Cytoprotective Effect of Pentagastrin and Epidermal Growth Factor on Stress Ulcer Formation

Possible Role of Somatostatin

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This study was designed to test the effects of pentagastrin and epidermal growth factor (EGF) on stress-induced ulceration and on the antral content of gastrin and somatostatin (SLI) in rats. Four groups of 14 to 15 rats had been prepared for 7 days by one of the following methods: saline injection (control); injection of pentagastrin (250 µg/kg, 3 times/day); injection of EGF (10 µg/kg, 3 times/day); or injection of EGF plus pentagastrin. At the end of the treatment period, half of each group of rats were sacrificed (nonstress group). There were no ulcers in the nonstress control groups of rats. Stress was applied by water immersion in the remaining half of the rats. The injections of pentagastrin and/or EGF resulted in substantial increase in antral content of SLI. After 20 hours of stress, the ulcer index was 40.5 ± 3.3 in the controls, compared to 6.4 ± 1.2 and 16.2 ± 2.3 in rats that received pentagastrin or EGF, respectively. Injections of both pentagastrin and EGF resulted in an ulcer index of 26.2 ± 2.0, which was significantly lower than that in controls, but higher than that in rats treated with either peptide alone. The stress resulted in significant decrease in antral SLI in all groups of rats, whereas SLI content in rats treated with pentagastrin and/or EGF remained significantly higher than that of controls. Antral content of gastrin did not differ significantly in the four groups tested. The ulcer index was inversely correlated with antral SLI content. We confirm and extend previous observations that pentagastrin and EGF prevent stress ulcer formation, and suggest that endogenous SLI may account, at least in part, for their antiulcer activity.

Although they have opposite effects on gastric acid secretion,1 pentagastrin2 and epidermal growth factor (EGF)3,4 both exert cytoprotective activity on gastric mucosa by mechanisms that are not entirely understood. Takeuchi and Johnson2 reported that injections of pentagastrin protected rats, which had been depleted of gastrin, against stress ulcer and provided evidence that the cytoprotective effect of pentagastrin was related to its trophic action. Johnson and Guthrie5 compared the trophic effects of pentagastrin and EGF on gastrointestinal mucosa and found that both peptides stimulated synthesis of DNA, RNA, and protein in oxyntic gland mucosa in rats. Konturek and colleagues3,4 demonstrated that nonantisecretory doses of EGF, insufficiently large to inhibit acid secretion, prevented the ulcer formation that would have been expected as a consequence of administration of acetylsalicylate in rats. Although neither pentagastrin nor EGF in their study could stimulate the synthesis of gastric mucosal prostaglandin,3 both pentagastrin and EGF did increase the DNA synthesis in gastric mucosa. The antiulcer effects of pentagastrin and EGF may, therefore, be related in part to increased synthesis of DNA.

On the other hand, somatostatin, which is present in high concentrations in the gastrointestinal tract,6 especially in the stomach, has been shown to reduce stress ulcer formation in rats.7,8 Ligumsky and colleagues9 demonstrated that somatostatin is capable of potentiating carbamylcholine-induced synthesis and release of prostaglandin E2 in the rat stomachs. In addition, Chayvialle and colleagues10 found significant decrease in gastric somatostatin in duodenal ulcer patients. These observations, taken together, suggest the possibility that somatostatin may be involved in the pathogenesis of gastroduodenal ulceration. Although the pathogenesis of stress ulceration11,12 is different from those of peptic ulcer or chemically-induced ulcer, we have elected,
initially, to determine whether somatostatin has any role in stress ulcer. In the present study, we have attempted to determine whether EGF as well as pentagastrin can prevent stress ulcer formation in rats subjected to water immersion, and whether gastric somatostatin may account for the antiulcer activity of pentagastrin or EGF.

Materials and Methods

Male Sprague-Dawley rats, weighing approximately 150 g, were divided into four groups composed of 14 to 15 rats. Members of each group were given one of the following subcutaneous injections: saline (Group 1); pentagastrin (250 μg/kg) (Ayerst Laboratories, New York) (Group 2); EGF (10 μg/kg) (Collaborative Research, Waltham, MA) (Group 3); or pentagastrin (250 μg/kg) and EGF (10 μg/kg) (Group 4). Injections were given three times a day for 7 days, while the rats were housed individually in wire cages and fed with a regular diet of Purina® Rat Chow (about 70 cal/day).

Before a study, rats were fasted for 24 hours with free access to water, and then half of each group of rats were sacrificed by decapitation (nonstress control). The remaining half of rats were restrained in cylindrical plastic stress cages. Immobilized rats were immersed in a water bath at 23°C to the level of xiphoid process, for 20 hours, according to the method of Takagi and Okabe. Rats were then decapitated. Blood samples were collected for serum gastrin determinations. The stomach was removed through a midline incision, inflated with 10 ml of cold saline (in order to stretch and fix mucosal folds to the same extent), and opened along the greater curvature. The antrum was immediately separated from the oxyntic gland area, subdivided into two parts, and frozen on dry ice. The stomach was examined for lesions under a dissecting microscope by an examiner who did not know the treatment regimen for that stomach. The length of each lesion was measured in millimeters and the lengths of all ulcers were added. The total length of all the lesions in millimeters was expressed as the ulcer index.

Tissue Extraction

Specimens of total antral tissue were weighed while frozen and then boiled either in distilled water or acetic acid. Tissue extraction was done by mincing one-half of the antrum and boiling it in water (10 mg/ml) for 30 minutes; the supernatants were kept frozen for measurement of gastrin by radioimmunoassay. The other half of the tissue specimen was boiled in 0.2 M acetic acid (10 mg/ml) for 15 minutes and homogenized with a tissue grinder (Kontes size AA, T. M. Kontes, Vineland, NY). The homogenate was centrifuged (10,000 g for 10 minutes) and the clear supernatant was kept frozen at −40°C until the radioimmunoassay for somatostatin was done. Just before assay, the extracts were neutralized with 0.2 M NaOH to pH 7.5, and then duplicate samples were assayed in three dilutions. When the results differed by more than 10%, the samples were reassayed.

Radioimmunoassay

Gastrin concentrations in serum and tissue extracts were measured by a specific double-antibody radioimmunoassay (the antibody used recognized all known molecular forms of gastrin). Somatostatin-like immunoreactivity (SLI) was measured by a radioimmunoassay technique that used antisera R-101, obtained from A. Arimura (Tulane University School of Medicine, New Orleans, LA). Synthetic Tyr-1-somatostatin (Peninsula Laboratories Inc., San Carlos, CA) was radioiodinated at room temperature (25°C), by means of the chloramine-T method. Tyr-1-somatostatin, dissolved in 0.01 M acetic acid (1 μg/10 μl), was mixed with 20 μl of 0.5 M phosphate buffer, pH 7.4, 1.0 mCi Na125I, and chloramine-T (5 μg in 10 μl H2O) for 15 seconds. Then, 100 μl of bovine serum albumin (10% w/v in 0.05 M phosphate buffer, pH 7.4) was added to stop the reaction. In order to remove unreacted iodine, the reaction mixture, supplemented with 1.0 ml of human outdated plasma, was shaken briefly with 20 mg QUSO G 32 (Philadelphia Quartz Co., Philadelphia, PA). After centrifugation, the pellet was washed twice with 1.0 ml of distilled water. The labeled somatostatin that had been absorbed was eluted with acetone/acetic acid/water (39:10:40) by the method of McIntosh and colleagues. Before assay, the tracer was repurified on a CM cellulose (CM 52) column (1 × 5 cm), with 0.002 M ammonium acetate, pH 4.6, as starting buffer, and 0.2 M ammonium acetate, pH 4.5, used for elution of the label.

Fractions from the descending slope of the second radioactive peak gave a specific activity of 470–650 μCi/μg. The maximum binding of label to excess of antiserum was between 82% and 89%, and the label was stable for 6–8 weeks at −40°C. Ion-exchange chromatography was reproducible, although the first radioactive peak showed an increase over a 2-month period. Non-specific binding was less than 5% in most radioiodinations.

Antiserum R-101, directed towards positions 5 to 13 of the somatostatin 14 molecule, was used routinely at a final titer of 1:40,000 (35–40% binding). The assay buffer consisted of 0.05 M phosphate buffer, containing 0.08 M NaCl, 0.2 mg/ml sodium azide, and 0.25% bovine serum albumin. Volumes of 100 μl of test sample (or synthetic cyclic somatostatin standard, Beckman),
along with 100 μl of 0.25 M ethylene-diamine-tetraacetic acid at pH 7.5, 100 μl antiserum, and buffer, were added to make a total volume of 900 μl. After 24 hours of incubation at 4 C, 100 μl of freshly purified $^{125}$I Tyr-1-somatostatin (4000–4500 cpm corresponding to 2–4 pg) was added and the incubation was continued for 3 more days at 4 C. Free labeled somatostatin was separated from the bound by use of secondary antibody (100 μl of goat antirabbit gamma globulin serum) and normal rabbit serum (10 μl per tube). (A representative standard curve is depicted in Fig. 1.)

The detection limit of the assay, calculated as the lowest concentration of somatostatin that produced a significant inhibition (p < 0.05 by Student's t-test, n = 10) of binding in comparison to control, was 2.0 pg/ml of incubate. Serial dilutions of antral tissue extracts gave curves that were parallel to those obtained with standard somatostatin. The within-assay and between-assay variations were less than 12% (coefficient of variation). The mean recovery of 2, 5, and 15 ng of standard added to tissue samples before extraction was 73% (range, 65–79%, n = 10 for each dose).

**Statistical Analysis**

All values are expressed as the mean plus or minus one standard error. The Student's t-test was used to analyze the data for statistical significance of differences between means. Differences with a p value of less than 0.05 were considered significant.

**Results**

During the pretreatment period, all rats were growing normally; body weights did not differ among the four groups of rats (Table 1). No ulcers were observed in the groups of nonstress controls. The weight of the gastric antrum and the serum gastrin levels did not differ significantly among the four groups in the nonstress controls (Table 1), whereas the antral content of gastrin was significantly decreased in rats treated with pentagastrin (Group 2). Significantly higher contents of somatostatin-like immunoreactivity (SLI) were observed in rats from Groups 2, 3, and 4 in comparison to that of controls (Group 1), while the content of antral SLI did not differ among Groups 2, 3, and 4.

The water-immersion stress produced linear ulcers of various length, and punctuate ulcers in the oxyntic gland area (Fig. 2). The ulcer indices for the four groups were as follows: controls, 40.5 ± 3.3; pentagastrin, 6.4 ± 1.2; EGF, 16.2 ± 2.3; and pentagastrin and EGF, 26.2 ± 2.0 (Table 2). Injections with pentagastrin alone (Group 2) and EGF alone (Group 3) resulted in significantly lower ulcer indices, in comparison to the controls (Group 1). The ulcer index in rats receiving both pentagastrin and EGF injections (Group 4) was also significantly decreased from controls, although it was substantially higher than that in either Group 2 or Group 3 (Table 2).

Neither serum gastrin nor antral gastrin contents differed significantly among the four groups after exposure to the water immersion stress and were not different from those of nonstress controls. In contrast, antral SLI contents were found to decrease significantly during exposure to the stress. Antral SLI content in rats treated with pentagastrin and/or EGF (Groups 2, 3, and 4) was

### Table 1. Effect of Pentagastrin and EGF on the Four Nonstressed Control Groups of Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pentagastrin</th>
<th>EGF</th>
<th>Pentagastrin + EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>183 ± 3</td>
<td>177 ± 5</td>
<td>177 ± 3</td>
<td>176 ± 3</td>
</tr>
<tr>
<td>Antral weight (mg)</td>
<td>117 ± 6</td>
<td>118 ± 4</td>
<td>137 ± 6</td>
<td>142 ± 9</td>
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<tr>
<td>Ulcer index (mm)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum gastrin (pg/ml)</td>
<td>50 ± 4</td>
<td>50 ± 3</td>
<td>45 ± 2</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>Antral gastrin (ng/antrum)</td>
<td>673 ± 80</td>
<td>330 ± 52*</td>
<td>512 ± 44</td>
<td>534 ± 84</td>
</tr>
<tr>
<td>Antral SLI (ng/antrum)</td>
<td>17.8 ± 2.7</td>
<td>60.3 ± 4.3</td>
<td>56.1 ± 5.9</td>
<td>68.1 ± 6.3</td>
</tr>
</tbody>
</table>

* Probability < 0.05 compared to control.
significantly higher than that of controls. Antral SLI in rats (Group 4), however, was substantially lower than that in rats treated with pentagastrin alone (Group 2).

**Discussion**

The results of this study demonstrated that chronic treatment with pentagastrin and EGF reduced the gastric ulcer formation that would be expected upon water-immersion stress in rats, although pentagastrin and EGF did not differ significantly in their antiulcer activities. In the rats that received both pentagastrin and EGF, the ulcer index was higher than that in rats treated with either pentagastrin or EGF alone, which indicates that the protective actions of each agent are not additive. Since injections of pentagastrin and EGF had been completed 8 hours prior to exposure to stress, their transient effects on gastric acid secretion may not directly account for these antiulcer activities.

Takeuchi and Johnson observed that chronic injection of pentagastrin prevented stress ulcer formation in rats and demonstrated that the formation of ulcers was correlated with decreases in mucosal DNA synthesis.

EGF has been shown to have a similar structure and similar biologic activities to human urogastrone, with equipotential inhibition of gastric acid secretion. EGF has also been shown by Konturek and colleagues to have cytoprotective activity against aspirin in the stomach of rats and cats. This cytoprotective action was accompanied by a stimulation of DNA synthesis in fundic mucosa. It is, therefore, conceivable that these antiulcer effects provided by pentagastrin or EGF may be in part attributed to their trophic action on gastric mucosa.

**TABLE 2. Effect of Pentagastrin and EGF on Four Groups of Rats Subjected to 20-Hour Water-Immersion Stress**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pentagastrin</th>
<th>EGF</th>
<th>Pentagastrin + EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>167 ± 5</td>
<td>168 ± 4</td>
<td>172 ± 5</td>
<td>175 ± 5</td>
</tr>
<tr>
<td>Antral weight (mg)</td>
<td>118 ± 8</td>
<td>123 ± 10</td>
<td>129 ± 7</td>
<td>118 ± 6</td>
</tr>
<tr>
<td>Ulcer index (mm)</td>
<td>40.5 ± 3.3</td>
<td>6.4 ± 1.2*</td>
<td>16.2 ± 2.3*</td>
<td>26.2 ± 2.0*†‡</td>
</tr>
<tr>
<td>Serum gastrin (pg/ml)</td>
<td>67 ± 8</td>
<td>101 ± 15</td>
<td>112 ± 15</td>
<td>74 ± 7</td>
</tr>
<tr>
<td>Antral gastrin (ng/antrum)</td>
<td>621 ± 119</td>
<td>517 ± 122</td>
<td>707 ± 84</td>
<td>693 ± 148</td>
</tr>
<tr>
<td>Antral SLI (ng/antrum)</td>
<td>4.1 ± 0.5</td>
<td>14.6 ± 2.2*</td>
<td>10.5 ± 2.7*</td>
<td>7.2 ± 1.0*†</td>
</tr>
</tbody>
</table>

* Probability < 0.01 compared to control.
† Probability < 0.01 compared to pentagastrin alone.
‡ Probability < 0.05 compared to EGF alone.
Conclusion

The new information provided by our study is that the antiulcer effects provided by both peptides were accompanied by a significantly higher amount of somatostatin in the antrum. Chronic administration of pentagastrin and EGF resulted in significantly higher antral SLI content in comparison to controls. During exposure to stress, antral SLI was markedly decreased in all groups. Although food deprivation causes a decrease in antral content of somatostatin,\textsuperscript{18,19} stress may cause a further decrease in synthesis and release of somatostatin. When values of antral SLI content were plotted against the corresponding ulcer index for each rat, a significant inverse relationship was found (Fig. 3). This correlation indicates that a reduction in tissue content of somatostatin may be associated with greater susceptibility to stress ulcer.

Indeed, the role of somatostatin in the pathophysiology of gastric and duodenal ulcers has been the subject of many studies. Schwille\textsuperscript{8} and Zierden\textsuperscript{7} and their colleagues observed that exogenous somatostatin prevented the formation of stress ulcers in rat gastric mucosa in a dose-dependent manner. The significance of gastric somatostatin seems to be more apparent in clinical studies on duodenal ulceration. Chayville and colleagues\textsuperscript{10} showed, for example, that duodenal ulceration is associated with a reduction of somatostatin in the antrum, and Sumii and colleagues\textsuperscript{20} observed a substantial decrease in antral somatostatin associated with a high gastrin/somatostatin ratio in duodenal ulcer patients. Whether the antiulcer effect of somatostatin is, however, attributable to inhibition of gastric secretion or to reduction in release of gastrin, or both,\textsuperscript{8,21,22} has not been clearly established.

Ligumsky and colleagues\textsuperscript{9} have demonstrated that somatostatin can potentiate the synthesis and release of endogenous prostaglandin E2 (PGE2) from the isolated rat stomach in the presence of carbamylcholine. Prostaglandins are well known as cytoprotective agents and a potent inhibitor of gastric acid secretion.\textsuperscript{23–25} It is, therefore, conceivable that pentagastrin and EGF may stimulate somatostatin synthesis and release, which may, in turn, result in synthesis of endogenous PGE2. In fact, Hiraishi and colleagues\textsuperscript{26} demonstrated that EGF-stimulated prostaglandin synthesis in rat gastric mucosal cells. We measured only antral SLI content, but we should note that somatostatin might act on the gastric fundus in both a paracrine and an endocrine manner. The mechanism by which both pentagastrin and EGF increased SLI content in the rat antrum is difficult to explain. Chiba and colleagues\textsuperscript{27} studied the effects of various peptides on the release of somatostatin from the isolated rat stomach. In their study, secretin evoked the greatest release of somatostatin, whereas the effect of pentagastrin on somatostatin release was found to be less remarkable. Plasma somatostatin is also increased by acidification of the antral mucosa.\textsuperscript{28} Pentagastrin, therefore, appears to stimulate somatostatin in a direct manner by stimulation of D cells, and in an indirect one by inducing gastric acid secretion. The latter mechanism may enhance secretin release, with subsequent release of antral somatostatin.

The effect of EGF on release of somatostatin is not known. Unpublished preliminary studies in our laboratory indicated that infusion of graded doses of EGF into the gastroepiploic artery in dogs results in a dose-dependent release of somatostatin into the gastric vein. Hence, it is possible that continuous stimulation of the release of somatostatin, evoked by pentagastrin or EGF, may result in increase in antral somatostatin content, as ACTH stimulates the content of corticosterone in the rat adrenal gland.\textsuperscript{29} The exact chain of events, however, that leads to higher antral somatostatin concentrations in response to pentagastrin and EGF is not known.

In conclusion, chronic injection of pentagastrin and EGF resulted in increase in resistance to stress-induced ulceration in rats. Although pentagastrin and EGF may prevent stress ulcer by means of their trophic action on gastric mucosa, the significant relationship between ulcer index and antral content of somatostatin suggests that endogenous somatostatin may be involved in prevention of stress ulcer formation.

Acknowledgments

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References


