Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3844–3846, June 1986
Cell Biology

Transforming growth factor α inhibits secretion of gastric acid

(epidermal growth factor/Ussing chamber/parietal cell)

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Communicated by Elizabeth D. Hay, February 3, 1986

ABSTRACT **Transforming growth factor α (TGF-α), a protein secreted by transformed cells and related to epidermal growth factor (EGF), was tested for its effects on gastric acid secretion. Guinea pig gastric mucosae were mounted in Ussing chambers and the rate of acid release was monitored by the pH-stat method. When administered prior to the secretagogue, TGF-α prevented the histamine-induced increase in the rate of acid secretion. Similarly, TGF-α caused a decrease in the rate of acid release in tissues that had already been stimulated with histamine. These data show that TGF-α inhibits gastric acid secretion in a manner similar to EGF and that the two growth factors share at least one physiological action unrelated to their mitogenic properties.**

Transforming growth factor α (TGF-α) is a protein that is structurally and functionally similar to epidermal growth factor (EGF). The overall amino acid sequences of these two peptides are about 33% homologous, and there is an even more highly conserved core region (1–4). TGF-α competes with EGF for binding to a receptor on the surface of cultured cells and isolated membranes (5–10), and an antibody to the EGF receptor blocks the mitogenic activity of conditioned medium containing TGF-α (11). TGF-α (or a partially purified preparation containing TGF-α) stimulates phosphorylation of tyrosine residues on the EGF receptor and other substrates (9, 12, 13). TGF-α, like EGF, is mitogenic for cultured cells (5, 9, 14) and in conjunction with transforming growth factor β permits anchorage-independent growth of NRK cells (7–10). *In vivo*, both TGF-α and EGF cause precocious separation of eyelids of newborn mice (15, 16).

In addition to these growth-related phenomena, EGF is known to inhibit gastric acid secretion (17–22). We have previously (22) used an Ussing-chamber preparation of guinea pig mucosae to study EGF's antisecretory properties. This system has the advantages over whole-animal models that the concentrations of test substances can be precisely controlled, that secondary effects arising from interaction of the test substances with other organs are eliminated, and that much smaller quantities of reagents are required. These properties make the *in vitro* guinea pig model ideal for studying the effects of peptides related to EGF.

One possible explanation for the coexistence of EGF and TGF-α is that, besides their shared mitogenic properties, they might also have biological activities unique to each peptide. To determine whether inhibition of acid secretion is one of these unique properties, we examined the effect of TGF-α on the Ussing-chamber preparation of guinea pig mucosa. We report here that TGF-α is capable of inhibiting histamine-stimulated acid secretion in a manner similar to EGF.

MATERIALS AND METHODS

Stomachs from young (200- to 250-g) female guinea pigs were hemisected along the greater and lesser curvatures and placed mucosal-surface down on a piece of Parafilm. The outer muscle layers were removed with fine forceps and both halves of the mucosa were mounted separately in Lucite Ussing chambers (diameter 1.6 cm). The assembled chambers were connected to water-jacketed gas-lift circulators maintained at 37°C. The nutrient surface was bathed in 15 ml of a solution containing 122 mM NaCl, 25 mM $NaHCO_3$, 5.0 mM KCl, 1.3 mM $MgSO_4$, 2.0 mM $CaCl_2$, 1.0 mM KH_2PO_4, 20 mM glucose, and gentamycin (Shering, Kenilworth, NJ) at 100 μg/ml. The solution was gassed with water-saturated 95% O_2/5% CO_2. The luminal surface was bathed in 15 ml of unbuffered saline (150 mM NaCl), and gassed with 100% O_2 that had been bubbled through 500 mM NaOH to remove CO_2. Acid secretion was measured by the pH-stat method; the solution bathing the luminal surface was titrated with 50 mM NaOH and the amount of base necessary to maintain the pH at 5.0 was monitored on a chart recorder. We have previously shown (22) in this system that EGF (at concentrations as low as 60 ng/ml) inhibited histamine-induced acid secretion and that this inhibition was specific in that nerve growth factor (at concentrations as high as 5.3 μg/ml) had no effect.

These Ussing-chamber preparations were used in two series of experiments: first to determine whether TGF-α can prevent histamine from increasing the rate of acid secretion and second to determine whether TGF-α can reduce the rate of acid secretion in tissue already stimulated by histamine. In both series of experiments, we used TGF-α that had been synthesized by a modification of the method of Merrifield and has been characterized previously (9, 16). EGF was isolated from male mouse submandibular glands by the method of Savage and Cohen (23) and its purity was characterized earlier (24). Data were analyzed by two-way (animal and treatment) analysis of variance (ANOVA) and (where appropriate) by Tukey's range test, using the SAS statistical system (SAS Institute, Cary, NC) run on an IBM 4341 computer.

In the first series of experiments, after the tissue had been mounted in the Ussing chamber and allowed to stabilize for 1.5 hr, the luminal and serosal fluids were replaced with fresh solutions. Thirty minutes later, TGF-α or EGF (final concentrations 120 ng/ml) in saline (0.1 ml), or saline alone was added to the serosal bath and, after another 30 min, histamine dihydrochloride (ICN) (in 0.1 ml of saline) was added to the nutrient solution at a final concentration of 100 μM. The treatment each mucosal half received was determined by a design similar to a Latin square design: the treatments were randomized with the restrictions that tissue halves from the same animal received different treatments and that the

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Abbreviations: TGF-α, transforming growth factor α; EGF, epidermal growth factor; ANOVA, analysis of variance.

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number of halves receiving any particular treatment were approximately equal. The acid secretion rate was measured at 10-min intervals, and these measurements were pooled to yield 30-min averages.

In the second series of experiments, after the 1.5-hr stabilization period, the luminal and serosal solutions were changed, and 30 min later histamine was added to the serosal solution (final concentration 100 μM). Forty-five minutes later TGF-α (final concentration 120 ng/ml) or an equivalent volume of saline (0.1 ml) was added to the serosal solution. One half-mucosa from each animal was randomly chosen to receive TGF-α, and the other half received saline. The rate of acid secretion was calculated from 10-min intervals and these were pooled to yield 30-min averages (the period preceding addition of TGF-α/saline was only 15 min).

RESULTS

In the first series of experiments (Fig. 1), histamine caused a 7-fold increase in the rate of acid secretion in control (saline-treated) tissues. Pretreatment with either TGF-α or EGF at 120 ng/ml prevented this increase. During the 30 min between 1 and 1.5 hr after adding histamine, the mean acid secretion rate for tissues treated with TGF-α was 2.0 ± 0.3 μeq/cm^2·hr (±SEM, n = 9), while that for control tissues was 4.2 ± 0.4 μeq/cm^2·hr (±SEM, n = 10); this difference was significant ($P < 0.05$ by Tukey's range test). Similarly, the difference between saline-treated tissues and mucosae treated with EGF (1.6 ± 0.2 μeq/cm^2·hr; ±SEM, n = 9) was significant during the same time period ($P < 0.05$ Tukey's test). The difference at 1–1.5 hr between EGF and TGF-α was not significant ($P > 0.05$, Tukey's test). There were no significant differences between any of the groups prior to addition of histamine ($P > 0.05$ by ANOVA). In both this series of experiments and in the second set, the variability due to animals was not significant ($P > 0.05$ by ANOVA).

In the second series of experiments (Fig. 2), histamine produced a 4-fold increase in acid secretion rate in both groups, and TGF-α reduced the secretion rate to near basal levels. In the 30-min period from 70 to 100 min after adding histamine, the mean acid secretion rate for TGF-α-treated tissue was 1.6 ± 0.3 μeq/cm^2·hr (±SEM, n = 6), while the mean secretion rate for tissues that had received saline was 3.9 ± 0.3 μeq/cm^2·hr (±SEM, n = 6); this difference in acid secretion rate was significant ($P < 0.05$ by ANOVA). Prior to addition of TGF-α and saline, the differences between the two groups were not significant ($P > 0.05$ by ANOVA).

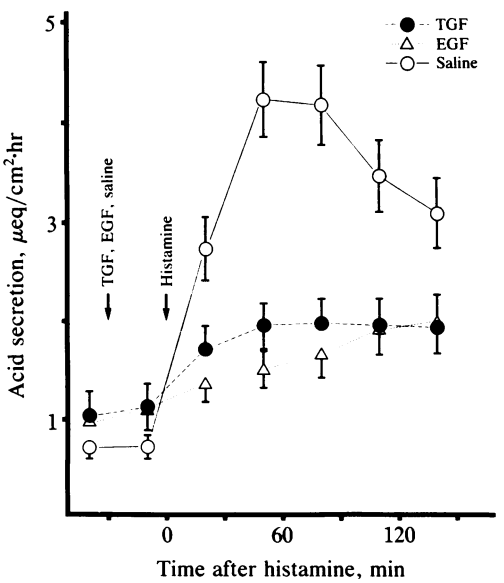

FIG. 1. Effects of EGF and TGF-α pretreatment on histamine-induced acid secretion from guinea pig gastric mucosae maintained in Ussing chambers. At the indicated times TGF-α or EGF (final concentration 120 ng/ml) or saline (0.1 ml) and histamine (final concentration 100 μM) were added to the serosal solution. Acid secretion was measured by the pH-stat method; each point is the average of a 30-min time interval, and error bars indicate SEM.

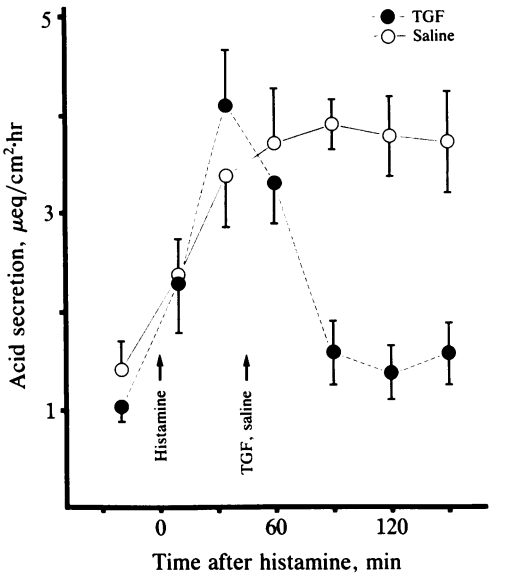

FIG. 2. Effect of TGF-α on acid secretion after histamine treatment of guinea pig gastric mucosa. Mucosae were mounted in Ussing chambers and at the indicated times histamine (final concentration 100 μM) and TGF-α (final concentration 120 ng/ml) or an equivalent volume of saline (0.1 ml) were added to the serosal solution. Acid secretion was measured by the pH-stat method; each point is the average of a 30-min interval (except for the 15-min interval immediately prior to TGF-α/saline addition), and error bars indicate SEM.

DISCUSSION

These results indicate that TGF-α is capable of inhibiting histamine-stimulated secretion of gastric acid. A direct comparison of inhibition by EGF and TGF-α was attempted in the first (pretreatment) experiments, in which (at a concentration of 120 ng/ml) both TGF-α and EGF caused a dramatic (75% and 85%, respectively) inhibition of histamine-stimulated acid secretion. Since it would be useful to know if TGF-α is as effective as EGF at lower doses (where inhibition would be less) we have tested the effects of doses ranging down to 30 ng/ml. It appears that the inhibitory responses to these two growth factors are similar but not identical.

While this work demonstrates that TGF-α (and EGF) inhibit acid secretion by acting on cells within the gastric mucosa, it does not identify which cell type is the target. It is possible that TGF-α (and/or EGF) acts directly on parietal cells. Alternatively, the growth factors could interact with other cells, which then modulate parietal cell activity by a paracrine mechanism. Identifying the cellular site of TGF-α and EGF action will be important in understanding the role of growth factors in regulating acid secretion.

EGF is a puzzling protein in that a wide range of cells have receptors for and respond to EGF, yet its richest sources appear to be the submandibular gland and saliva of mouse. Why, if this peptide is functioning as a general growth-regulating hormone, is it secreted into saliva? The discovery of EGF's effects on gastric secretion and its similarity to TGF-α raised the possibility that for cells outside the gastrointestinal tract, the physiological ligand for the EGF

receptor may not be EGF but instead TGF-α or some other member of the EGF/TGF-α family of peptides. As a corollary, it might be supposed that EGF's effects on the gastric mucosa are unique and perhaps limited to salivary EGF. We (22) and others (21, 25) have shown previously, however, that EGF does not inhibit acid secretion from the luminal surface of the gastric mucosa, which is presumably where salivary EGF would contact the gastric mucosa (the serosal surface was used in the current experiments). This observation, coupled with the present evidence showing that EGF's antisecretory properties are shared by TGF-α, argues against the hypothesis that salivary EGF's unique role is to regulate acid secretion. Despite this, it seems unlikely that there would be an evolutionary advantage to producing two growth factors with nearly identical functions. Further study will need to uncover the differences in the biological properties of these two peptides, and from these differences we should be able to learn much more about the physiological roles of both EGF and TGF-α.

This work was supported by National Institutes of Health Grants CA 36544 (to J.P.T.), AM 15681 (to W.S.), DE 06955 (to R.A.M.), and AM 34854 (to Harvard Digestive Disease Center). A portion of this work has been presented (26).

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