

## Interaction between Epidermal Growth Factor and Gastrin on DNA Synthesis of the Gastrointestinal Mucosa in Rats

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### ABSTRACT

Interaction between epidermal growth factor (EGF) and gastrin on DNA synthesis of the rat gastrointestinal tract was examined. Fasted male rats were divided into four groups and injected with 10  $\mu\text{g}/\text{kg}$  human EGF, 300  $\mu\text{g}/\text{kg}$  pentagastrin, human EGF plus pentagastrin and saline (control), respectively. The animals were sacrificed 8 or 16 hr thereafter and the incorporation of [ $^3\text{H}$ ]thymidine into the gastrointestinal mucosa was measured. Human EGF increased DNA synthesis of the fundus, antrum and cecum, while pentagastrin stimulated that of the fundus, duodenum, ileum and proximal colon. Synchronous administration of these peptides also stimulated DNA synthesis of the fundus, antrum and cecum. However, in the ileum and proximal colon, DNA synthesis stimulated by pentagastrin was suppressed by the administration of both agents synchronously. These data suggest that these growth factors regulate the growth of gastrointestinal tract with complex interactions.

**Key words:** Human epidermal growth factor, DNA synthesis, Gastrointestinal mucosa

Mouse epidermal growth factor (mouse EGF), which was first isolated from male mouse submandibular glands<sup>5</sup>, is a polypeptide containing 53 amino acids and stimulates the growth of many tissues *in vitro* and *in vivo*<sup>4,6,7,37</sup>. Human  $\beta$ -urogastrone, a potent inhibitor of gastric acid secretion<sup>15</sup>, was subsequently isolated from human urine and is probably identical to human EGF<sup>8,29</sup>. In the gastrointestinal mucosa, EGF stimulation of DNA synthesis has been reported with some contradictory results<sup>12,20,21,26,27</sup>. Some investigators have reported that mouse EGF is a trophic substance for only oxyntic gland mucosa of the stomach and the duodenum in rats<sup>11,20</sup>. But in the mouse system, trophic effect of mouse EGF has been observed in most part of gastrointestinal tract<sup>26,27</sup>. Al-Nafussi and Wright<sup>1</sup> had also found that the response of gastrointestinal mucosa to EGF differed in rats and mice. The differential growth response of the gastrointestinal mucosa to exogenous EGF is considered to be due to the specificity of animal species used in the experiments.

On the other hand, Gastrin, a classic and well-established gastrointestinal hormone, shows a trophic influence on the gastrointestinal mucosa and stimulates gastric acid secretion and pancreatic enzyme secretion<sup>18,19</sup>. We have also demonstrated that gastrin promotes the growth of a human gastric cancer cell line as well as a xenotransplantable human gastric carcinoma in nude mice<sup>24,30</sup>.

Johnson and Guthrie<sup>20</sup> had examined the effects of EGF and gastrin individually on the growth of rat oxyntic glands and hypothesized that acid secretion and mitogenesis are the results of two separate mechanism. However, the effect of synchronous administration of EGF and gastrin or the interaction between EGF and gastrin on DNA synthesis of the gastrointestinal mucosa has not been reported.

The present study was made to investigate the trophic effect of human EGF on the rat gastrointestinal mucosa. Moreover, the interaction between human EGF and gastrin on DNA synthesis of gastrointestinal mucosa was examined.

### MATERIALS AND METHODS

#### Animals and Treatments

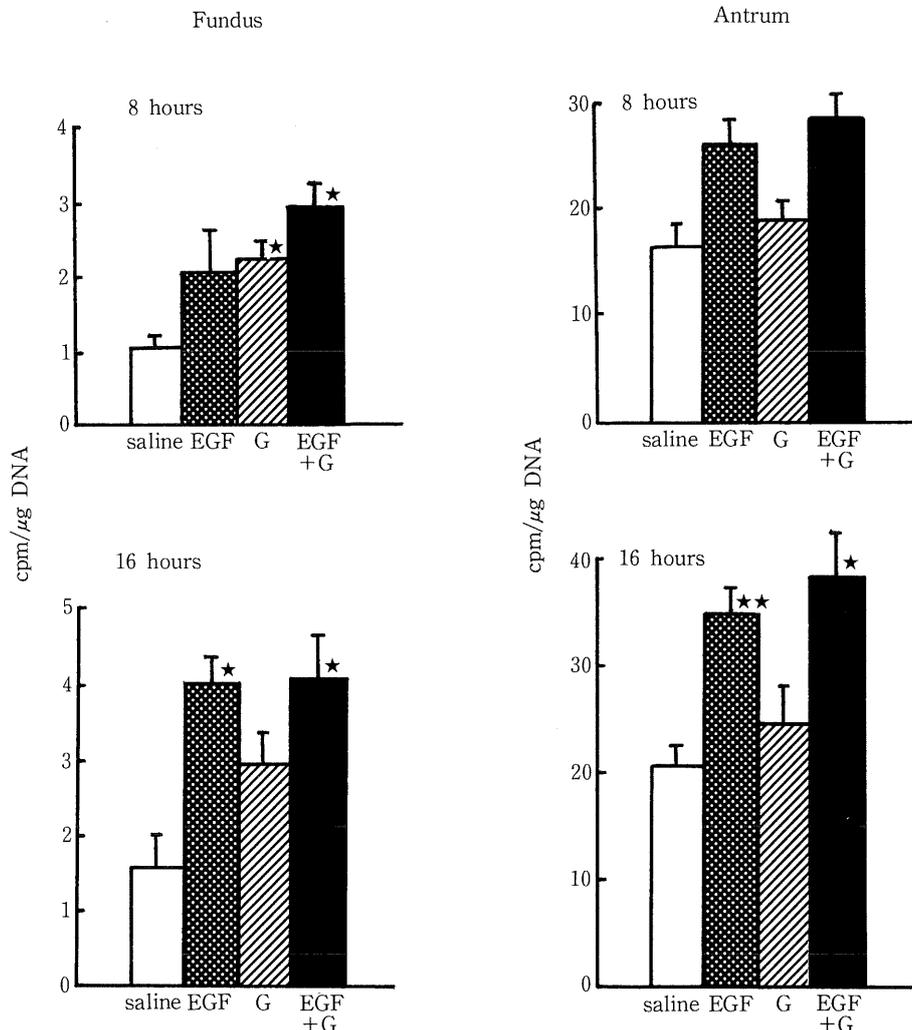
Six-week-old male Wistar strain rats (JCL Wistar rat, Japan Clea Co., Osaka, Japan) weighing 120-140 g were employed in the present study. Forty rats were randomly divided into four equal groups and fasted for 24 h. During this time they had free access to water. The first group received 10  $\mu\text{g}/\text{kg}$  body weight human EGF intravenously through the tail vein. Highly purified hEGF which was prepared from a genetic-engineered *E. coli* host and had full bioactivity was kindly supplied by Wakunaga Pharm. Co., Hiroshima, Japan<sup>16</sup>. It had been confirmed that 2-25  $\mu\text{g}/\text{kg}$  body weight of human EGF showed a maximal trophic influence on rat various organs<sup>36</sup>. The second group received pentagastrin (300  $\mu\text{g}/\text{kg}$  body weight, Sumitomo

Chemical Industries Ltd., Tokyo, Japan) intravenously. The dosage of pentagastrin used was based on our previous reports<sup>18,35</sup>. The third group was injected with 10  $\mu\text{g}/\text{kg}$  body weight human EGF and 300  $\mu\text{g}/\text{kg}$  body weight pentagastrin synchronously and the fourth as control was given the same volume of physiological saline solution in the same manner as the first group. Eight or 16 hr after the administration, five rats in each group were sacrificed. DNA synthesis stimulated by both agents was confirmed to occur 5hr after the injection and continue at least for 13hr<sup>36</sup>. All rats were injected intravenously with 1 mCi of [<sup>3</sup>H]thymidine (Amersham Japan, Ltd., Tokyo, Japan) per kg body weight 1 hr before sacrifice. The gastrointestinal tract was removed and fixed in 10% buffered formalin. After fixation for 2–3 days, representative specimens of the glandular stomach (fundus and antrum), duodenum, jejunum, ileum, cecum, proximal colon and distal colon were taken and mucosal tissues were prepared by careful scraping using a surgical knife. In this manner, mucosal tissues were able to be easily obtained and confirmed histologi-

cally. The mucosal scrapings were preserved at  $-20^{\circ}\text{C}$  for less than a week before use.

### DNA Synthesis

DNA synthesis was determined by the incorporation of [<sup>3</sup>H]thymidine into DNA in the mucosa. DNA was extracted from each scraped mucosa by the method of Burns et al<sup>3</sup>. Frozen tissue (30–40 mg wet weight of the antrum and 100–150 mg of the others) was hydrolyzed in 2 ml of 1N NaOH at  $60^{\circ}\text{C}$  for 15 hr. After cooling on ice, the mixture was neutralized with 1 N HCl and the same volume of 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 min and the supernatant was discarded. The pellet was solubilized in 1 ml of 6% perchloric acid heated to  $70^{\circ}\text{C}$  for 30 min. After recentrifugation, the radioactivity in 0.5 ml of the supernatant was counted in a liquid scintillation counter. With calf thymus DNA as a standard, the DNA content of the samples was determined by the fluorometric assay using 3,5-diamino-benzoic acid<sup>17</sup>. DNA synthesis was expressed as cpm [<sup>3</sup>H]thymidine per  $\mu\text{g}$  DNA.



**Fig. 1.** Effects of human EGF and pentagastrin on DNA synthesis in rat glandular stomach (fundus and antrum). Each value represents mean  $\pm$  S.E. of 5 experiments. G; pentagastrin. ★ Significant difference from saline group (★  $p < 0.05$ , ★★  $p < 0.01$ ).

### Statistics

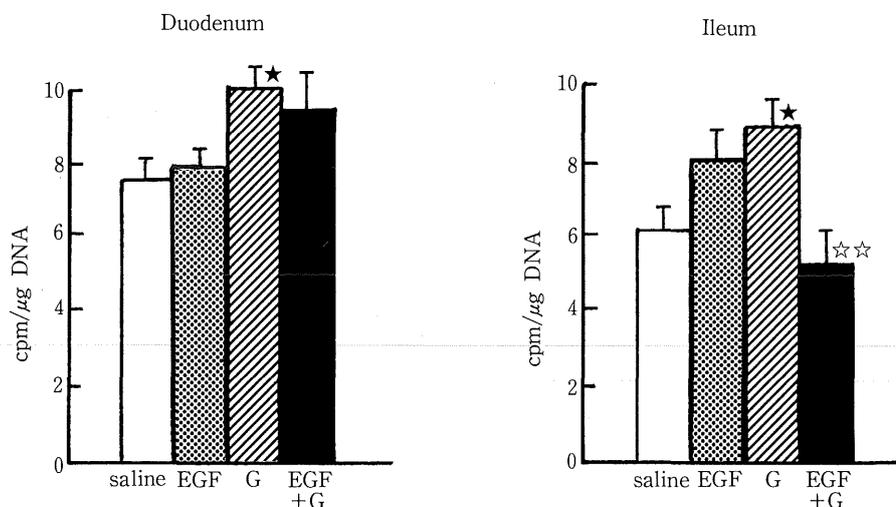
All data are shown as means and standard errors of the means. Means were compared by the Student's *t* test for unpaired data and considered significantly different if  $p < 0.05$ .

### RESULTS

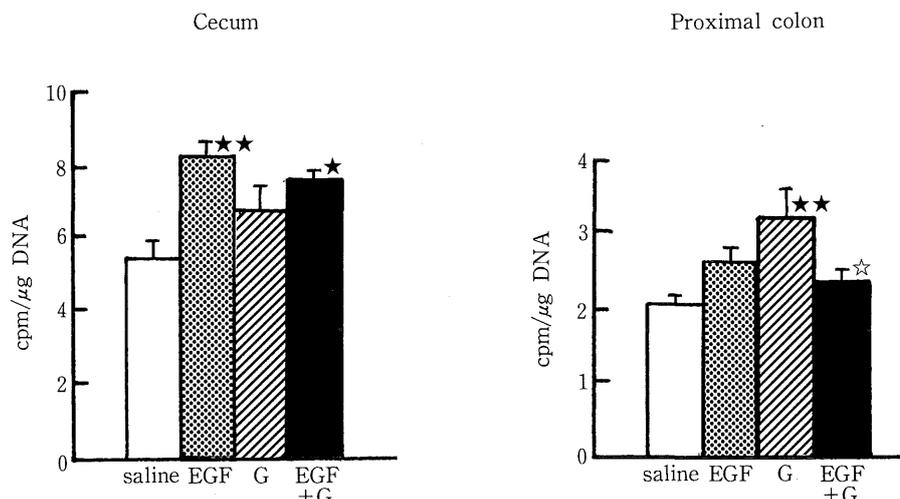
Effects of human EGF and pentagastrin on DNA synthesis in the rat stomach mucosa are shown in Fig. 1. Only in fundic mucosa, the responses to human EGF and pentagastrin differed at different times after the injections. Pentagastrin increased DNA synthesis approximately 100% compared to the control 8 hr after administration, the difference being significant ( $p < 0.05$ ). Human EGF increased DNA synthesis but not significant. Synchronous injection of human EGF and pentagastrin significantly stimulated DNA synthesis ( $p < 0.05$ ). At 16 hr after administration, significant increase of DNA

synthesis was observed in human EGF treated group and both agents treated group ( $p < 0.05$ ). But, the injection of pentagastrin alone was less effective. In the antral mucosa, either human EGF or human EGF plus gastrin significantly stimulated DNA synthesis at 16 hr (human EGF group,  $p < 0.01$ ; human EGF plus pentagastrin group,  $p < 0.05$ ). Pentagastrin did not show any effect on this area.

Fig. 2 and 3 illustrate the DNA synthetic response of the mucosa of the duodenum, ileum, cecum and proximal colon at 8 hr after the injection. In the duodenum, only pentagastrin significantly stimulated DNA synthesis ( $p < 0.05$ ), whereas the effects of either human EGF or human EGF plus pentagastrin were not significant (Fig. 2). In the ileum, the injection of pentagastrin significantly stimulated DNA synthesis ( $p < 0.05$ ) (Fig. 2). However, synchronous injection of human EGF and



**Fig. 2.** Effects of human EGF and pentagastrin on DNA synthesis in rat duodenum and ileum. Values were obtained from rats killed at 8 hr. Each value represents mean  $\pm$  S.E. of 5 experiments. G; pentagastrin. ★ Significant difference from saline group ( $p < 0.05$ ). ☆ ☆ Significant difference from pentagastrin treated group ( $p < 0.01$ ).



**Fig. 3.** Effects of human EGF and pentagastrin of DNA synthesis in rat cecum and proximal colon. Values were obtained from rats killed at 8 hr. Each value represents mean  $\pm$  S.E. of 5 experiments. G; pentagastrin. ★ Significant difference from saline group (★  $p < 0.05$ , ★★  $p < 0.01$ ). ☆ Significant difference from pentagastrin treated group ( $p < 0.05$ ).

pentagastrin showed no influence on DNA synthesis, the values being significantly different between in pentagastrin treated group and in human EGF plus pentagastrin treated group ( $p < 0.01$ ). That is, synchronous administration of human EGF and pentagastrin suppressed pentagastrin stimulated DNA synthesis. In the cecum, injections of both human EGF alone and human EGF plus pentagastrin showed significant increase of DNA synthesis ( $p < 0.05$ ) (Fig. 3). The same response as observed in the ileum was evident in the proximal colon (Fig. 3). Pentagastrin stimulated DNA synthesis disappeared by synchronous injection of human EGF and pentagastrin, the difference between pentagastrin treated group and human EGF plus pentagastrin treated group being significant ( $p < 0.05$ ). The responses to various treatments in the four sites of the gastrointestinal tract described above at 16 hr after the injection showed the same tendency as at 8 hr shown in Fig. 2 and 3, but were less effective. DNA synthesis in the jejunum and distal colon was not affected by any treatment.

### DISCUSSION

EGF is one of the most biologically potent and best characterized growth factors because of its physiological, chemical, and biological properties<sup>7,8</sup>. EGF has been shown to have a trophic influence on the mucosa of the gastrointestinal tract<sup>12,21,28</sup>. Scheving et al<sup>26,27</sup> reported that mouse EGF increases DNA synthesis of the tongue, esophagus, stomach, small intestine and large intestine in CD2F<sub>1</sub> mice. Johnson et al<sup>20</sup> have observed the stimulatory effect of mouse EGF on DNA synthesis only in the fundus of the stomach using male Sprague-Dawley rats. To our knowledge, this is the first report demonstrating a trophic effect of human EGF on the gastrointestinal mucosa using animal model. In the present study, human EGF showed trophic effect on the mucosa of the fundus, antrum and cecum. These findings are comparable to the results of Scheving et al<sup>26,27</sup> except for small intestine. Therefore, the differences in animal species and kinds of EGF are assumed to be present in the trophic effect of EGF.

EGF is known to inhibit gastric acid secretion, and decreased acidity in the stomach may cause increased gastrin release<sup>15,32</sup>. However, it has been considered that the trophic effect of EGF is independent of that of gastrin or acid secretion<sup>20</sup>. That is, the target organs of trophic influence are different between EGF and gastrin. Further, secretin, which is a potent inhibitor of the trophic action of gastrin in the gastrointestinal tract, has not altered the response of EGF. Also in the present study, the affected organs were different between human EGF and pentagastrin. Human EGF increased DNA synthesis of the fundus, antrum and cecum while pentagastrin stimulated that of the fundus, duodenum, ileum and proximal colon. These

observations support the idea that two separate mechanisms are present in the effects of EGF and gastrin. In the present study, however, synchronous administration of human EGF and pentagastrin suppressed pentagastrin stimulated DNA synthesis in the ileum and proximal colon. These findings indicate that the growth of the gastrointestinal tract may be regulated not only by EGF and gastrin individually but also by these peptides with complex interactions.

EGF has been shown to bind to EGF receptor, a glycoprotein with a molecular weight of 170-kDa, following autophosphorylation, enhancement of its tyrosine-specific kinase activity and finally stimulation of cell growth<sup>9,10,31</sup>. Isolated small intestinal cells have been shown to have EGF receptor<sup>2,14</sup>. EGF also stimulates ornithine decarboxylase activity in the digestive tract<sup>13</sup>. It has been demonstrated that EGF receptor complex stimulates calcium uptake and phosphatidylinositol turnover and activates calcium-activated phospholipid-dependent protein kinase in human epidermoid carcinoma cell lines<sup>25</sup>. Recently, we have confirmed that calcium-activated phospholipid-dependent protein kinase activity is present in either gastric mucosa or gastric carcinoma<sup>33</sup>. On the other hand, gastrin binds its specific membrane receptor, a glycoprotein with a molecular weight of 74 or 78-kilodalton which was more recently identified<sup>22,23</sup>. We have reported that the trophic effect of gastrin on gastric carcinoma cell is linked to cAMP induction and activation of cAMP-dependent protein kinase particularly its type I isoenzyme<sup>24,30,34</sup>. Therefore, protein phosphorylation must play an important role in the trophic effects of EGF as well as gastrin on the gastrointestinal tract. Further studies should be conducted to clarify the precise mechanism of the growth control by these growth factors in the gastrointestinal tract.

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