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EPIDERMAL GROWTH FACTOR STIMULATES PROSTAGLANDIN E RELEASE FROM ISOLATED PERFUSED RAT STOMACH

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SUMMARY: Effect of epidermal growth factor, a potent stimulator of growth in many tissues, on immunoreactive-prostaglandin E secretion from isolated perfused rat stomach was investigated. Both mouse and human epidermal growth factor (10^{-7} M) evoked a marked increase in immunoreactive prostaglandin E secretion from the rat stomach. Since prostaglandin E is a potent inhibitor of gastric acid secretion, the present study suggests that the increase in prostaglandin E secretion from the stomach may be responsible for the antisecretory effect of epidermal growth factor on gastric acid.

Epidermal growth factor (EGF), a potent stimulator of growth in a variety of tissues, is a 53-amino acid residue single-chain polypeptide, first isolated from the submandibular glands of male mice (1). It has subsequently been isolated from human urine (2,3) and appears to be identical to human β-urogastrone, a potent inhibitor of gastric acid secretion (4), in view of the similar amino acid composition and biological properties; EGF has gastric antisecretory activity and urogastrone stimulates precocious eyelid opening of the newborn mice (5-8). However, the precise mechanism of gastric acid inhibition by EGF/urogastrone still remains unknown. On the other hand, several bioactive substances with potent inhibition of gastric acid secretion have recently been identified in the stomach, such as somatostatin as demonstrated in isolated perfused rat stomach

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(9,10), and prostaglandin E (PGE) from the stomach of rats (11) and humans (12,13). Thus, these bioactive substances may play an important role in the regulation of gastric acid secretion under physiological conditions.

In this study, the effect of both mouse EGF (mEGF) and human EGF (hEGF) on immunoreactive (IR)-PGE release from isolated perfused rat stomach was investigated in order to ellucidate the mechanism of inhibitory action of EGF on gastric acid secretion.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing between 300 and 350g were used. The animals were kept in a temperature-controlled and air-conditioned room under artificial lighting (lights on 0600-1800h), fed Oriental laboratory chow (Oriental Yeast Co., Tokyo, Japan), and given tap water ad libitum.

Perfusion study: The perfusion of the isolated rat stomach was performed as previously described (9). In brief, all perfusions were accomplished with 4.6% DKRBG[1] gassed with 95% O_2-5% CO_2, and perfused into the left gastric artery by a peristaltic pump at a flow rate of 2 ml/min without recirculation. Both the perfusate and the stomach preparation were kept at 37°C throughout the experiment. After the prestimulation period (20 min) of perfusion with 4.6% DKRBG alone, mEGF or hEGF (10^{-7} M) diluted in DKRBG was infused for 25 min. The final pH of the perfusate was not influenced by infusion of the test materials and maintained at pH 7.4. In the control experiment, the stomach was perfused with 4.6% DKRBG alone throughout the experiment. Only one perfusion was done in each stomach preparation. The gastric venous effluents were collected during every 5 min into tubes containing a mixture of a bacitracin (2×10^{-5} M: Sigma Chemical Co., St. Louis, MO)-Trasylol (1000 KIU/ml: Bayer, Leverkusen, FRG) and stored at -20°C until assayed.

Assay procedures: Determination of PGE in the perfusate was performed by radioimmunoassay (RIA) using anti-PGE_2 serum as previously reported (14). Synthetic PGE_2 was used as a standard and also as the labeled tracer. The antiserum showed little or no cross-reaction with PGA, PGB or PGFα, but a 68% cross-reaction with PGE_1. Therefore, IR-PGE represented the total content of PGE group in this study. The minimum detectable amount of PGE_2 was 50 pg/tube. The antiserum did not cross-react with either human gastrin I, somatostatin, mEGF, or hEGF.

Origin of materials: Human EGF was isolated from human urine according to the method of Cohen et al. (2,3), and mEGF was purchased from Collaborative Research Inc. (Waltham, MA). Synthetic PGE_2 was kindly donated by Ono Pharmaceutical Co. (Osaka, Japan), and [^{3}H]-PGE_2 was purchased from Radiochemical Center (Amersham, England). Human gastrin I (a gift from I.C.I.

[1] Abbreviation: DKRBG, dextran Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose.

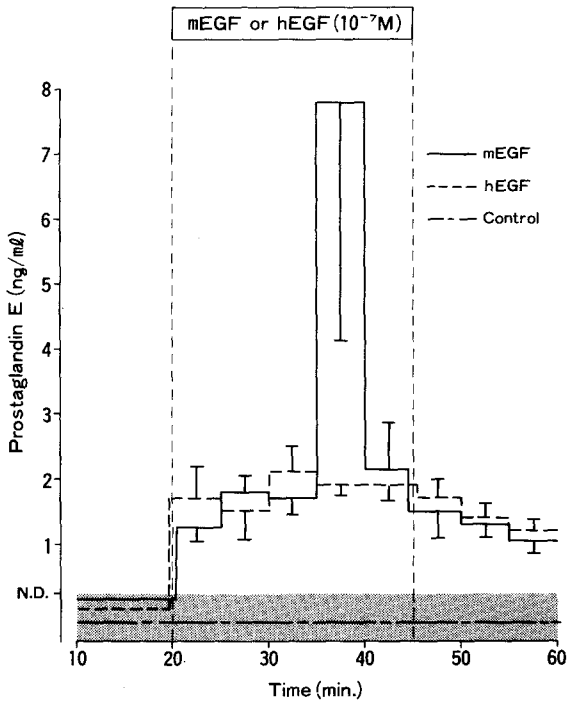

Figure 1. Effects of mEGF and hEGF (10^{-7} M) on immunoreactive PGE secretion from isolated perfused rat stomach. Both mEGF and hEGF were perfused for 25 min after a pre-stimulation period (20 min). Each value represents the mean ± SEM of six experiments.
N.D. ------ not detectable

Ltd., Macclesfield Cheshire) and somatostatin (purchased from Protein Research Institute, Osaka, Japan) were of synthetic origin.

RESULTS

A serial dilution curve generated by the perfusate after stimulation was quite parallel to the standard PGE_2 (data not shown), suggesting that it is immunologically indistinguishable from PGE_2.

During perfusion with 4.6% DKRBG alone, IR-PGE in the perfusate was undetectable throughout the experiment. In contrast, both mEGF and hEGF evoked a profound increase in IR-PGE secretion (Figure 1); the peak value induced by 10^{-7} M mEGF and hEGF were 7.8 ± 3.7 ng/ml (15 min) and 2.1 ± 0.4 ng/ml (10 min), respectively. After the end of the infusion of mEGF or hEGF, IR-PGE levels gradually decreased.

DISCUSSION

In this study, both mEGF and hEGF significantly stimulated the total IR-PGE secretion from isolated perfused stomach of rats. It has been shown that both PGE_1 and PGE_2 are released from the gastric mucosa (12,15). The present study, however, does not indicate which PGEs predominate in the perfusate because the anti-PGE_2 serum used in this experiment does not distinguish PGE_1 from PGE_2.

It has recently been shown that mEGF and hEGF are potent inhibitors of gastric acid secretion induced by a variety of stimuli in animals and humans (3,5), thereby suggesting their possible identity with β-urogastrone. Furthermore, exogenously administered prostaglandin E markedly inhibited the stimulated gastric acid secretion and a possible role of PGEs in the gastric mucosa as endogenous inhibitor for gastric acid secretion has been suggested (16). It has also been reported that, in addition to the mitogenic effect, EGF stimulates PGE_2 production in canine kidney cells (17). Therefore, the increase in PGE secretion in gastric perfusate induced by EGF probably results from its direct stimulation of prostaglandin production by the gastric mucosa. PGE production by gastric mucosa may indeed mediate the inhibitory action of EGF on gastric acid.

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