Gastroprotective and vasodilatory effects of epidermal growth factor: the role of sensory afferent neurons

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The possible protective effect of intragastric EGF on the gastric mucosa of urethane-anesthetized rats was examined. EGF was dissolved in 0.01 M PBS at the appropriate doses. Gastric mucosal injury was induced by the intragastric application of 60% ethanol (5 ml/kg) through a nasogastric tube. The increase of the gastric mucosal blood flow have been suggested to be the possible mechanisms (12, 16).

Recent studies have shown that capsaicin-sensitive afferent neurons, as well as prostaglandins, play an important role in the gastric mucosal defensive mechanisms in rats. The stimulation of these neurons by intragastric capsaicin alters the gastric mucosal blood flow (27, 28), motility (33), and acid and HCO3 secretion (27, 32) and thus reduces gastric mucosal damage (11, 17, 38, 41). EGF increases gastric mucosal blood flow and induces gastric mucosal protection, possibly via capsaicin-sensitive afferent neurons (16). However, the precise mechanisms of the gastric hyperemic and protective effects of EGF are still not fully understood. The aims of the present experiments were to elucidate 1) whether intragastric EGF protects the gastric mucosa against ethanol injury, 2) whether topically applied EGF dilates the gastric mucosal microvessels, and, if so, 3) what are the mechanisms involved.

MATERIALS AND METHODS

Animal preparation. The experiments were reviewed by the Committee on the Ethics of Animal Experiments at the Graduate School of Medical Sciences, Kyushu University and were done according to the Guidelines for Animal Experiments of the Graduate School of Medical Sciences, Kyushu University and the law (no. 105) and notification (no. 6) of the Japanese Government.

Male Wistar rats (conventional, 250 g) were fasted for 24 h. Free access to tap water was allowed before experiments. After anesthesia with intraperitoneal urethane (1.25 g/kg), the rectal temperature was continuously monitored and maintained between 37 and 38°C with a heating lamp. Systemic blood pressure was monitored via a catheter inserted in the left femoral artery. To avoid dehydration, saline was continuously infused at a rate of 1.5 ml/h via a catheter inserted in the left femoral vein.

Experiment I. The possible protective effect of intragastric EGF on the gastric mucosa of urethane-anesthetized rats was examined. EGF was dissolved in 0.01 M PBS at the appropriate doses. Gastric mucosal injury was induced by the intragastric application of 60% ethanol (5 ml/kg) through a nasogastric tube.

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plastic cannula intubated orally. Sixty minutes after anesthesia, 10 or 30 \( \mu \)g of EGF in 1 ml PBS or vehicle were orally intubated (\( n = 5 \) group). Fifteen minutes later, the ethanol was applied topically. The stomach was removed 60 min thereafter and fixed in 0.5% formalin for 30 min. Then the stomach was cut along the greater curvature and photographed. The percentage of injured corpus mucosa was calculated by computerized image analysis (NIH Image, v. 1.61).

The effect of pretreatment with sensory desensitization by capsaicin, human calcitonin gene-related peptide (CGRP)1 antagonist hCGRP-(8–37), or nitric oxide (NO) synthase inhibitor \( \text{N}^2\text{-nitro-L-arginine methyl ester (L-NAME)} \) was investigated in animals treated with 30 \( \mu \)g EGF or vehicle (\( n = 5 \) group). Capsaicin-sensitive afferent neurons were desensitized through the systemic and functional ablation by capsaicin. As previously described (42), capsaicin was injected subcutaneously in three consecutive doses of 25, 50, and 50 mg/kg (total of 125 mg/kg) during the 2-wk period before the experiment. Sensory desensitization was confirmed by instilling a drop of capsaicin solution (0.1 mg/kg) into an eye of each rat. The instant response with wiping movements toward the eyes was regarded as inadequate desensitization. The capsaicin-pretreated rats with a negative wiping movement test were regarded as functionally ablated and used for the experiment. Either hCGRP-(8–37) (100 nmol/kg) or L-NAME (10 mg/kg) was bolus injected intravenously 10 min before the intragastric administration of EGF or vehicle. L-NAME was given either alone or in combination with L-arginine (300 mg/kg iv) as a substrate for NO synthase.

**Intravital microscopy.** Intravital microscopy was applied by the method reported by Ohono et al. (29), with a slight modification. Briefly, the stomach was exposed through a ventral midline abdominal incision and cut along the greater curvature. After the anterior wall was resected with an electric cautery scalpel (B-3396; Summit Medical, Tokyo, Japan), the posterior wall of the glandular stomach was fixed in a plastic chamber with the mucosal surface facing the window every 9 min (\( n = 6 \) group). Changes in arteriolar or venular diameters of 25–50 \( \mu \)m were measured using the intravital microscopic technique. After surgery, a resting period of at least 15 min was allowed before the application of EGF and then was resumed for 6 min until the next application to wash out the preceding compound.

The effect of pretreatment with sensory desensitization by capsaicin, hCGRP-(8–37), or L-NAME was tested (\( n = 6 \) group). For sensory desensitization, capsaicin at 5 mM was superfused for 10 min after the confirmation of arteriolar dilatation by topical capsaicin at a concentration of 160 \( \mu \)M and then by modified Krebs solution for 60 min. The arteriolar dilatation reached a maximum within 1 min after initiation and then remained at that level for at least 10 min. The arteriolar diameter gradually returned to the basal value within 60 min after the removal of capsaicin. In a preliminary experiment, capsaicin desensitization was confirmed by the second topical capsaicin application 70 min later at a concentration of 160 \( \mu \)M, which has been demonstrated to induce a maximal response (40). Arteriolar dilatation by the second application of capsaicin was <10\%, and therefore the capsaicin-sensitive afferent neurons were considered to be desensitized. Either hCGRP-(8–37) (100 nmol/kg) or L-NAME (10 mg/kg) was bolus injected intravenously 10 min before the topical EGF application (100 \( \mu \)g/ml). L-NAME was given either alone or in combination with L-arginine (300 mg/kg iv).

**Chemicals and treatments.** The following chemicals were used: EGF (kindly provided by Dr. B. Nakajima, Hitachi Chemical, Japan), capsaicin (Wako Chemical, Osaka, Japan), hCGRP-(8–37), L-NAME, L-arginine (Sigma, St. Louis, MO), and ethanol (Wako Chemical, Osaka, Japan). EGF was dissolved in 0.01 M PBS (Sigma) in experiment I and in modified Krebs buffer in experiment II. Capsaicin was dissolved in a solvent composed of 10% ethanol, 10% Tween 80 (Sigma), and 80% vol/vol normal saline (0.15 N NaCl). hCGRP-(8–37), L-NAME, and L-arginine were dissolved in saline containing 0.1% BSA. Ethanol was diluted in distilled water. All chemicals were freshly prepared just before the experiments.

**Statistics.** Values are expressed as means ± SE. Student’s \( t \)-test was used for comparisons of two groups. Significance of differences was determined with a one-way ANOVA followed by Fisher’s protected least significant difference (PLSD) for the comparison of multiple groups. A two-factor repeated-measures ANOVA followed by Fisher’s PLSD was used for the data of serial measurements. \( P \) values <0.05 were considered statistically significant.

**RESULTS**

**Experiment I.** Gastric mucosal lesions 60 min after ethanol injection occupied 24.3 ± 2.6% of the glandular area in vehicle-treated rats. The intragastric application of EGF (10 or 30 \( \mu \)g) significantly reduced the gastric mucosal lesions (12.0 ± 3.5% in the 10 \( \mu \)g group, \( P < 0.01 \), and 7.3 ± 1.3% in the 30 \( \mu \)g group, \( P < 0.001 \), Fig. 1).

Pretreatment with either capsaicin desensitization, hCGRP-(8–37), or L-NAME slightly, but not significantly, increased the gastric mucosal lesions (33.9 ± 5.1\%, 31.1 ± 6.7\%, and 38.2 ± 6.6\%, respectively) in the vehicle-treated rats. All of these pretreatments inhibited the protective effect of intragastric EGF (30 \( \mu \)g) against ethanol-induced mucosal lesion (38.6 ± 3.9\% in the capsaicin desensitization group, 33.0 ± 3.7\% in the hCGRP-(8–37) group, and 36.9 ± 5.1\% in the L-NAME group, \( n = 5 \), Fig. 2). There was no significant difference in the gastric mucosal lesions between the vehicle-treated and EGF-treated rats with...
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Fig. 1. The effect of intragastric epidermal growth factor (EGF) on macroscopic gastric mucosal damage induced by 60% ethanol (n = 5/group). Lesion index (%erosions in glandular stomach) was significantly lower in the groups treated by EGF. *P < 0.05 and **P < 0.001 vs. vehicle-treated group. Error bars represent SE.

DISCUSSION

Our results showed that in urethan-anesthetized rats 1) intragastric EGF prevents ethanol-induced gastric mucosal injury and topically applied EGF dilates the arterioles but not the venules in the basal part of gastric mucosa dose dependently and 2) these effects of EGF are mediated through the capsaicin-sensitive afferent neurons via CGRP- and NO-dependent mechanisms. Because EGF was applied to the serosal side of the glandular stomach in the second experiment, the effect of EGF on the arteriole observed in the experiment may be slightly different from that under physiological conditions. However, our observations suggest that EGF does dilate arterioles in damaged gastric mucosa that lacks an epithelial layer (i.e., gastric ulcer and erosion).

The EGF receptor (EGF-R) has been shown to belong to the type 1 tyrosine kinase receptor family and to be located in the gastric tissue of both rodents and humans (31, 34). At the acute and healing stage of gastric mucosal damage, EGF-R has been shown to be overexpressed in the epithelia (19, 35). It has also been confirmed in rats that the main source of EGF in the gastric contents is the submandibular glands (20, 22), and that growth factor exists at a concentration of 19.6 μg/l in the rat (14). Furthermore, EGF in the salivary glands (9) and in the gastric juice (23) increases by

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severalfold that of the basal value under conditions of gastric mucosal damage induced by various stimuli. Whereas we applied EGF to rats at extremely high concentrations compared with those in physiological conditions, a similar preventive effect of large amounts of EGF against mucosal injury has been shown in other experiments (12, 16).

Intragastric EGF protects the gastric mucosa against various stimuli such as stress, ethanol, hypertonic saline, and aspirin (12, 16, 21, 24, 26). Although parenteral EGF has been shown to decrease gastric acid secretion (25), intragastric EGF revealed a protective effect against aspirin- and stress-induced mucosal damage without reducing acid secretion (24). It thus seems likely that acid suppression alone is not the significant mechanism of the protective effect of EGF in our experiments. The trophic action to the gastric mucosa characterized by increase in DNA, RNA, protein, and mucus secretion (15, 18) has been confirmed, but this effect seems to be unrelated to the preventive effect of intragastric EGF. Cytoprotection through the stimulation of prostaglandin production by EGF (5, 8) has been suggested to be another mechanism for the preventive effect. However, the role of prostaglandin synthesis in the protective effect of EGF still remains controversial because intragastric EGF exhibited a preventive effect even against aspirin-induced mucosal injury of the rat stomach without affecting prostaglandin production (26).

It has been shown in several experiments that intragastric EGF increases the mucosal blood flow of the
stomach. Hui et al. (12) demonstrated that intragastric EGF increased the blood flow of the rat gastric mucosa after topical ethanol treatment, and it also dose-dependently reduced the degree of mucosal damage. Although Hui et al. (12) measured the mucosal blood flow by laser-Doppler flowmetry, we directly observed the arterioles in the gastric mucosa induced by intragastric EGF by using intravital microscopy. Whereas an increase in the gastric mucosal blood flow has been shown in rats treated by subcutaneous EGF (16), the vasodilatory action of EGF seems to be attributed to an extremely topical response, because the arterioles dilated even after the removal of submucosal tissue in our experiment.

Recently, the interaction of EGF and NO in the gastric protection has been investigated in animal experiments (1, 36, 37). Tripp and Tepperman (37) reported in sialoadenectomized rats that subcutaneous EGF did not influence NO synthase activity in ethanol-treated gastric lesions, whereas EGF reduced ethanol-induced mucosal lesions. However, Brzozowski et al. (1) reported that in stress-induced mucosal lesions an increase in the mucosal blood flow induced by subcutaneous EGF was inhibited by either capsaicin desensitization or NO synthase inhibitor. Our results also indicated a close interaction between EGF and NO in unsialoadenectomized rats. The discrepancy in the role of NO in EGF-treated animals may relate to sialoadenectomy or differences in the route of EGF administration. On the basis of these previous data and our results, it seems obvious that EGF induces hyperemia through a NO-dependent mechanism, although the protective effect of EGF may not be explained by an increase of gastric mucosal blood flow alone.

Fig. 5. Comparison of the dilatation of the gastric mucosal arterioles induced by topical EGF (100 μg/ml, n = 6/group). Capsaicin desensitization, hCGRP-(8–37) (100 nmol/kg iv), or L-NAME (10 mg/kg iv) significantly inhibited the dilatation of arterioles. Concomitant treatment with L-arginine (300 mg/kg iv) restored the inhibition induced by L-NAME. *P < 0.05 and **P < 0.001. Error bars represent SE.

In past experiments, capsaicin desensitization for sensory neurons was completed by systemic administration of high dose capsaicin (42), as in our first experiment. The procedure of desensitization seems to induce a systemic functional depletion of capsaicin-sensitive neurons. In experiment II, however, we intentionally applied a high dose of capsaicin directly on the gastric wall, and a substantial desensitization could thus be achieved. The method of desensitization coupled with intravital microscopy may be a model for investigating the role of capsaicin-sensitive afferent neurons in the regulation of mucosal blood flow.
In conclusion, intragastric EGF plays a protective role against gastric mucosal injury induced by ethanol, and the effect may be attributable to hyperemia through stimulation of capsaicin-sensitive afferent neurons and subsequent CGRP- and NO-dependent mechanisms. It is presumed that the dilatation of the arterioles may thus be an essential event in the protective effect of EGF against gastric mucosal injury.

REFERENCES


