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Short communication

Epidermal growth factor protects mouse ileal mucosa from Triton X-100-induced injury

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Abstract

Luminal exposure of mouse ileal mucosal sheets in vitro to 0.3% Triton X-100 for 10 min resulted in a 45.3 ± 4.4% decrease in tissue resistance (R_t). Luminal pretreatment of tissue with epidermal growth factor (EGF, 30 nM) reduced the Triton X-100-induced decrease in R_t to 10.2 ± 3.5%. This effect of luminal EGF was concentration-related with an A_{50} value of 7.4 nM. EGF applied to the basal buffer also produced a similar protective effect, but it was 5.3-fold less potent than luminal EGF. Morphological analysis indicated the formation of denudations at villi tips by Triton X-100 which was prevented by EGF pretreatment. These results suggest that EGF may play a role in the protection of ileal mucosal integrity.

Keywords: EGF (epidermal growth factor); Mucosal protection; Tissue resistance; Receptor; Epithelium

1. Introduction

Epidermal growth factor (EGF) is present in various gastrointestinal secretions (Rao, 1991), and has been shown to regulate a number of gastrointestinal functions, indicating its physiologic and/or pathophysiologic implications for the gastrointestinal tract. In addition to its growth promoting activity (Lebenthal and Leung, 1987) EGF also produces a number of acute effects in the gastrointestinal tract. EGF regulates gastric acid secretion (Rao et al., 1993), duodenal bicarbonate secretion (Marotta et al., 1990), gastrointestinal motility (Hollenberg et al., 1989), intestinal ion transport (Rao and Porreca, 1995), nutrient absorption (Opleta-Madsen et al., 1991) and protects gastrointestinal mucosa from luminal irritants (Konturek et al., 1991). EGF when administered orally or systemically was cytoprotective in gastric ulceration induced by aspirin, stress, ethanol and acid, and duodenal ulceration by cysteamine (Konturek et al., 1991). It is suggested that EGF plays an important role in the maintenance of a healthy epithelial barrier and hence protects the gastrointestinal mucosa from various injurious substances.

The role of EGF in protecting small intestinal mucosa is not well understood. We examined the protective role of EGF in small intestinal mucosa, in mouse ileum in vitro using the Triton X-100-induced mucosal injury model (Moore et al., 1989). The present studies show that EGF pretreatment prevents Triton X-100-induced decrease in R_t and mucosal damage in mouse ileum in vitro.

2. Materials and methods

2.1. Animals

ICR male mice (40 g body weight) fed with a normal diet and maintained at a 12 h light/dark cycle were used for these studies. Animals were fasted in a metabolic cage for 18 h prior to the experiment.

2.2. Preparation of ileal mucosal sheets

The small intestine was rapidly excised from mice under pentobarbital (75 mg/kg b.w.; i.p.) anesthesia. The ileum was cut longitudinally along the mesenteric border and opened into flat sheets. Mucosal sheets were prepared by pinning one edge of a sheet of a whole ileum in bathing media (serosal side up), holding the opposing edge of the tissue taut with a glass slide, and making an incision in the

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serosal surface with the edge of another glass slide. The outer neural and muscular layers are then removed by grasping the exposed edge of the muscularis externa with forceps and removing all components in a single sheet. This dissection methodology was verified experimentally (i.e., a lack of effect with the direct nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium on short circuit current) and by histological examinations.

2.3. Measurements of bioelectric parameters

Intact mucosal sheets of the ileum were mounted to Ussing chambers. Luminal and basal surfaces of the tissue are independently bathed with 10 ml of Krebs-Ringer bicarbonate buffer containing 10 mM mannitol and 10 mM glucose, respectively. Bathing media are oxygenated and maintained at 37°C via a high-impedance dual voltage clamp (World Precision Instruments, Sarasota, FL) and recorded on a 4-channel Gould polygraph recorder.

2.4. Induction of mucosal damage by Triton X-100

After 30 min equilibration time, different doses of EGF (3–100 nM) or the carrier alone were administered to the luminal or basal buffers. 15 min after EGF administration, Triton X-100 was introduced into the luminal buffer to achieve a final concentration of 0.3%. Following a 10 min exposure, the luminal buffer was withdrawn and washed with 50 ml of buffer and finally replaced with a fresh Triton X-100-free buffer. The potential difference that results from the application of a constant current (100 μA) was recorded at different time intervals before and after Triton X-100 treatment. R_t was calculated from the potential difference and the applied current using Ohm's law.

2.5. Microscopic analysis

In separate experiments mucosal sheets were removed immediately after Triton X-100 treatment, washed with the buffer and fixed in buffered formalin. Paraffin sections (1 μm) of these tissues were then stained with hematoxylin and eosin and examined under light microscopy.

2.6. Chemicals

EGF was purchased from Bioscience Products (Madison, WI) and purified by high performance liquid chromatography as described before (Rao, 1995). All other chemicals were of the analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

2.7. Statistics

The concentration-effect curves for EGF were noncumulatively constructed, whereby each tissue was exposed to only one concentration of EGF. The concentration of EGF eliciting 50% of the maximal response (A_{50} values) was obtained from least-square analysis of the linear portions of the concentration-response curves using a computer program (Pharmacological calculations). Comparison between two groups was made by the Student's t tests for grouped data. The significance in all tests was derived at the 95% or greater confidence level.

3. Results

Mucosal sheets of mouse ileum mounted to Ussing chambers maintained a steady state R_t of 31 ± 3 Ω/cm^2 that was stable at least for 5 h. Luminal exposure to 0.3%

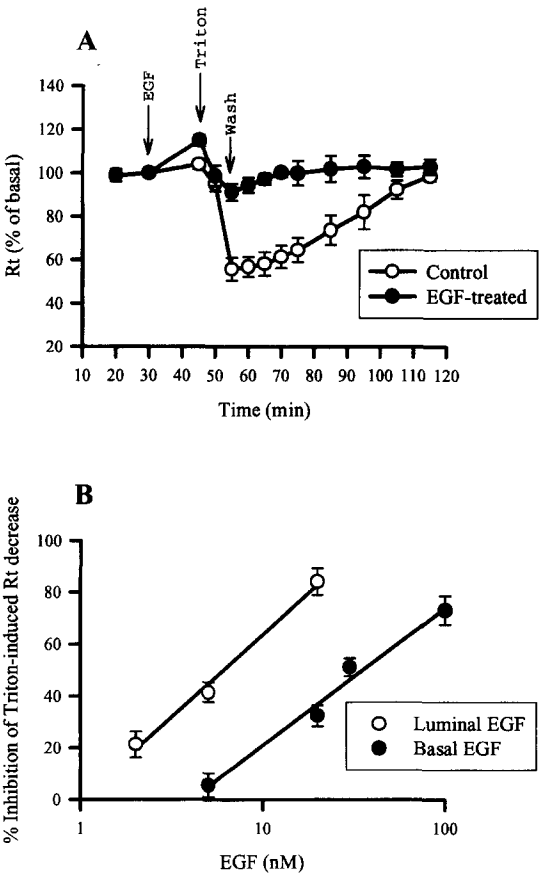

Fig. 1. A: Effect of luminal exposure (between time −10 and 0 min) to 0.3% Triton X-100 on the R_t of mouse ileal mucosa, control or EGF-pretreated (added to luminal buffer at time −15 min to achieve a final concentration of 30 nM). The x axis represents the time after tissue was mounted to Ussing chambers; time used for washing the Triton X-100 off from luminal surface and replacing with fresh buffer containing EGF (which took nearly 1 min) is not included. Values are mean ± S.E.M. of four tissues from four different mice. * Values that are significantly ($P < 0.01$) different from corresponding control values. B: Non-cumulative concentration-effect relationship curves for luminal and basal EGF. Tissues were pretreated with different concentrations of luminal or basal EGF and their mucosal protective effects are evaluated as % inhibition of Triton X-100-induced decrease in R_t. Values are mean ± S.E.M. of 5–7 tissues (each tissue within the group was derived from a different mouse).

Triton X-100 resulted in a 45.3 ± 4.4% decrease in R_t, with a gradual recovery following the removal of detergent (Fig. 1A). The time for 50% recovery was 26.5 ± 5.3 min. In tissues that were luminally treated with EGF (30 nM) 15 min prior to Triton X-100 administration, detergent exposure produced only an 8.2 ± 3.5% decrease in R_t (Fig. 1A); the time for 50% recovery was 5.6 ± 0.9 min. This effect of luminal EGF was highly potent (Fig. 1B) with A_{50} (95% confidence limits) values of 7.4 (5.2–10.5) nM. EGF added to the basal buffer also reduced the Triton X-100-induced decrease in R_t (Fig. 1B), but A_{50} (95% confidence limits) values for basal EGF were 39.1 (25.3–52.5) nM. Luminal or basal pretreatment of mucosal sheets with transforming growth factor-α (TGF-α) (30 nM) inhibited the Triton X-100-induced decrease in R_t by 75 ± 6.3% ($n = 5$) and 59 ± 4.8% ($n = 5$), respectively. On the other hand, pretreatment of mucosal sheets with neuropeptide Y (30 nM) produced no significant effect on the Triton X-100-induced change in R_t.

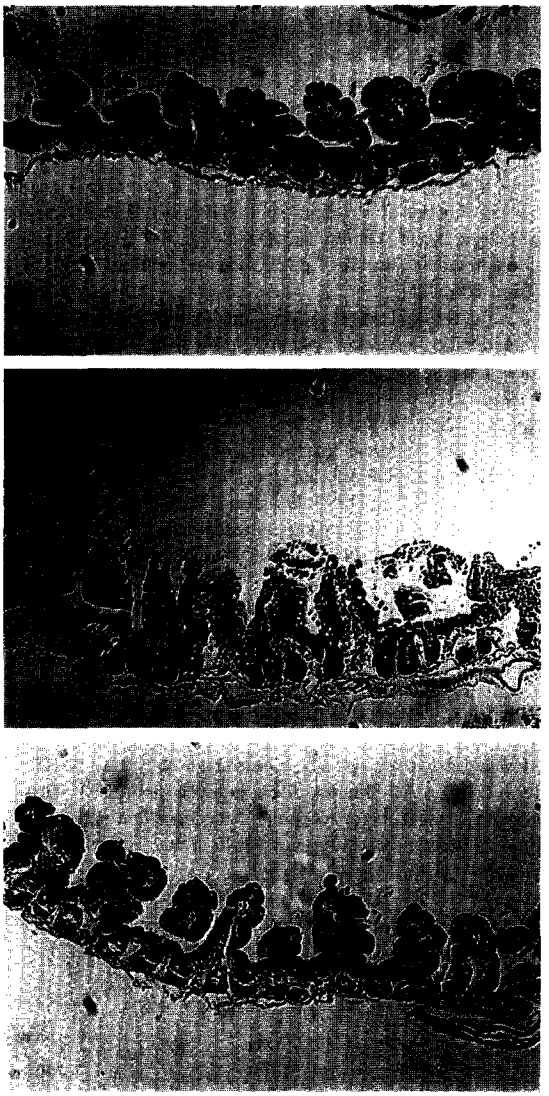

Fig. 2. Light microscopy of cross sections of ileal mucosal sheets, control (top), exposed to Triton X-100 (middle) and pretreated with EGF (30 nM, luminal) and exposed to Triton X-100 (bottom).

Fig. 2 shows the light microscopy of sections of control ileal sheets and those ileal sheets that were exposed to Triton X-100 with or without EGF pretreatment. Denudation at tips of villi was observed in ileal mucosa that was exposed to Triton X-100, while villi in control ileum and EGF-pretreated ileum exposed to Triton X-100 appeared intact.

4. Discussion

The results of the present studies indicate that EGF protects ileal mucosa from Triton X-100-induced damage in vitro. As previously shown in guinea pig ileal mucosa (Moore et al., 1989), a brief exposure to dilute Triton X-100 on the luminal surface resulted in a decreased transileal potential difference and R_t in mouse ileal mucosa. Morphological analysis demonstrated that this decrease in bioelectric properties was previously shown to be caused by development of denudations at villi tips (Moore et al., 1989). The present studies also show that pretreatment of ileal mucosa with EGF 15 min prior to exposure to Triton X-100 significantly reduced the detergent-induced decrease in potential difference and R_t, suggesting that EGF acutely protected ileal mucosa from this luminal insult. A number of in vivo studies have previously demonstrated the mucosal protective action of EGF in the gastroduodenal mucosa (Konturek et al., 1991). EGF administration to rats reduced the ulcer index in gastroduodenal ulcers induced by stress, alcohol, acid or cysteamine (Konturek et al., 1991). The role of EGF in protecting intestinal mucosa however is not clear. In the present study, we show that EGF can protect intestinal mucosa from induced damage, and that this effect of EGF is an acute effect. The mechanism involved in this protection is not clear, but it may involve a stimulated secretion of mucin from goblet cells, bicarbonate secretion, or EGF may acutely modulate cellular functions of villus cells, thus increasing the resistance of these cells against Triton X-100 damage. Histological analysis indicated that the EGF-mediated prevention of Triton X-100-induced decrease in R_t is associated with the prevention of morphological damage of ileal villi. The present studies also show that EGF is highly potent in preventing Triton X-100-induced mucosal damage. The potency of luminal EGF appears to be fivefold greater than that of basal EGF. Previous studies have demonstrated that EGF is released in various gastrointestinal secretions at high concentrations, and is under neurohormonal regulation (Rao, 1991). The protective effect of apical EGF in the present study suggests that luminally released EGF may modulate the intestinal epithelium by binding to the apical surface. However, this interpretation is complicated by the failure to consistently localize EGF specific receptors on the brush

border membranes of the intestine (Thompson, 1988; Scheving et al., 1989). Although it has been repeatedly demonstrated that luminal EGF is biologically active the mechanism responsible is unclear. A recent study demonstrated that luminal EGF protected intestinal mucosa from fatty acid-induced injury by stimulating mucus secretion (Ishikawa et al., 1994), which appears to involve the translocation of EGF to the basal surface possibly via the injured epithelium. Therefore, it remains to be established if luminal EGF played the physiological role in the intact epithelium. It is possible in the present study that luminal EGF may have diffused across the epithelium and acted on the receptors at the basal surface. It however does not explain the greater potency of luminal EGF over basal EGF. Further studies are required to address this issue.

Evidence for physiologic relevance of mucosal protection by luminally released EGF is provided by a number of previous studies investigating the role of salivary EGF in mucosal protection. Rats administered with cysteamine, a duodenal ulcerogen, failed to develop gastric ulcers. However, cysteamine produced gastric mucosal lesions in rats in which the submandibular glands (main source of EGF) had been surgically removed (Olsen et al., 1984). This cysteamine-induced gastric mucosal damage in sialoadenectomized rats was reduced by administration of saliva, but not by saliva from which EGF had been removed. Subsequent studies demonstrated that the ulcer index of gastric lesions induced by acid, stress, indomethacin and ethanol (Olsen et al., 1984; Konturek et al., 1991) was dramatically higher in sialoadenectomized rats, and the ulcer index was reversed by luminal administration of EGF, suggesting that luminal EGF has an important physiologic role in protecting gastric mucosa. Our recent studies (Rao et al., 1994) indicate that salivary EGF plays a significant role in maintaining normal ileal epithelial integrity in mice during fasting. Sialoadenectomy in mice reduced intestinal luminal EGF levels and lowered transileal R_t, while increasing the permeability to ^{51}Cr-EDTA by 15-fold, suggesting the increased paracellular conductance. The effects of sialoadenectomy on ileal permeability were reversed by EGF supplementation.

In summary, the present studies indicate that luminal EGF may have a role in protecting intestinal mucosa. These studies also suggest that one mechanism by which EGF protects the intestinal epithelium may involve its direct interaction with epithelial cell, and acute modulation of cellular function leading to increased resistance against injurious substances.

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