Human duodenogastric reflux, retroperistalsis, and MMC

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The aim of this study was to determine to what extent human migrating motor complex (MMC)-related secretory phenomena are influenced by a recently discovered period of duodenal retroperistalsis during late phase III. A constant-flow perfusion technique was used to measure gastric appearance of acid, bicarbonate, pepsin, bilirubin, IgA, and duodenally infused \(^{14}\)C polyethylene glycol (PEG) 4000 in 12 healthy volunteers. Interdigestive gastroduodenal motility was recorded by digital manometry. During late antral phase II and III, the gastric lumen was acidified (P < 0.005 phase III vs. phase I) together with a marked increase in luminal pepsin output (3.1 ± 1.2 during phase III vs. 0.25 ± 0.08 kU/min). This physiological duodenal-antral reflux phenomenon may play an important role in the chemical and immunological restitution of the antral mucosal barrier function after the exposure to high acid and pepsin concentrations during antral phase III activity.

METHODS

Twelve healthy volunteers (7 males, 5 females; mean age 28 yr, range 19–53 yr) participated in the experiments. The study was approved by the Ethical Committee of Sahlgrenska University Hospital, and each subject gave informed consent. The experiments were performed in the morning after an overnight fast. The experimental setup, recording equipment, calibration procedures, and formulas used for calculation of acid and bicarbonate output rates and bilirubin reflux have been described in detail in a recent validation study (5). The technique for motility recording and analysis of retroperistaltic activity has also been described previously (2). The reader is referred to these publications for technical details and further references to methodology-related original publications. A brief description of the methods used follows.

Gastric perfusion technique. The principle behind the gastric technique is gastroduodenal perfusion during approximately constant flow conditions, with continuous measurements of \(\mathrm{pH}\) and \(\mathrm{PCO}_2\) in the mixed gastric effluent. An additional polyethylene catheter (1.4 mm ID) was loosely attached to the tip of the nasogastric tube and subsequently withdrawn 15 cm. This catheter was used for infusion of saline (150 mM). The perfusion rate was set at 31 ml/min and was checked in each separate experiment. The perfusate also contained phenol red (3 mg/l) as a nonabsorbable volume marker. The aspirate was diverted through a specially designed Perspex chamber equipped with one \(\mathrm{pH}\) electrode and one \(\mathrm{PCO}_2\) electrode, thus enabling continuous measurements. The Perspex chamber and the aspiration tube were heated in a water jacket at 37°C, i.e., the same temperature as that of the preheated perfusion fluid. The signal from the electrodes were analyzed every 15 s by a computerized sampling system (LabView, National Instruments) connected to a Macintosh personal computer. After aspiration was passed through the measuring chamber, it was collected in separate flasks, which were changed at 5-min intervals, for measurement of phenol red, \(^{14}\)C polyethylene glycol (PEG), pepsin, bilirubin and IgA concentrations. The flasks were suspended from a Statham force-displacement transducer, and recovered volume (equal...
to weight) as a function of time was continuously registered on a Grass polygraph (Grass Instruments, Quincy, MA). Calculations of acid and bicarbonate outputs were performed according to previously published principles (5, 6).

Measurement of pepsin, bilirubin, and IgA outputs. The pepsin activity assay used was a modified version of the method described by Lee and co-workers (19). The pepsin activity was calibrated against a standard phenol control, and the results were expressed as units per milliliter. The bilirubin concentration in the samples was measured by a technique based on the accelerated diazo reaction (5). The IgA concentration in the aspirates was determined by a sandwich ELISA, using goat anti-human immunoglobulin (Jackson Immuno Research Laboratories) for coating and goat anti-human IgA (Jackson Immuno Research Laboratories) for detection of immunoglobulins (9, 33). This assay does not distinguish between polymeric (secretory) IgA and monomeric (serum derived) IgA.

Output rates were calculated by multiplying concentration by total volume flow, analogous to the calculation of acid and bicarbonate outputs (5, 6).

Occlusion of the pylorus. Duodenogastric reflux was prevented by means of a transpyloric barostatic balloon system (5) in all experiments. Briefly, two latex balloons, 25 mm in length with an interballoon distance of 30 mm, were placed on either side of the pylorus, as repeatedly checked by fluoroscopy. The balloons were filled with air and were connected to a third, larger balloon that was submerged 15 cm below water to generate an isotonic system (see Ref. 5 for technical details).

Motility recordings. Gastroduodenal pressure was registered by open-tip recording (side holes) using a low-compliance pneumohydraulic flow system. In the experiments with a nonoccluded pylorus, an eight-channel catheter (Arndorfer) was positioned, in addition to the gastric perfusion tubes, with its tip in the proximal part of the jejunum. Correct location and preservation of the position of the tube was confirmed by fluoroscopy. The pressure-recording side holes were situated 2, 17, 30, 34, 45.5, 47, and 48.5 cm from the distal end of the tube. Thus three ports were situated 1.5 cm apart in the antral region, three were situated 2, 17, 30, 32, 34, 45.5, 47, and 48.5 cm from the pyloric occlusion, duodenal phase III (i.e., late phase I and early phase II). The duodenogastric reflux was expressed as the percent reflux occurring in the gastric effluent in relation to the amount infused into the duodenum during the same (5 min) time period.

Experimental protocol. In the seven experiments with an open pylorus, gastroduodenal motility was registered together with measurements of PCO₂, pH, IgA, pepsin, bilirubin, phenol red, and [14C]PEG concentration in gastric effluent. In the six experiments with pyloric occlusion, duodenal motility was registered with concomitant assessment of the same components in gastric effluent as mentioned, except for IgA and pepsin. Each experiment was performed without any further experimental intervention and was pursued for 3 h.

Analysis of temporal coordination between motor and secretory events. The duration of the full MMC cycle varies markedly due to differences in the duration of phase II. The intraindividual difference from cycle to cycle is at least as large as the interindividual variability (12). Therefore, to analyze the results against a common absolute time scale, we restricted our calculations to the time period starting 30 min before and ending 30 min after the end of proximal duodenal phase II. In the ensuing presentation, “time 0” denotes the end of proximal duodenal phase II, phase II activity. Phase II was defined as motor activity below the phase III frequency but >2 contractions/10 min. Phase I was defined as a period >3 min with <2 contractions/10 min and preceded by phase II. A commercially available computer program (Polygram, version 5.06 X1, Synectics Medical, Stockholm, Sweden), in addition to manual calculations, was used in the quantitative and qualitative analysis of the MMC.

The direction of migration of phase III-related pressure waves was determined as previously described (1, 2). The analysis of the motility pattern was performed in a singleblind design, i.e., the investigator was unaware of the secretory patterns.

Determination of duodenogastric reflux. As a marker for duodenogastric reflux, a trace amount of [14C]PEG (93 kBq/μL, NEN Products, Du Pont Scandinavia, Stockholm, Sweden) dissolved in saline (150 mM), to which was added 2 g/L of cold PEG (mol wt ~4000; Sigma, St. Louis, MO), was infused continuously at a rate of 0.3 ml/min into the proximal part of the duodenum via one of the side holes of the motility recording tubes. The total radiation dose administered in each experiment thus never exceeded 5 kBq. PEG 4000 is a virtually nonabsorbable compound, and most of this minute radiation dose was consequently excreted via feces. In the experiments with an open pylorus, the PEG solution was infused 6 cm caudal to the pylorus. In the pyloric occlusion experiments, the infusion port was located 3 cm distal to the distal balloon, i.e., at approximately the same level as in the control experiments.

One-milliliter samples of gastric effluent were added to 4 ml of scintillation counter liquid (Pico-Aqua, Packard Instruments, Downers Grove, IL), and the [14C] β-emission was measured in a liquid scintillation counter with automatic photometric quench correction (Packard, Tri-Carb 1900). Reflux was expressed as the percent β-activity occurring in the gastric effluent in relation to the amount infused into the duodenum during the same (5 min) time period.

Statistics. Only periods decided in advance were compared with “baseline” values, which were defined as the mean value during the time period between 10 and 20 min after the end of duodenal phase III (i.e., late phase I and early phase II). The analysis was performed with the nonparametric Wilcoxon signed-rank test, and a P value of <0.05 was regarded as statistically significant. In the figures, data are expressed as means ± SE for the sake of clarity.
RESULTS

Patterns of MMC-related secretion and duodenogastic reflux. Altogether, 12 phase III periods, all but 1 originating in the stomach, were registered during a 3-h experiment in the 7 subjects. Technically successful simultaneous recordings of acid output (µmol/min), pepsinogen secretion (kU/min), IgA output (µg/min), and duodenogastric reflux of bilirubin (nmol/min) and PEG (percentage of infused amount) were obtained during eight phase III periods in six individuals, with four individuals being represented by one period and two subjects with two periods.

The time course of the secretory phenomena before, during, and after phase III is illustrated in Fig. 1. The gastric components (acid secretion and pepsin secretion) had a common pattern, with a significantly increased release during late antral phase II and phase III (Fig. 1, A and B), a swift decrease in late duodenal phase III (Fig. 1C), and stable, low levels after the end of the motor activity front (antral phase I and early phase II; Fig. 1, D and E). Bicarbonate output also exhibited a statistically significant increase in late phase II (Fig. 1, A and B), but a characteristic peak also occurred during early antral phase I (Fig. 1C). The reflux parameters (bilirubin, PEG) differed from this “gastric” pattern in some important respects: if occurring at all, bilirubin reflux started in mid and/or late phase II (Fig. 1, A and E), but always ended before the start of phase I. Occasional bursts of PEG reflux occurred in late phase II (Fig. 1, A and B), but a characteristic peak was invariably seen during late duodenal phase III, i.e., equivalent to early antral phase I (Fig. 1C). The IgA output curve, finally, exhibited characteristics of both the gastric and “reflux” patterns, with one peak during late antral phase II and phase III (Fig. 1, A and B) and another short-lasting peak during early antral phase I (Fig. 1C).

Effects of pyloric occlusion. During pyloric occlusion, nine duodenal phase III periods, fulfilling defined criteria and visually ordinary, were registered in six subjects. The MMC-related acid secretion pattern in the presence of the balloon system was similar to that seen with an open pylorus, but there was a nonsignificant tendency to generally lower acid secretion rates in the pyloric occlusion experiments (Fig. 2).

During three of these nine recording periods, substantial reflux of PEG occurred (11/12 5-min periods in 2 cases and 10/12 5-min periods in 1 case), i.e., complete pyloric occlusion was not achieved. Only the six periods (6 different individuals) with complete occlusion were included in the analysis. In five of these six subjects, complete occlusion was maintained during the entire experiment. In one subject, however, an episode of PEG reflux occurred during early antral phase I. All included fractions, including those corresponding to the PEG reflux period, were devoid of bilirubin. Bicarbonate output and PEG reflux rates before, during, and after phase III are summarized in Fig. 3. With complete...
pyloric occlusion, bicarbonate output remained relatively stable at a mean rate of 6.6 ± 2.3 µmol/min (range 4.7–10.5 µmol/min) before, during, and after phase III.

Phase III-related retrograde peristalsis and its effects on duodenogastric reflux and secretory patterns. In the experiments with a nonoccluded pylorus, retrograde peristalsis during the later one-half of duodenal phase III, i.e., after the end of the antral activity front, occurred in 10 of 12 phase III periods recorded in the seven subjects. In nine of these ten phase III periods, all waves were retrograde, whereas in the remaining subject, ~80% of the waves were retrograde. One subject exhibited one phase III with and one phase III without retroperistalsis.

MMC-related bicarbonate output curves before, during, and after activity fronts with or without retroperistalsis are shown in Fig. 4. With retroperistalsis, the bicarbonate output curve was biphasic, the first peak coinciding with the onset of duodenal phase III (−7.7 ± 1.1 min before the end of duodenal phase III; Fig. 4A) and a second peak occurring immediately after (+1.7 ± 0.5 min; Fig. 4A) the end of duodenal phase III, i.e., in early antral phase I. Both peaks were statistically significantly different from values during duodenal phase I and early phase II (10–20 min after end of duodenal phase III activity, *P < 0.05*). The second (postantral phase III) bicarbonate output peak was absent in the two subjects lacking retroperistalsis during late phase III (Fig. 4B). During the period of retroperistalsis, there was also a significant increase in PEG reflux (*P < 0.05*), a phenomenon that was not seen in subjects lacking retroperistalsis (Fig. 5).

MMC-related output of pepsin and IgA. Pepsin secretion closely followed the MMC-related acid secretion curve, reaching maximal values in association with antral phase III (Fig. 6). The mean output rate during the last 20 min preceding the end of antral phase III

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**Fig. 2.** Mean acid secretion during 15-min periods preceding and following end of duodenal phase III activity during pyloric occlusion (hatched bars, n = 8) and with an open pylorus (filled bars, n = 9). Values are means ± SE. MMC, migrating motor complex. ***Significant difference (*P < 0.005*) between phase III and phase I. Pyloric occlusion did not significantly affect acid secretion rate during either period.

**Fig. 3.** MMC-related duodenogastric reflux of PEG (A) and gastric bicarbonate output (B) in 5 subjects during efficient pyloric occlusion. Time 0, indicated by a dashed line, denotes end of duodenal phase III activity. PEG reflux is expressed as percent of total amount of duodenally infused [14C]PEG 4000, and bicarbonate output is expressed in µmol/min. □, PEG reflux; ○, bicarbonate output. Values are means ± SE.

**Fig. 4.** MMC-related bicarbonate output in presence (A; n = 6, means ± SE) and absence (B; n = 2, means) of phase III-related retrograde peristalsis.
was 3.1 ± 1.2 kU/5 min, compared with 0.25 ± 0.08 kU/5 min during phase I (P < 0.01). There was no significant postantral phase III increase in pepsin secretion.

The MMC-related IgA output curve is shown in Fig. 7A. The curve was biphasic, with a period of increased output during late antral phase II and phase III and a distinct but short-lasting peak during early antral phase I (in both cases, P < 0.05 vs. phase I values). The second peak was only seen in subjects exhibiting retroperistalsis (Fig. 7B).

**DISCUSSION**

The results of this study illustrate that the antrum, pylorus, and proximal duodenum behave like an integrated, functional, secretomotor unit rather than as segregate regions. Since Frederick Hoelzel in 1925 observed an increase in gastric acid output coinciding with “hunger contractions” (11), a number of “secretory components” of the MMC have been described: duodenal release of bile and pancreatic juices (8), increased gastric acid secretion (35), increased secretion of bicarbonate in both the stomach (16) and the proximal duodenum (17), and increased intestinal chloride secretion (10, 25). The integrative physiology of this complex system is incompletely understood. The rhythm itself seems to be generated by cholinergic neurons of the enteric nervous system (28), but the coordination of the different secretory phenomena depends on intact extrinsic innervation (for additional references, see Refs. 6 and 26). Phase II and phase III activity is accompanied by release of motilin from endocrine cells in the duodenal mucosa, but it is not altogether clear if motilin release actually generates the cycles (20, 27). In the small intestine, the motor component of the program is generated by the enteric nervous system (28), and the stimulation of chloride secretion at the end of the cycle is probably due to activation of a motility-triggered noncholinergic reflex that is relayed in the submucosal plexus (22, 31).

Considerably less is known about the gastric components of the system, possibly due to the fact that “basal” acid secretion in the most common animal model, the awake dog, is low and highly variable without a consistent and clear-cut MMC rhythm (4, 21). With regard to bicarbonate secretion, the study of the integration between motility and this secretory parameter has been hampered by lack of appropriate methodology.
The major advantage with our system is its high time resolution. However, at an acid pH, absolute levels of gastric bicarbonate secretion are underestimated by as much as 50% (5). On the other hand, recovery rate of exogenously administered bicarbonate is constant and concentration independent within each individual. The problem of subtotal recovery of bicarbonate is less important in the present context, because the conclusions are based on release patterns rather than absolute steady-state levels.

In the present study, we confirmed that acid secretion increases during late antral phase II and phase III. In a previous series of similar experiments (6), we obtained an 8.1 ± 0.8-fold difference between basal and peak acid secretion levels. The seemingly smaller difference in the present study is due to a different mode of expression of the secretory data, the cycles being not exactly "in phase." The acid secretion cycle remained grossly unaffected during pyloric occlusion with an isotonic balloon system. The absolute acid secretion rate was, however, slightly lower in the pyloric occlusion group, but the difference was not statistically significant. It cannot be excluded that the antral balloon to some extent activated inhibitory antral reflexes (29). The magnitude of this effect was not sufficiently great to extinguish the MMC rhythm.

A 12-fold increase in pepsin release also occurred during late antral phase II and phase III. Our perfusion system artificially generates a "common cavity" situation in the stomach and also dilutes the normally occurring concentration changes. In the absence of perfusion, some of the acid and pepsin secreted by the oxyntic mucosa during phase I and early phase II will probably remain in the "fundic pool" until the start of propulsive phase II motility. When this fluid is transported distally by antral phase III activity, the antrum may therefore be exposed to high concentrations of both acid and pepsin.

Another phenomenon with a potential damaging effect on the antral mucosa is bile. In the present study, we never saw any bile reflux during this particular period, reproducing previous results (6). These findings in humans contrast with those obtained in dogs, in which bile reflux seems to occur during the activity front (3). In our study, bile reflux, if present at all, occurred irregularly, often starting approximately in the middle of the cycle. In both dogs and humans, the gall bladder contracts in late phase II, and no release occurs after the activity front (14, 18, 24, 30, 32). Efficient antegrade peristalsis occurs in late phase II and early phase III (2, 15), and it therefore seems likely that most of the bile present in the lumen will be transported in the distal direction before the start of retroperistalsis in late phase III. Because retroperistalsis only occurs in the duodenum (2), lack of remaining bile in this segment of intestine may account for the finding that no bile reflux occurred during this particular period. This explanation is supported by the finding that PEG, which was continuously infused, did reflux during the postantral phase III period. The reason for the different composition of the refluxed material in dogs and humans is unknown. One possibility is that the longer distance between the papilla and the antrum in humans makes antegrade peristalsis more efficient, leading to virtually complete emptying of the duodenum before the start of retrograde peristalsis.

The third secretory phenomenon, bicarbonate output, was biphasic, with one fairly variable peak during late antral phase II and early phase III and one very distinct peak that always occurred after the end of antral phase III and during the second one-half of duodenal phase III, reproducing previous findings (6, 7). Both these peaks were abolished by pyloric occlusion that was confirmed by lack of PEG reflux. Consequently, we have no evidence that gastric bicarbonate secretion participates in the MMC rhythm, a finding that is at variance with one previous study in which bicarbonate secretion was measured at an alkaline pH by back titration during occlusion of the pylorus with a nonisotonic balloon (16). The reason for the discordant findings is unknown.

Analysis of the retroperistaltic patterns during phase III provided further evidence for a reflux mechanism.
behind the postantral phase III bicarbonate peak. The bicarbonate peak was accompanied by PEG reflux, and both phenomena were exclusively seen in subjects exhibiting retroperistaltic activity during phase III. Furthermore, in one subject with one phase III with and one phase III without retroperistalsis, the first but not the second phase III period was followed by a bicarbonate peak.

The most likely source of the refluxing bicarbonate seems to be the duodenal bulb. In the canine duodenum, duodenal bicarbonate secretion is stimulated in association with the activity front (17). Retrograde propulsion of secreted bicarbonate therefore seems to be a possible explanation for the postantral phase III peak. In humans, basal duodenal bicarbonate secretion rate is ~5 µmol·cm⁻¹·min⁻¹ and increases ~10-fold during acid stimulation (13). The duration of the period of retroperistaltic activity is <5 min (2). If one assumes a 5-cm functional production segment and if all secreted bicarbonate regurgitates, one would expect a total refluxed amount of ~125 µmol. The approximate area under the postantral phase III peak is 50 ± 8 µmol of bicarbonate (range 10–113 µmol, n = 16; data from Ref. 6), values that are clearly compatible with the reflux hypothesis. These numerical considerations further support that the postantral phase III bicarbonate peak is indeed due to retroperistalsis-driven duodenogastric reflux.

The last studied secretory component, IgA, also exhibited a biphasic pattern: one initial increase during late phase II/early phase III resembling the gastric release pattern, and one peak during antral phase I matching the reflux pattern. In previous reports, we described a gastric component of MMC-related IgA output (9), a finding that was reproduced in the present study. The total duodenal mucosal IgA production is ~200 µg/min and increases by ~50% during late phase II (23). The area under the second component of the IgA output curve in the present study was in the 300- to 500-µg range, corresponding to ~1–2 min of basal duodenal IgA production, which again is compatible with a reflux phenomenon during the period of retroperistaltic activity. The fact that this peak was abolished in two subjects lacking retrograde peristalsis further supports this hypothesis (Fig. 7). Very little is known about the mode of control of gastric IgA release (9). It is tempting to speculate that retroperistalsis sweeps IgA produced by the duodenal mucosa in the retrograde direction and thereby indirectly contributes to antral immunological defense. The importance of retroperistalsis for antral immune function needs to be validated by further experiments.

In conclusion, the antrum, pylorus, and proximal duodenum constitute an integrated, functional secretomotor unit in the interdigestive state. Our results suggest that retroperistalsis-generated reflux of bicarbonate and IgA at the start of antral phase I may contribute to the restoration of antral mucosal pH and immunological barrier function after passage of the activity front.

Perspectives

The current findings further support the concept that the MMC acts like an interdigestive “gastrointestinal housekeeper,” integrating mechanical (high phase III motor activity), physical (water secretion and “bile detergent”; see Ref. 6), biological (pepsin), chemical (acid), and immunological (IgA) components into an effective “rinsing program.” Several of these abrasive forces are, however, also potential mucosal aggressors and need a careful regulation to prevent damage of the mucosal lining. Furthermore, restoration of the mucosa after the period of “abrasion” may well be equally important. Any disturbance in any part of this secretomotor program may lead to, directly or indirectly, mucosal injury. An increased knowledge and understanding of these phenomena could add important information about the underlying pathophysiological processes behind mucosal diseases in both the stomach and the duodenum, including those associated with infection of Helicobacter pylori. Integrative physiology is mandatory in further studies of these secretomotor phenomena as they encompass motility-related as well as secretory and immunological parameters.

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