Divergent Effects of Bombesin and Bethanechol on Stimulated Gastric Secretion in Duodenal Ulcer and in Normal Men

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To further investigate differences in the responses of normals and patients with duodenal ulcer with respect to gastrin release and acid and pepsin secretion, we infused bombesin (1 μg/kg·h) or bethanechol (40 μg/kg·h) during the middle hour of a 3-h infusion of pentagastrin and compared the results with a pentagastrin injection without added drug. Pentagastrin dosage (0.1 μg/kg·h) was set to give about half-maximal response, to detect either inhibition or further stimulation of gastric secretion, whereas the dose of bombesin was chosen to give maximal gastrin but less than maximal acid secretion. Serum gastrin and somatostatin were also measured. In all subjects tested, bethanechol produced no effects on acid, gastrin, or somatostatin release but increased pepsin output. By contrast, bombesin inhibited pentagastrin-stimulated acid output in all 6 normal men by an average of 55%, whereas it inhibited acid output in only 2 of the 9 men with duodenal ulcer. Serum gastrin increases after bombesin in duodenal ulcer were three to four times greater than in normals. Although bombesin stimulates acid only by releasing gastrin, we postulate that bombesin may also simultaneously limit acid and pepsin secretion and speculate that this effect could be mediated by bombesin-induced somatostatin release. The causes for differences between duodenal ulcer and normals remain speculative.

Vagal stimulation of gastric secretion, acting through postganglionic pathways of the gastric enteric nervous system, involves several diverse elements including acetylcholine, gastrin-releasing (bombesin-like) peptide, gastrin, and, probably, somatostatin (1-5). While there is muscarinic stimulation of parietal and peptic cells as well as gastrin release (6), there is also a vagal nicotinic (atropine resistant) pathway that releases a bombesin-like peptide present in postganglionic gastric nerves of many species, including humans (7). Bombesin, in turn, also releases gastrin into the circulation. The action of gastrin is entirely hormonal, i.e., confined to circulating gastrin levels (8-11).

In various models, both stimulation and inhibition of gastric secretion, as well as gastrin release, have been demonstrated for the vagus (5,6) as well as for acetylcholine (1,2,6), histamine (8), gastrin (9), and bombesin (2,8,10). In the isolated rat stomach (1,2,12), it has been shown that bombesin and somatostatin are crucial and reciprocal intermediates of the stimulation/inhibition balance of cholinergic or nervous actions on the stomach.

The strategic location of somatostatin-secreting D cells in close proximity to both G cells and fundic secretory cells (3,4) suggests that somatostatin acts locally (paracrine effect) to modulate gastrin release and also acid and pepsin secretion.

To attempt to define potential inhibitory mechanisms in humans, we tested the effects of both bombesin and bethanechol, a cholinergic agonist, given during a submaximal infusion of pentagastrin. We studied men, both with and without duodenal...
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June ulcer (DU), and measured acid and pepsin secretion as well as circulating levels of gastrin and somatostatin.

Materials and Methods

Studies were performed in 9 adult male patients with endoscopically proven uncomplicated and currently inactive DU (age 39 ± 4.3 yr, weight 70 ± 4.6 kg; mean ± SE) and 6 healthy male controls (age 33 ± 3.8 yr, weight 81 ± 6.0 kg; mean ± SE) without ulcer history. Each participant gave informed written consent for the studies, which were approved by the Institutional Review Board of the University of Alabama.

All antisecretory drugs were withdrawn for at least 48 h. In each test, after a 10-h fast, an Argyle sump nasogastric tube (Nashville Surgical Supply, Anniston, Ala.) was positioned fluoroscopically in the most dependent part of the stomach, fasting residual contents were removed, and secretion was collected in 10-min consecutive periods for 4 h at constant 90–120 mmHg suction. Saliva was aspirated by continuous suction. The aspirated gastric sample volume was measured to the nearest 1 ml, and 2-ml aliquots were titrated with 40 mM NaOH to pH 7.0 using a Radiometer autoburette (Radiometer American, Westlake, Ohio). Pepsin concentration was measured in each sample by an automated technique (13) using hemoglobin substrate (Eastman Kodak Co., Rochester, N.Y.) at pH 2.0 with reference to crystalline pepsinogen (Sigma Chemical Co., St. Louis, Mo.) as the standard.

Blood was drawn at 5–30-min intervals. The serum was separated and stored at −20°C for later measurement of (a) serum gastrin concentration by radioimmunoassay (14), using antibody 1296 donated by Dr. John Walsh, University of California, Los Angeles, Calif., and (b) serum somatostatin concentration by radioimmunoassay, performed by Dr. Michael Berelowitz, University of Cincinnati, Cincinnati, Ohio. Results are given in picograms per milliliter. For the somatostatin assay, serum was treated with aprotinin and heparin and shipped frozen on dry ice in batches. (One batch of sera was accidentally thawed and rendered invalid.) The somatostatin assay, which measures both free and total somatostatin, uses a rabbit serum antibody at a dilution of 1:15,000 and 125-I labeled tyrosine-l-somatostatin. Interassay and intraassay variations are 5% (15).

Each subject underwent three studies on separate days in random order. In all studies, after a basal period of 40 min, 0.1 μg/kg · h pentagastrin was given by constant intravenous infusion for a period of 200 min. This dosage was therefore selected as likely to demonstrate an action of the putative inhibitor. Bombesin was made up fresh in saline from sterile freeze-dried stock for each study. Both the bombesin and pentagastrin were infused intravenously, using a Harvard syringe pump (Harvard Apparatus, Millis, Mass.) for each, through a single intravenous catheter with a connector close to the needle. None of the subjects were nauseated or had vomited, no subjects experienced abdominal cramps during the bombesin infusion, and, as previously found (9), heart rate and blood pressure were not altered. Acid and pepsin outputs were calculated as the product of concentration and volume per unit time.

In all 6 normal controls and 5 of the 9 DU subjects, another study was undertaken using the same protocol, but bombesin was replaced by a 1-h infusion of 40 μg/kg · h bethanechol chloride (Merck, Sharpe & Dohme, Philadelphia, Pa.).

For the same subject and the same time period, the output in the pentagastrin control infusion was compared with the experiments in which bombesin or bethanechol were additionally given. Significance was determined by the paired Student’s t-test. The results are presented in 20-min outputs, representing the sum of two consecutively measured 10-min samples, and, where appropriate, for longer periods (e.g., for the full hour of test drug infusion). A probability value <0.05 was considered significant.

Results

Reproducibility

Both acid and pepsin outputs in the first hour of the pentagastrin infusion were reproducible in the three different studies—pentagastrin, pentagastrin + bombesin, and pentagastrin + bethanechol (Figures 1 and 3).

Bombesin

Gastric secretion. NORMALS. In all 6 normal subjects, acid output was strikingly and progressively reduced during the 1-h bombesin infusion to a mean value of 45% of the control infusion in the last third of the hour (p < 0.01, Figure 1, Tables 1 and 2). Secretion recovered to control pentagastrin values within 20 min after the bombesin infusion was discontinued. Pepsin output was also significantly (p < 0.01) depressed during the bombesin infusion (Tables 1 and 2), recovering rapidly to control levels after bombesin was stopped (Figure 1).

DUODENAL ULCER. Basal and pentagastrin-stimulated acid and pepsin output in the DU group were almost twice as high as in the normal men. In sharp contrast to the inhibition in all the normal controls, stimulated acid secretion was inhibited in
Figure 1. Top. Acid output (left panel) and pepsin output (right panel) in 6 normal men in the basal state (40 min) and during an intravenous infusion of 0.1 μg/kg·h pentagastrin for 3 h (100–240 min) (a) alone and (b) with 1 μg/kg·h bombesin infused during the period 100–160 min (mean ± SEM). Bottom. Acid output (left panel) and pepsin output (right panel) in 9 men with DU studied with the same protocol as above (mean ± SEM). BB, bombesin; pg, pentagastrin.

Table 1. Control Values 20 Minutes Before Bombesin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Acid (mEq/20 min)</th>
<th>Pepsin (PU/20 min)</th>
<th>Fasting serum gastrin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.84 ± 0.92</td>
<td>115,000 ± 23,000</td>
<td>23.1 ± 12.6</td>
</tr>
<tr>
<td>DU</td>
<td>7.09 ± 0.84</td>
<td>179,000 ± 28,000</td>
<td>55.7 ± 11.9</td>
</tr>
</tbody>
</table>

DU, duodenal ulcer; PU, peptic units. Values are expressed as mean ± SEM. Only 2 of the 9 DU subjects and in those 2 subjects inhibition of acid occurred in the face of increased serum gastrin (+30 and +121 pg/ml, respectively, Tables 1 and 2). Acid increased or remained unchanged in the other 7 (Tables 1 and 2). The mean values for the group given bombesin with pentagastrin showed no difference from the control pentagastrin infusion in the same subjects (Figure 1) for either acid or pepsin. The decreases in acid and pepsin outputs in normals during the bombesin infusion
Table 2. Individual Changes in Acid and Pepsin Output During the Last 40 Minutes of Bombesin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Δ Acid (%)</th>
<th>Δ Pepsin (%)</th>
<th>Δ Serum gastrin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-58</td>
<td>-46</td>
<td>+9</td>
</tr>
<tr>
<td>2</td>
<td>-62</td>
<td>-39</td>
<td>-167</td>
</tr>
<tr>
<td>3</td>
<td>-66</td>
<td>-53</td>
<td>+23</td>
</tr>
<tr>
<td>4</td>
<td>-22</td>
<td>-52</td>
<td>+3</td>
</tr>
<tr>
<td>5</td>
<td>-50</td>
<td>-58</td>
<td>+40</td>
</tr>
<tr>
<td>6</td>
<td>-69</td>
<td>-77</td>
<td>+26</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>-54.5 ± 7.0</td>
<td>-54.2 ± 5.3</td>
<td>44.7 ± 25</td>
</tr>
<tr>
<td>Significance of Δ</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

DU

<table>
<thead>
<tr>
<th></th>
<th>Δ Acid (%)</th>
<th>Δ Pepsin (%)</th>
<th>Δ Serum gastrin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+40</td>
<td>+56</td>
<td>+200</td>
</tr>
<tr>
<td>2</td>
<td>+6</td>
<td>-29</td>
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<td>3</td>
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<td>-24</td>
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</tr>
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<td>8</td>
<td>-45</td>
<td>-44</td>
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</tr>
<tr>
<td>9</td>
<td>-32</td>
<td>-53</td>
<td>+121</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>+7.5 ± 9.4</td>
<td>-2.7 ± 13.8</td>
<td>193 ± 36.5</td>
</tr>
<tr>
<td>Significance of Δ</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

DU vs normals

<table>
<thead>
<tr>
<th></th>
<th>t = 5.29</th>
<th>3.48</th>
<th>3.35</th>
</tr>
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<tbody>
<tr>
<td>p &lt; 0.001</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

DU, duodenal ulcer; NS, not significant. a For control values before bombesin infusion see Table 1.

were significantly different from the lack of change seen in DU (p < 0.001, p < 0.01, respectively) (Tables 1 and 2). In the normal subjects the fall in pepsin was congruent with that of acid, whereas in DU subjects pepsin output fell in 5 of the 9 compared to only 2 of the 9 subjects in whom acid output was inhibited.

During the 20–30 min after bombesin was discontinued in the DU subjects, acid and pepsin secretion both rebounded to levels above control and above the bombesin hour (p < 0.05 by paired t-test) (Figure 1).

**Serum Gastrin and Somatostatin.** Serum gastrin (Figure 2) values were unchanged during the basal period and the infusion of pentagastrin. The infusion of bombesin produced a rapid increase within 5 min in both normal and DU subjects. The increase was much smaller in normal subjects, ranging from 3 to 40 pg/ml in 5 of the 6 subjects. In the sixth subject, serum gastrin increased by 167 pg/ml. In the DU patients, serum gastrin peaked at 5 min and remained elevated during the bombesin infusion. The increase in DU subjects was four times greater than in normal subjects (p < 0.01) (Tables 1 and 2). After bombesin was discontinued, serum gastrin fell slowly but was still elevated after 1 h. Serum somatostatin (Figure 2) was not systematically or significantly affected by bombesin, falling slightly in normal subjects and rising transiently in DU subjects.

**Bethanechol**

Using the same experimental protocol, we gave the 6 normal subjects and 5 of the DU subjects the cholinergic agonist bethanechol instead of bombesin to determine whether cholinergic inhibition could be demonstrated in either group.

**Normals.** Acid output in response to the pentagastrin infusion was not altered by the bethanechol infusion (Figure 3). Pepsin output, however, was increased during the hour of bethanechol infusion (by paired t-test, t = 3.2, p < 0.02). No significant change in either serum gastrin or somatostatin was found (Figure 2).

**Duodenal ulcer.** Bethanechol did not alter pentagastrin-stimulated acid output in the 5 DU subjects so studied (Figure 3). Pepsin output was elevated during bethanechol infusion in 4 of the 5 subjects. The values were not quite significantly different (p = 0.055) from the control pentagastrin output. The pattern of increase resembled the change seen in normal subjects. Serum gastrin and somatostatin levels were measured in 4 and 3 subjects, respectively, in this section of the study. No significant alterations of either were seen (Figure 2).

**Discussion**

The present results amply confirm, in a different setting, two effects of bombesin that we have previously reported (9). First, bombesin stimulates levels of serum gastrin three to four times greater in men with DU than in nonulcer normal controls, and second, despite the release of gastrin, this dosage of bombesin inhibited gastric secretion of acid and pepsin in normal subjects, as it does in dogs (8), but not in most DU patients. In the present study, the bombesin effects were examined in the presence of an infusion of a half-maximal dosage of pentagastrin so that both positive and negative effects of bombesin on gastric secretion might be observed. If the inhibition represented the net effect between two opposing events (stimulation by gastrin and inhibition by a coreleased inhibitor), the effect of the latter must be very potent, because it not only prevented a
rise in secretion with the added gastrin, but quite potently inhibited ongoing stimulation by pentagastrin. Inhibition of acid secretion even occurred in 2 of the men with DU who had a large additional increase in serum gastrin. In the other 7 DU patients, the increases in serum gastrin resulted in additional stimulation of gastric acid secretion.

Although the stimulation of acid by bombesin is well defined by the sequential events of gastrin release through direct action on the C cells (17) and a hormonal effect of circulating gastrin on secretory cells, the mechanism of inhibition of the action of gastrin by larger dosages of bombesin (8,9) is undefined. The most probable candidate for such inhibi-
Figure 3. Top. Acid (left panel) and pepsin (right panel) output in 6 normal men with 40 min basal secretion followed by a 3-h infusion of pentagastrin, 0.1 μg/kg-h, (a) alone and (b) with superadded bethanechol, 40 μg/kg-h, in the period 100–160 min (mean ± SEM). The total pepsin output for the hour of bethanechol infusion was significantly greater than in the paired pentagastrin infusion without bethanechol (t = 3.2, p < 0.02). For serum gastrin and somatostatin values, see Figure 2. Bottom. Acid output (left panel) and pepsin output (right panel) in 5 men with DU studied with the same protocol as above. For serum gastrin and somatostatin values in these experiments, see Figure 2.
tion is the strategically located D cell system (3,4), acting via the local release of somatostatin (1,3,4). There is good evidence for involvement of somatostatin in modulating the response of the stomach to various stimuli, including bombesin (1,2,10,12), and exogenously given somatostatin can clearly inhibit gastrin release (1,10) as well as basal and stimulated gastric secretion (10,18–20). Though somatostatin levels in the blood have been reported to increase after various stimuli (21–25), circulating somatostatin does not necessarily reflect localized paracrine events. Furthermore, the failure to find a change in circulating somatostatin in the present studies does not rule out a significant local role for somatostatin in the proposed model. Because DU patients are not less or more sensitive to exogenous somatostatin (26,27), a decreased somatostatin content in antral mucosa of DU patients (28,29) would be consistent with our finding of a greater than normal release of gastrin by bombesin in DU. Richelson et al. (17) have also shown that whereas bombesin released gastrin, it did not release somatostatin from in vitro dispersed glands from human antral mucosa obtained from peptic ulcer patients at surgery. We do not have evidence regarding somatostatin release in antral mucosa of normal humans or in fundic mucosa of either DU or normal humans, where we postulate the existence of local somatostatin effects on acid- and pepsin-secreting cells.

The output of acid and pepsin increased significantly in DU patients after bombesin was discontinued, as noted before (9), despite failing—though still elevated—serum gastrin levels. The most plausible explanation for this finding is that the inhibitor dissipates quickly, while an increased level of the stimulant, gastrin, is still circulating. An inhibitor with a short half-life would fit the circumstances well. Somatostatin, for which other circumstantial evidence is cited above, would be a likely candidate. Experimentally such a rebound is seen after somatostatin infusions (10).

The difference between normal and DU subjects with respect to gastrin release by bombesin was not found by Delle Fuve et al. (30), the only other group to examine this question. The rapid rise to peak levels at 5 min in our DU patients (Figure 2) rules out a delayed elimination of gastrin, because that would produce progressively rising blood levels. Thus, higher levels in DU probably result from greater release of gastrin. The relative mean increases in normal and DU subjects were 2 × basal and 3.5 × basal, respectively. Inasmuch as antral gastrin content in DU is not greater than normal (27), the difference between DU and normal subjects most logically would be due to a lesser suppression of gastrin release in DU by lower effective amounts of antral somatostatin (28,29). For now, such a mechanism remains unproved, but possible.

In isolated human and rat antral glands, carbachol inhibits the release of somatostatin and thereby promotes the release of gastrin (17). Similarly, in intact perfused isolated rat (1,12) and pig stomachs (5) with intact intramural nerves, somatostatin release is suppressed and gastrin release is stimulated by cholinergic agents or vagal stimulation. The potent effects of the cholinergic agents on gastrin and somatostatin in the isolated rat stomachs (1,2) and on gastrin and gastric secretion of acid and pepsin in dogs (6) and other animals led us to restudy bethanechol in the present experiments. As previously reported by others (31,32), no evidence was found for inhibition of acid secretion or of release of gastrin by cholinergic agonists in either normal or DU subjects. Whether these results indicate a lack of stimulation in humans or the presence of a neatly balanced inhibition is unknown. Gastrin levels are higher in DU subjects after stimulation by food (30,33) and, in this case, bombesin (9). The larger gastrin response in DU with repetitive physiologic stimulation, such as food, may result in a trophic effect on fundic secreting mucosa, with a greater basal and maximal output of acid and pepsin (16); these findings are typical of many, if not most, chronic DU patients (16,34). Whether somatostatin (26) or, for that matter, bombesin is involved in the physiologic responses, as proposed in our model, remains to be proven.

References


