum gastrin, and gastric juice gastrin output in healthy volunteers ($n=15$) under basal conditions (BAO) and during perfusion of the stomach with 0.15 M sodium phosphate buffer (pH 7) and 10% peptone solution (pH 7). Flow rate 50 ml/15 min. Bars represent mean $\pm$ SE. For acid secretion, serum gastrin and gastric juice gastrin the differences at all time intervals after 75 minutes were statistically significant by Wilcoxon's test ($P < 0.001$).

Fig. 6 Same as Fig. 5 for duodenal ulcer patients ($n=14$). After 75 minutes, differences for acid secretion, serum gastrin, and gastric juice gastrin were statistically significant by Wilcoxon's test ($P<0.001$).

exposure to untreated gastric juice. Our results support the conclusion that this is due to pH-dependent peptic cleavage of gastrin. We found that recovery of gastrin 1-17 from untreated gastric juice is inversely proportional to the pH-dependency of peptic activity as reported by Piper and Fenton,¹⁰ and we were unable to find any gastrin-like immunoreactivity in untreated gastric juice. Perfusion of the

stomach with sodium bicarbonate and peptone solution at pH 5.5 resulted in an increase of intragastric pH which was apparently inadequate to abolish peptic activity entirely, so that trace amounts of gastrin-like immunoreactivity were detected in only a few samples of gastric juice. However, gastric perfusion with sodium phosphate and peptone pH 7 produced adequate alkalinity in the gastric contents to protect gastrin from degradation, and all samples collected this way contained considerable amounts of gastrin-like immunoreactivity. It seems possible that the failure of Stadil *et al.*⁵ and Straus and Yalow⁶ to detect gastrin in gastric juice might have been caused by complete intragastric degradation of the gastric juice gastrin by pepsin and other proteolytic enzymes.

There was no difference between synthetic human gastrin 1-17 and gastric juice immunoreactivity as regards size, charge, and immunoreactivity with the antibodies used. Small amounts of immunoreactivity similar to endogenous human gastrin 1-34 were also detected.

Our data showed that a protein solution is suitable for the stimulation of gastrin output into gastric juice. The simultaneous increase of gastrin output into blood and gastric juice on stimulation of secretion, suggests that gastrin is bidirectionally released from the antral G-cells into the circulation and the lumen of the stomach. The absence of gastrin 1-17 in the gastric juice of antrectomised patients (Hengels, unpublished data) supports this assumption. Anderson¹¹ reported the proof of gastrin in buffer perfusates from the isolated gastric antrum of dogs. This was confirmed by Uvnäs-Wallensten¹² in cats. Our results are in agreement with the experiments of these investigators. The biological significance of gastric juice gastrin in man and its route of secretion are under investigation.

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