Antral release of gastrin and somatostatin in duodenal ulcer and control subjects

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SUMMARY Organ culture was used to compare gastrin and somatostatin release from cultured antral mucosa obtained from duodenal ulcer and non-ulcer (control) subjects. In response to dibutyryl cyclic AMP (DBCAMP) cultured antral mucosal explants from patients with a history of duodenal ulcer released a greater proportion of antral gastrin into the medium than did antral mucosal explants from non-ulcer subjects. Somatostatin release from antral mucosa from duodenal ulcer patients was substantially less than somatostatin released by antral explants from non-ulcer subjects. In the non-ulcer subjects there was a direct positive correlation between the amounts of antral somatostatin and gastrin released into the culture medium (r=0.64, <P0.01). In the duodenal ulcer patients, however, there was no correlation between gastrin release and somatostatin release from antral mucosa (r=0.09; P>0.2). Results of these studies identify enhanced gastrin release in response to stimulation and decreased release of somatostatin from antral mucosa of duodenal ulcer patients. These alterations in paracrine relationships of antral somatostatin and gastrin in duodenal ulcer subjects may contribute, at least in part, to the pathogenesis of duodenal ulcer disease.

The pathogenesis of duodenal ulcer disease is complex and is believed to involve interactions of multiple factors.1-4 In vivo human studies designed to define differences between duodenal ulcer patients and non-ulcer subjects have dealt principally with comparisons of gastric acid secretory rates,5-7 clearance of acid from the stomach8 and duodenum9 and acid mediated feedback inhibition of antral gastrin release.10 Parietal cell function has been examined to determine acid secretory capacity and responsiveness to exogenously administered stimuli11 and to endogenously released gastrin.12 Somatostatin has been shown to possess the capacity to inhibit both gastrin release and gastric acid secretion. Somatostatin is present in large concentrations in antral mucosa in cells intimately proximate to gastrin-containing and -releasing cells and is also released into the circulation. It is as yet uncertain to what extent gastrin release and gastric acid secretion are regulated by local (paracrine) and/or systemic effects of somatostatin.13 Recent studies have suggested that alterations in local actions (paracrine functions) of somatostatin and gastrin in the antral mucosa may, at least in part, contribute to enhanced gastrin release and increases in gastric acid secretion in duodenal ulcer.14-15

The present study was directed to examine and compare potential paracrine relationships between gastrin and somatostatin in antral mucosa of duodenal ulcer and non-ulcer subjects. In vitro organ culture16-18 of antral mucosa was utilised as an experimental model for the study of similarities and differences between duodenal ulcer and non-ulcer subjects in antral gastrin and somatostatin release during short term culture.

Methods

SUBJECTS
The volunteer subjects were nine men with chronic duodenal ulcer disease and nine healthy men without history of peptic ulcer disease. The mean age of the duodenal ulcer subjects was 51 years (range 23-64 years) and the mean age of control individuals was 39 years (range 28-61 years). Individuals with
chronic duodenal ulcer disease had the diagnosis established endoscopically during previous evaluation of recurrent symptomatic disease. At the time of study no patient with chronic duodenal ulcer disease had endoscopic evidence of active duodenal or gastric ulcer. None of the study subjects had serious accompanying medical illnesses, previous gastric surgery, or evidence of gastric outlet obstruction. Fasting serum gastrin concentrations were normal (<150 pg/ml) in all ulcer and non-ulcer subjects. All oral medications were discontinued three days before oesophagogastroduodenoscopy.

Complete oesophagogastroduodenoscopy examination was done in all subjects before obtaining antral tissue. Individuals with endoscopic evidence of active duodenal or gastric ulcer, duodenitis or gastritis were excluded from the study. All subjects volunteered for the study and gave written informed consent. The study was approved by The Human Research Committee of the University of Florida College of Medicine.

**Endoscopic Procurement of Antral Mucosal Explants**

After overnight fast, subjects underwent oesophagogastroduodenoscopy with an ACM1 F-8 end-viewing panendoscope. The posterior pharynx was anaesthetized with topical 4% lidocaine. When required to achieve mild sedation, diazepam was administered intravenously before and during the procedure. Atropine or other anticholinergic medications were not administered. Oral secretions were controlled during the procedure by oropharyngeal suction. Antral mucosal biopsies for use as explants were obtained by use of standard Olympus endoscopic biopsy forceps without central tissue spike. Sixteen to 30 consecutive antral biopsies were obtained from each subject and were transferred immediately to the organ culture system. The majority of antral mucosal specimens were obtained from the immediate prepyloric area along the greater and lesser curvatures. Both duodenal ulcer and non-ulcer subjects tolerated the procedure well, without significant symptoms and/or complications.

**Organ Culture Techniques and Analytical Procedures**

A total of 410 antral mucosal biopsies were obtained and cultured as individual antral explants using the organ culture technique previously described. Antral mucosal explants were cultured in Trowell-T8 culture medium (Grand Island Biological Co, Grand Island, NY) to which were added penicillin (100 U/ml) and streptomycin (100 μg/ml). Explants were incubated for an initial hour of stabilisation after which the culture medium was removed and exchanged with fresh Trowell-T8 culture medium. Culture of the antral explants was continued for an additional six hours after the culture medium exchange. In vitro gastrin release by antral mucosa from duodenal ulcer and control subjects was determined under basal culture conditions and in response to dibutyryl cyclic AMP (DBCAMP; 10 mM) (Sigma Chemical Co, St Louis, MO). The culture medium was sampled (10 μl) at 0.5, 2, 4, and six hours for determination of gastrin content. Somatostatin content of the medium was measured at the termination of the six hour culture period. Recovery from the media of added gastrin and somatostatin after six hour organ culture was 95.2±1.4 and 90.2±2.1%, respectively. At completion of six hours of culture the explants were weighed promptly, frozen on dry ice and were then maintained at −20°C, until peptide hormone extraction. 18 19 Residual tissue sediments were preserved for protein determination. Media and antral tissue gastrin and somatostatin contents were determined by radioimmunoassay.19-21 Radioimmunoassay of gastrin and somatostatin showed intraassay coefficients of variation of 5 and 6% and interassay coefficients of variation of 10 and 12%, respectively. Gastrin and somatostatin release were expressed as nanogram peptide per milligram tissue or per cent of antral explant peptide released into the culture medium. Values were represented as the mean±1 standard error of the mean. Statistical significance was assigned when p<0.05. Data were analysed by paired and unpaired Student’s t test and linear regression analysis.

**Results**

**Gastrin Content of and Release from Cultured Antral Explants**

Gastrin contents of antral mucosal specimens from individual subjects and from different subjects in both groups varied considerably: range of antral mucosal gastrin content 0.1–29.7 ng/mg explant (Table 1). The mean gastrin content of antral mucosa from duodenal ulcer patients was 2.2±0.4 and that for the non-ulcer subjects was 6.7±1.9 ng gastrin/mg explant (p<0.05). Explant gastrin contents, with basal culture medium alone or in the presence of DBCAMP, did not change during the duration of culture (p>0.1).

Despite variability in antral tissue gastrin contents the per cent of antral gastrin released into the medium under basal (control) culture conditions was similar for both non-ulcer and duodenal ulcer subjects: 6.9±1.0% and 5.8±1.2% total gastrin per hour, respectively (p>0.1). Dibutyryl cyclic AMP (DBCAMP) stimulated gastrin release from antral
Table 1. Gastrin contents of cultured antral explants

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Non-ulcer subjects tissue gastrin content (ng/mg explant)</th>
<th>Duodenal ulcer patients tissue gastrin content (ng/mg explant)</th>
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<tbody>
<tr>
<td>n*</td>
<td>Mean±SEM</td>
<td>Range</td>
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<tr>
<td>1</td>
<td>15</td>
<td>9.6±2.4</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>2.4±0.7</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.2±0.3</td>
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<tr>
<td>5</td>
<td>7</td>
<td>1.3±0.5</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>1.7±1.9</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>4.8±1.9</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>9.7±1.9</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td></td>
<td>6.7±1.9</td>
</tr>
</tbody>
</table>

*n equals the number of individual antral explants cultured.

Table 2. Antral mucosal gastrin release in the presence or absence of dibutyryl cyclic AMP (DBCAMP)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Non-ulcer subjects and antral gastrin released</th>
<th>Duodenal ulcer patients and antral gastrin released</th>
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<tr>
<td></td>
<td>Basal</td>
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<tr>
<td>1</td>
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</tr>
<tr>
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<tr>
<td>Mean±SEM</td>
<td></td>
<td>41.9±5.9</td>
</tr>
</tbody>
</table>

*p was calculated by paired t-test to estimate significance of difference in per cent gastrin release during six hours of culture in the presence or absence (basal) of DBCAMP.

Antral mucosal explants from both duodenal ulcer and non-ulcer subjects (Table 2). The cumulative proportion of gastrin release stimulated by DBCAMP during the six hour culture was substantially greater in the duodenal ulcer patients than control; gastrin release stimulated by DBCAMP from antral mucosa from duodenal ulcer patients was 2-5 times greater than DBCAMP stimulated gastrin release from antral mucosa of non-duodenal ulcer subjects: 16.8±3.3 vs 6.5±1.3% change from basal (p<0.01), respectively (Fig. 1).

ANTRAL SOMATOSTATIN CONTENT

Antral mucosal explants from duodenal ulcer patients contained less somatostatin (129.8±53.4 pg/mg explant) than antral mucosa from the non-ulcer subjects (260.5±56.1 pg/mg explant) (p<0.03). The amounts of antral somatostatin relative to antral gastrin, however, were similar in antral mucosal explants from duodenal ulcer and non-ulcer patients.

Fig. 1 Dibutyryl cyclic AMP stimulated gastrin release from antral mucosal explants from non-ulcer subjects (n=9) and from duodenal ulcer patients (n=9) is expressed as percent change from basal (non-stimulated) gastrin release. *p<0.01.
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subjects (5.9 and 3.9%, respectively). Culture of antral mucosa in the presence of DBCAMP did not alter the mean tissue somatostatin content of antral mucosal explants from the duodenal ulcer patients (123.9±42.3 pg/mg explant, p>0.1) nor from the non-ulcer subjects (242.2±52.7 pg/mg explant, p>0.1).

Somatostatin release

Figure 2 compares somatostatin release from antral mucosal explants from duodenal ulcer and non-ulcer subjects. Antral mucosal explants from duodenal ulcer patients released substantially less somatostatin than antral mucosal explants from non-ulcer subjects: antral mucosa from duodenal ulcer patients released 11.5±5.8% compared with 34.9±5.9% explant somatostatin for the non-ulcer subjects (p<0.02). Addition of DBCAMP to cultures of antral tissue from non-ulcer subjects was associated with substantial increases in somatostatin release into the culture media in five of nine experiments. Analysis of the combined data by paired t test, however, did not show statistically significant stimulation by DBCAMP of somatostatin release from antral mucosal cultures of non-ulcer subjects. In contrast with these findings, DBCAMP did not affect somatostatin release from antral mucosal explants of any of the nine duodenal ulcer patients.

In the non-ulcer subjects there was a direct (r=0.64) relationship (p<0.01) between the release of antral explant somatostatin and gastrin into the culture medium under basal and stimulated (DBCAMP) conditions (Fig. 3). In contrast, however, with cultures of antral explants of duodenal ulcer patients there was no correlation between antral somatostatin release and antral gastrin release into the culture medium (r=0.09; p>0.1).

Discussion

Most investigators have found that many, but not all, patients with duodenal ulcer release more gastrin into the circulation in response to a standard protein meal than do non-ulcer subjects. Enhanced gastrin release in patients with duodenal ulcer has not been accounted for by increased amounts of gastrin in the antral mucosa and/or greater numbers of antral gastrin cells. Studies examining the gastrin content of antral mucosa of duodenal ulcer patients have yielded variable results, being reported as low, normal or high in comparison with non-ulcer individuals.

In the present study, in order to minimise error because of variations in gastrin distribution in the antral mucosa, large numbers of antral mucosal...
samples (16 to 30 were obtained from each subject and each antral mucosal explant was examined individually. Gastrin release in these studies was expressed as a function of antral gastrin content.

It has been proposed that increased gastrin responsiveness in duodenal ulcer patients may be due, at least in part, to enhanced functional activity of gastrin cells.\textsuperscript{30} \textsuperscript{31} This study represents the first \textit{in vitro} comparison of functional cellular responsiveness and potential paracrine interrelationships between antral mucosal gastrin and somatostatin of duodenal ulcer and non-ulcer subjects. Previous studies from our laboratory, using rat antral mucosa in organ culture, demonstrated the capacity of cyclic AMP to stimulate gastrin release and provided evidence in support of a role for endogenous cyclic AMP in stimulating release of antral gastrin.\textsuperscript{32} In the present study cultured antral mucosal explants from duodenal ulcer subjects were found to release a larger proportion of antral gastrin into the medium in response to DBCAMP stimulation than did antral mucosal explants from non-ulcer subjects. It is not possible to compare directly these \textit{in vitro} observations to those results of \textit{in vivo} human investigations. Nonetheless, these studies define demonstrable differences in gastrin cell responsiveness to stimulation \textit{in vitro} which may reflect fundamental alterations in gastrin cell responsiveness and/or disturbances in antral paracrine influences on gastrin cells of duodenal ulcer patients.

Somatostatin has been proposed as a locally acting paracrine regulator (inhibitor) of antral mucosal gastrin release.\textsuperscript{33} \textsuperscript{35} In the present study, somatostatin release from antral mucosa from duodenal ulcer subjects was substantially reduced. Antral mucosa from the duodenal ulcer patients released less than one-third the amount of somatostatin released by antral explants from non-ulcer subjects. In the non-stimulated (basal) state, however, this reduced level of somatostatin release did not affect basal gastrin release from antral mucosa from duodenal ulcer subjects. These findings are contrasted with the significant differences observed in DBCAMP stimulated gastrin release from antral mucosa from duodenal ulcer subjects when compared with control subjects.

These studies support the hypothesis that inhibitory paracrine influences on antral gastrin cells by somatostatin may be disordered in duodenal ulcer, thereby reducing or removing normal somatostatin mediated inhibition of gastrin release. In the non-ulcer subjects we found a direct correlation between antral release of somatostatin and gastrin in the culture medium, whereas this correlation was not found with culture of antral mucosa from duodenal ulcer patients (Fig. 3). The direct relationship between antral gastrin release and somatostatin release by antral mucosa in the non-ulcer subjects may represent one limb of a feedback control mechanism, in which release of gastrin may be modulated by released somatostatin through its paracrine action on the G cell, effecting inhibition of gastrin release.\textsuperscript{36} In normal individuals antral somatostatin containing (D) cells positioned in intimate proximity to gastrin containing and releasing (G) cells may respond to alterations in local environmental concentrations of gastrin. Occupancy of gastrin receptors on the surfaces of somatostatin cells would result in stimulation of somatostatin synthesis and release. Reduced occupancy of these receptor sites by gastrin would reduce somatostatin synthesis and release. In this manner, somatostatin is proposed to serve a local modulating role (suppression) in regulation of further gastrin release. The direct correlation between antral gastrin and somatostatin release observed in the non-ulcer subjects is consistent with this proposed mechanism for somatostatin-gastrin regulation. The lack of a direct relationship between antral mucosal gastrin and somatostatin release, associated with decreased antral mucosal somatostatin release, in the duodenal ulcer patients suggests a defect in this regulatory mechanism. The nature of this defect is as yet not defined. Studies have shown that the number and ratio of antral G and D cells in duodenal ulcer subjects are the same as in control individuals.\textsuperscript{37} Functional differences in D cells of duodenal ulcer subjects may be reflected by a decrease in the number or affinity of gastric receptors on antral mucosal somatostatin cells, a defect in the transmission of gastrin, or access of gastrin, to those receptors, or a defect in the postgastrin receptor responsible for promoting somatostatin synthesis and release. A defect in this proposed paracrine somatostatin-gastrin control mechanism in duodenal ulcer would be expressed by an absolute reduction in somatostatin release, increased gastrin in response to stimulation, and a dissociation between gastrin release and somatostatin release, as identified in this study.

Results of these experiments have shown decreased release of somatostatin as well as increased release of gastrin by antral mucosa of duodenal ulcer subjects; these were associated with an absence of the direct relationship between antral somatostatin and gastrin release found in non-ulcer subjects. These results suggest alterations in paracrine relationships between antral mucosal somatostatin and gastrin in patients with duodenal ulcer, which may contribute to abnormalities in regulatory peptide release in duodenal ulcer.
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