Regulation of Epidermal Growth Factor Receptors in Rat Gastric Mucosa: Effect of Gastric Ulceration

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The effect of experimental ulcers on the binding of human epidermal growth factor (hEGF), β -urogastrone, to plasma membranes isolated from rat gastric mucosa was studied in order to develop hEGF as an anti-ulcer drug. The binding of [125 I]hEGF to the gastric plasma membranes was significantly decreased 1 h after treatment with aspirin (300 mg/kg) and after 12 h of water-immersion stress. However, the number of EGF binding sites was increased 12 h after aspirin administration. There was little change in the binding of [125 I]insulin to the gastric plasma membranes in response to water-immersion stress. These results indicate that changes in EGF binding to its receptors occur on plasma membranes of the rat gastric mucosa during ulcer.

Keywords — epidermal growth factor (EGF); urogastrone; receptor; rat gastric mucosa; plasma membrane; aspirin; ulcer; stress

Introduction

Epidermal growth factor (EGF, β -urogastrone), a 53 amino acid polypeptide, is structurally and functionally similar to transforming growth factor α (TGF- α). EGF has several effects on gastrointestinal function; (a) inhibition of gastric secretion in several species,¹⁾ (b) stimulation of the proliferative process in digestive tract tissues,²⁾ and (c) promotion of the healing of experimental gastric or duodenal ulcers.³⁾ These effects of EGF suggest that it is responsible for maintaining the structural integrity of the gastrointestinal mucosa and for preventing mucosal injury caused by various irritants. Therefore, this peptide may be a useful therapeutic agent for ulcer healing.

We studied secretin receptors in rat gastric mucosa and pancreas.⁴⁾ Forgue-Lafitte *et al.*⁵⁾ studied EGF receptors in the gastric glands of guinea pigs. Zimmerman *et al.*⁶⁾ have demonstrated EGF receptors in the canine antrum mucosa by autoradiography. In our previous study,^{7,8)} EGF binding to gastric membrane receptors was demonstrated in rats and that bind-

ing was found to be decreased during fasting.

However, the changes in EGF receptors during production and healing of ulceration remains to be studied. The pathogenesis of stress ulceration is different from that of chemically-induced ulcers.⁹⁾ In this paper we examine the effect of ulcer formation induced by aspirin or by water-immersion stress on gastric EGF receptors in order to determine the usefulness of EGF as an anti-ulcer cytoprotective agent.

Materials and Methods

Materials — Highly purified human EGF (more than 99%) was kindly provided by Wakunaga Pharmaceutical Co. (Hiroshima, Japan). Porcine insulin was obtained from Novo Industri A/S (Copenhagen, Denmark). Radioiodinated human EGF ([125 I]hEGF) and [125 I]labeled human insulin ([125 I]insulin) labeled at tyrosine-A14 were obtained from Amersham Japan (Tokyo). The specific activities of [125 I]hEGF and [125 I]insulin were about 1000—1200 and 1800—2000 Ci/mmol, respectively. Percoll was purchased from Pharmacia

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Fine Chemicals (Uppsala, Sweden). Aspirin was JP XI grade. All other chemicals were obtained from commercial sources and were reagent grade.

Experimental Animals — Male Wistar albino rats were purchased from SLC (Shizuoka, Japan). Standard rat chow (Type MF; Oriental Yeast, Tokyo, Japan) and water were provided. Gastric plasma membranes were always prepared from normal control rats and experimental ulcer rats simultaneously.

Aspirin-Induced Ulcer¹⁰: Fasted rats were given orally 300 mg/kg aspirin suspended in 0.5% carboxymethylcellulose (CMC). Control rats were given 0.5% CMC solution. Animals were sacrificed 1, 6, 12, and 24 h after aspirin administration to prepare gastric membranes.

Water-Immersion Restraint Stress Ulcer: Rats were restrained in stress cage compartments. Immobilized rats were immersed in a water bath at $21 \pm 1^\circ\text{C}$ to the level of the xiphoid process for 1 or 12 h according to the method of Takagi and Okabe.¹¹ Control rats were fasted for the same period as ulcer induced rats. Animals were then sacrificed and the stomachs were removed for preparation of gastric plasma membranes.

Preparation of Gastric Plasma Membranes

— Plasma membranes were isolated from the gastric mucosa of rats (180–260 g) by Percoll density gradient centrifugation as described previously.⁷ The stomachs were removed and the mucosa was homogenized with ice-cold buffer containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 100 U/ml Trasylol, and 0.1 μM pepstatin (buffer A). The homogenate was centrifuged according to a method described previously.⁷ Protein was assayed according to the method of Lowry *et al.*¹² using bovine serum albumin (BSA) as the standard.

Binding of [¹²⁵I]hEGF or [¹²⁵I]Insulin to Plasma Membranes — Binding studies were performed at 25 °C for 60 min in a buffer containing 100 mM Tris-HCl (pH 7.5), 0.1 mM PMSF, 100 U/ml Trasylol, and 0.1 μM pepstatin containing 2% BSA (incubation medium) as described previously.⁷ To determine the non-specific binding of EGF, 100 nM of unlabeled

hEGF was added to parallel incubations. Separation of bound radioactivity was performed by rapid filtration.⁷ The binding of [¹²⁵I]insulin was also studied under the same incubation conditions as in the EGF study. To determine the nonspecific binding of insulin, 1 μM of unlabeled insulin was added to the parallel incubation.

Statistical Analysis — The results are presented as means $\pm$ S.E. Results were statistically evaluated with Student's *t*-test taking $p < 0.05$ as the level of significance.

Results

In fasting rats, mucosal lesions were produced by the oral administration of 300 mg/kg aspirin suspended in 0.5% CMC as a chemically induced ulcer model. At 1 h after aspirin administration, mucosal lesions were evident and severe as described by Hashizume *et al.*¹⁰ The binding of [¹²⁵I]hEGF to these mucosa membranes was significantly decreased to 65% of that of controls at 1 h after the administration (Fig. 1, $p < 0.05$). However, the binding was apparently returned

Fig. 1. Effect of Aspirin-Induced Ulceration on the Binding of [¹²⁵I]hEGF to Gastric Plasma Membranes

Plasma membranes (30 μg /tube) were incubated with [¹²⁵I]hEGF (0.2–0.3 nM) for 60 min at 25 °C. Each column is the mean and S.E. of 1–4 separate experiments performed in triplicate. a) Significant ($p < 0.05$) change compared with the controls.

Fig. 2. Representative Scatchard Plots of [125 I]hEGF Binding to Gastric Plasma Membranes

Plasma membranes were incubated for 60 min at 25 °C with [125 I]hEGF plus various concentrations of unlabeled hEGF. The concentration range of EGF used was 0.03–30 nM. (A) ○, control; ●, 1 h after aspirin (300 mg/kg) administration. (B) ○, control; ●, 12 h after aspirin (300 mg/kg) administration. Their regression lines were provided.

to the control level 6 h after administration of aspirin. Furthermore, at 12 h after administration of aspirin, the binding of [125 I]hEGF was significantly increased to 132% of that of control rats ($p < 0.05$). Scatchard analysis showed a single class of binding sites and suggests that the observed differences in EGF binding were due to changes in the number of EGF binding sites rather than in the affinity of the receptors (Fig. 2). At 24 h after treatment, EGF binding had apparently returned to the control level and most ulcer lesions were not detected.

As stress-induced ulcer model, restrained rats were immersed in a water bath at 21 ± 1 °C.¹¹⁾ Mucosal lesions increased with the duration of water-immersion. The binding of [125 I]hEGF to mucosal membranes was slightly reduced to 85% of the control level after 1 h exposure to the stress (Fig. 3, $0.05 < p < 0.10$). The EGF binding was significantly decreased to 70% of the control level after 12 h exposure to the stress ($p < 0.05$). The EGF binding had not returned to the control level at 18 h after 6 h-exposure to the stress (data not shown). The binding of [125 I]insulin to gastric membranes was not significantly different from the control by exposure to 1 or 12 h of water-immersion stress.

Discussion

Anti-secretory and cytoprotective actions of

Fig. 3. Effect of Stress-Induced Ulceration on the Binding of [125 I]hEGF or [125 I]Insulin to Gastric Plasma Membranes

Plasma membranes (30 μ g/tube) were incubated with [125 I]hEGF (0.2–0.3 nM) or [125 I]insulin (0.1 nM) for 60 min at 25 °C. Each column is the mean and S.E. of four separate experiments done in triplicate. Hatched column: EGF binding, open column: insulin binding. a) Significant ($p < 0.05$) change compared with the controls.

EGF are considered to be initiated by the binding of EGF to membrane receptors. In previous reports, we have demonstrated EGF receptors on plasma membranes isolated from rat gastric mucosa.⁷⁾ Changes in the EGF receptors were observed in response to nutrient states such as fasting, in which the incidence of gastric ulcer is increased.¹³⁾ Therefore, the present study was undertaken to characterize the effect of ulcer on gastric EGF receptors in a fasting condition.

Functional and morphological alterations are observed in each ulcer model. Aspirin administered into the stomach stimulates gastric mucosa directly and causes a reduction in mucus secretion. Pretreatment with aspirin results in the virtual suppression of prostaglandin generation in the gastric mucosa.¹⁴⁾ Stress-induced ulcers result in a remarkable reduction in gastric blood flow.¹⁵⁾ Generally, several ulcer models are used to evaluate the pharmacological effects of an antiulcer agent. The time profiles of ulcer formation or repair of mucosal lesions in this study were similar to those of previous reports.^{10,11)} The findings in this study demonstrate that the binding property of EGF was changed by chemical or stress-induced ulceration, and that these alterations may be due to the changes in the number of EGF binding sites.

Aspirin-induced or stress-induced gastric ulcers caused a reduction of EGF binding to the plasma membranes. Freidenberg *et al.*¹⁶⁾ used insulin binding as a reference to study the effect of nutrient states on EGF binding to the plasma membranes from rat liver, and we also studied the effect of fasting on insulin binding (as a reference of EGF binding) to gastric plasma membranes in the previous study.⁸⁾ The receptors for insulin were also demonstrated in the rat stomach.¹⁷⁾ The binding of [¹²⁵I]insulin to the gastric membranes prepared from rats immersed in water was not significantly different from that of the control. Therefore the decrease in EGF binding by stress did not represent general phenomena of other receptors on the gastric cell surface. The healing of the mucosal lesions was accompanied with a significantly higher increase in EGF binding than the control level 12 h after aspirin administration. The reasons for this apparent correlation between changes in EGF bind-

ing and ulcer history are uncertain. Konturek *et al.*³⁾ observed that the formation of aspirin-induced ulcers resulted in a significant reduction in deoxyribonucleic acid (DNA) synthesis for a 3 h period and that this reduction was brought back to normal levels by the administration of EGF. It is possible to postulate that the increase in EGF binding 12 h after aspirin treatment causes an increase in DNA synthesis, which may be related to the cytoprotective action of EGF.

During 1–12 h exposure to stress by water-immersion, the formation of gastric ulcerations increased progressively and the duration of the stress was accompanied by a decrease in EGF binding to the gastric membranes. Recently, it has been reported that DNA synthesis reached approximately one half of the control value after 14 h of exposure to stress and the administration of EGF restored it to the value observed in the control rats.¹⁸⁾ There may be a relationship between the reduction in EGF binding and the reduction of DNA synthesis during stress.

Recent studies have revealed that the gastrointestinal levels of TGF- α , which shares the same receptors with EGF, are much higher than those of EGF.^{19,20)} In order to clarify the role of EGF receptors in the gastric mucosa in promotion of the ulcer healing process, it will be important to study TGF- α binding in various ulcer models and to study the changes in gastric EGF receptors by the administration of EGF or TGF- α in ulcer models.

The present study indicates that chemical-induced and stress-induced ulceration affects EGF binding to the plasma membrane receptors on rat gastric mucosa. These results support the hypothesis that the EGF receptors play an important role in ulcer healing and may be important for the development of EGF as an anti-ulcer medicine.

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