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The Effects of Gastrin, Epidermal Growth Factor, and Somatostatin on DNA Synthesis in a Small Intestinal Crypt Cell Line (IEC-6)[1] (42484)

CHRIS N. CONTEAS*,† AND ADHIP P. NANDI MAJUMDAR*,‡,[2]

Department of Medicine, Veterans Administration Medical Center, Martinez, California 94553, and Departments of †Medicine and ‡Biological Chemistry, University of California, School of Medicine, Davis, California 95616

Abstract. Exposure of IEC-6 cells for 24 hr to either gastrin (50–500 ng/ml) or EGF (100–500 ng/ml) significantly stimulated (100–165%) the rate of [^{3}H]thymidine incorporation into DNA (referred to as DNA synthesis) when compared with the corresponding basal levels. Somatostatin (10–500 ng/ml) produced no apparent change in DNA synthesis in IEC cells. On the other hand, somatostatin completely inhibited the EGF-induced rise in DNA synthesis. The gastrin-mediated stimulation in DNA synthesis was not affected by somatostatin. The rate of DNA synthesis in IEC cells in the presence of both gastrin and EGF was found to be greater (additive) than that caused by either of the peptides alone. A similar but less dramatic change in the actual number of cells (assessment of cell replication) was observed when the IEC cells were exposed for 24 hr to gastrin, EGF, and somatostatin, either alone or in combination. Whereas gastrin (250 ng/ml) and EGF (250 ng/ml) by themselves increased the number of cells significantly by 29 and 37%, respectively, together they caused a 72% stimulation, when compared with the basal levels. Somatostatin by itself caused no apparent change in IEC cell population, but it significantly inhibited the EGF- but not the gastrin-induced stimulation in IEC cell replication. It is concluded that both gastrin and EGF exert a direct proliferative effect on IEC cells, and the EGF action is regulated by somatostatin.

In recent years a variety of evidence has appeared to show that a number of gastrointestinal (GI) hormones/peptides including gastrin, somatostatin, and epidermal growth factor (EGF) regulate growth of the GI tract (1, 2). Whereas in rats a single or multiple injections of either gastrin or EGF stimulates GI mucosal cell proliferation (1–6), prolonged infusion of somatostatin has been found to inhibit gastric mucosal cell division (7). In addition, administration of somatostatin together with gastrin has been shown to diminish the gastrin-mediated stimulation of cell proliferation in the gastric mucosa (7), indicating an interaction between the two hormones. Whether such an interaction is the result of a direct effect of somatostatin or occurs indirectly through neural, endocrine, or paracrine system remains to be evaluated. In a number of cancerous cell lines, however, somatostatin has also been found to inhibit EGF-induced cell proliferation (8, 9). Little is known whether such an interaction occurs in normal cells. Thus to evaluate further the role of GI hormones/peptides in the regulation of GI mucosal cell proliferation, we have examined the changes in DNA synthesis in IEC-6 cells as well as IEC cell populations in culture in response to gastrin, somatostatin, and EGF, either alone or in combination. The IEC-6 cell line, originally derived from the mucosa of the proximal small intestine of germ-free Sprague-Dawley rats, is a nontumor continuous cell line. This cell line also retains many of the antigenic and physical properties of the small intestinal crypt cell (10, 11), and consequently offers the opportunity to study the role of various endo- and exogenous factors in the regulation of structural and functional properties of crypt cells.

Materials and Methods. IEC-6 cells, originally developed by Quaroni *et al.* (10), were obtained from the American Type Culture Collection (Rockville, MD). The cells were propagated in 75-cm polystrene flasks (Falcon Plastics, Oxnard, CA) in Dulbecco's modified Eagle's medium (DMEM) containing 5% di-

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[2] Present address: Research Service (151), VA Medical Center, Allen Park, MI 48101.

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alyzed fetal bovine serum (Gibco Laboratories, Grand Island, NY), 4 m*M* glutamine, and 10 μg/ml insulin. Dialyzed fetal bovine serum was used to reduce the level of various growth factors in the serum. The cultures were maintained in a water-saturated atmosphere with 5% CO_2 at 37°C. The cells were harvested by trypsinization and reseeded in wells at a density of 0.5–1.0 × 10^6 cells per well. The cells were allowed to reach 50% confluency and then were incubated further in the absence and presence of gastrin (G-17I, Peninsula Laboratory, Belmont, CA), somatostatin (Sigma Chemical Co, St. Louis, MO), or EGF (Collaborative Research, Waltham, MA), either alone or in combination, as stated in the legends to the figures. [*methyl*-^{3}H]Thymidine (40 mCi/mmol; Amersham, Arlington Heights, IL) was added (1 μCi/ml) 3 hr prior to termination of the incubation. The cells were harvested by trypsin–EDTA treatment (1 ml of 0.05% trypsin–0.02 *M* EDTA for 5 min at 37°C). The cells were washed with DMEM and finally dispersed with 0.2 *N* perchloric acid (12, 13). DNA and protein were extracted from the perchloric acid precipitates as described elsewhere (12, 13) and DNA was counted for radioactivity. Since the DNA content in the extracted material fell below the detection limit, the results were expressed as dpm/mg protein. Protein content was determined by the Coomassie blue method using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

In some experiments the harvested cells were suspended in DMEM and counted under a microscope employing a hemocytometer.

The data were statistically evaluated by Student's *t* test for nonpaired values, taking $P < 0.05$ as the level of significance.

Results. Exposure of IEC-6 cells for 24 hr to either gastrin (50–500 ng/ml) or EGF (100–500 ng/ml) resulted in a significant 100–165% increment in [^{3}H]thymidine incorporation into DNA (will also be referred to as DNA synthesis) when compared with the corresponding basal levels (Fig. 1). None of the concentrations of somatostatin (25–500 ng/ml) produced any significant change in the rate of DNA synthesis in IEC cells (Fig. 1).

The combined effects of gastrin, EGF, and somatostatin on DNA synthesis in IEC cells were then investigated. The results are shown in Fig. 2. In the presence of either gastrin or EGF (250 ng/ml), the rate of DNA synthesis was significantly increased by 150–175% when compared with the basal levels. Again somatostatin produced no apparent change in DNA synthesis. However, somatostatin completely abolished the EGF-induced rise in DNA synthesis. The gastrin-mediated stimulation in DNA synthesis was not affected by somatostatin. Addition of gastrin together with EGF produced an additive effect, resulting in a 300% enhancement in DNA synthesis when compared with the basal incorporation.

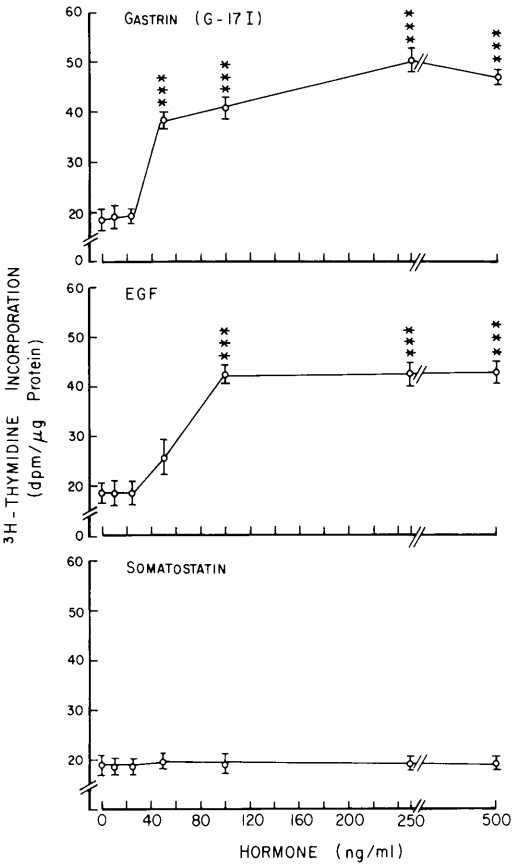

FIG. 1. Effects of increasing concentrations of gastrin, EGF, and somatostatin on [^{3}H]thymidine incorporation into DNA of IEC-6 cells. Incubations were performed for 24 hr in the absence (basal) or presence of one of the hormones. [^{3}H]Thymidine was added 3 hr prior to termination of the incubation. Each value represents the mean ± SEM of six observations. ***$P < 0.001$, when compared with the basal controls.

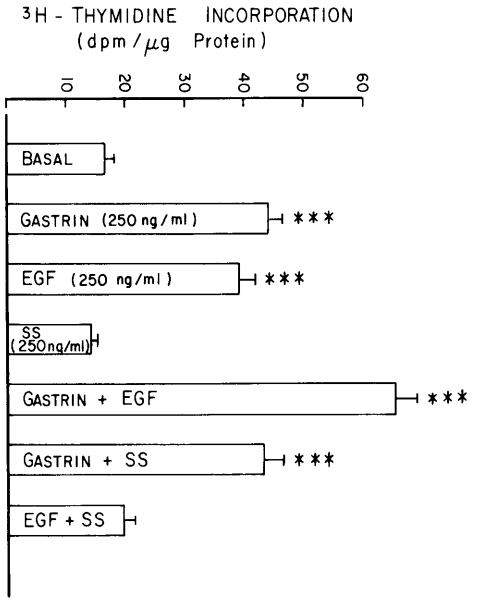

FIG. 2. Effects of gastrin, EGF, and somatostatin (ss), either alone or in combination on [^{3}H]thymidine incorporation into DNA of IEC-6 cells. Incubations were performed for 24 hr. [^{3}H]Thymidine was added 3 hr prior to termination of the incubation. Each value represents the mean ± SEM of six observations. ***$P < 0.001$ when compared with the basal controls.

The next experiment was undertaken to determine the responsiveness of the IEC cells to the length of exposure of gastrin and EGF. Although incubation of cells for only 1 hr with gastrin or EGF resulted in a 22–40% increment in DNA synthesis over the basal levels, the magnitude of stimulation increased with longer period of exposure (Table I). Thus, incubation of IEC cells for 24 hr with gastrin or EGF resulted in about 350% enhancement in DNA synthesis when compared with the basal values (Table I).

Cell replication is the end point of the cell cycle. The last experiment, therefore, was undertaken to determine the combined effects of gastrin, EGF, and somatostatin on cell replication. As observed for DNA synthesis, gastrin and EGF by themselves caused a significant (29–37%) stimulation in IEC cell replication, as evidenced by the increased number of cells (Table II). Again, EGF together with gastrin produced a greater stimulation (72%) in cell replication than that caused by either of the peptides alone when compared with the basal levels (Table II). Somatostatin by itself was ineffective but it significantly decreased the EGF-induced stimulation in IEC cell replication (Table II). The gastrin-mediated stimulation in cell replication was not affected by somatostatin (Table II).

TABLE I. EFFECT OF DURATION OF INCUBATION OF IEC-6 CELLS WITH GASTRIN OR EGF ON [^{3}H]THYMIDINE INCORPORATION INTO DNA

Incubation condition	Incubation time (hr)	[^{3}H]Thymidine incorporation (dpm/μg protein)
Basal	24	7.5 ± 0.2
+ Gastrin	1	10.3 ± 0.3*
	3	15.0 ± 0.3**
	24	34.0 ± 0.9**
+ EGF	1	9.2 ± 0.4
	3	15.8 ± 0.5**
	24	33.5 ± 1.5**

Note. IEC-6 cells were incubated with gastrin (250 ng/ml) or EGF (250 ng/ml) for 1 or 3 hr, washed subsequently, and reincubated for another 19 or 17 hr in the absence of the peptides. The basal and 24-hr samples were incubated in the absence and presence of hormones (gastrin or EGF), respectively, for 24 hr. To each sample [^{3}H]thymidine (1 μCi/ml) was added 3 hr prior to termination of the reaction. Each value represents the mean ± SEM of six observations.

* $P < 0.05$, ** $P < 0.001$, when compared with the basal levels.

TABLE II. EFFECTS OF GASTRIN, EGF, AND SOMATOSTATIN, EITHER ALONE OR IN COMBINATION OF IEC-6 CELL POPULATIONS

Incubation condition	Cell number (×10^4/ml)
Basal	59.5 ± 2.1
Gastrin	76.6 ± 1.7**
EGF	81.8 ± 1.9**
Somatostatin	58.9 ± 2.0
Gastrin + EGF	102.5 ± 2.1**
EGF + somatostatin	68.0 ± 1.9*,+
Gastrin + somatostatin	70.3 ± 2.0**

Note. Incubations were performed for 24 hr. At the end of the incubation period the cells were harvested by trypsin–EDTA treatment, washed with DMEM, and resuspended in the same medium. A small aliquot was counted under a microscope employing a hemocytometer. Each value represents the mean ± SEM of eight observations.

* $P < 0.005$, ** $P < 0.001$, when compared with the basal levels.

+ $P < 0.001$, when compared with the EGF-treated cells.

Discussion. The results of our present investigation demonstrate that IEC cells (small intestinal crypt cells) in culture are responsive to gastrin and EGF as evidenced by stimulation in DNA synthesis. Although somatostatin by itself caused no apparent change in DNA synthesis, it completely abolished the EGF-induced increment in DNA synthesis. However, somatostatin did not affect the gastrin-mediated stimulation in DNA synthesis.

Gastrin is now considered to be a trophic hormone for the mucosa of various regions of the gastrointestinal tract, including the oxyntic gland area of the stomach, and the small and large intestines (1, 2, 14–17). Most of the observations in support of this contention came from *in vivo* studies, where various parameters of growth-related processes were measured after administration of gastrin to experimental animals. Little effort has been made to determine whether gastrin exerts a direct growth-promoting effects on mucosal cells of the digestive tract. Using organ cultures of oxyntic gland mucosa, Sutton and Donaldson (18) showed that although most secretagogues stimulated pepsiongen synthesis, only gastrin was capable of stimulating the synthesis of structural protein. In a similar organ culture system, Shield *et al.* (19) observed increased protein synthesis by pentagastrin in the stomach but not in the duodenum, jejunum, and colon of rabbits. However, none of these studies were directed to evaluate the proliferative changes of the muscosa by gastrin. Our current data demonstrate that in IEC cells gastrin is able to stimulate DNA synthesis, indicating a direct cellular proliferative effect of the hormone on these cells. The proximal small intestinal origin of IEC cells further indicates that small intestinal cell proliferation is also affected by gastrin.

Like gastrin, EGF was also found to stimulate DNA synthesis in IEC cells. This is not surprising since EGF, a mitogenic peptide, has been shown to stimulate cell proliferation in a number of tissues, including the stomach and small intestine (3–6). We have recently demonstrated that incubation of small intestinal explants from pre- and neonatal rats with EGF stimulates protein and DNA synthesis, indicating a direct proliferative effect of the peptide on the small intestine (20). These findings are in complete agreement with the present observation.

Both gastrin and EGF also stimulated IEC cell replication. This interpretation came from the observation that the number of IEC cells was significantly increased following exposure of the cells to gastrin and EGF for 24 hr.

Unlike gastrin and EGF, somatostatin had no direct proliferative effect on IEC cells, since presence of this hormone produced no apparent change either in DNA synthesis or in cell number. However, when somatostatin was added together with EGF, it significantly decreased the stimulatory effect of EGF. Such an observation is similar to what has been noted in a variety of other systems, where EGF and somatostatin have been found to act in an antagonistic or competitive manner at biochemical (8, 9, 21) and functional (22) levels. This has been thought to be the result of alteration of the functional property of the EGF receptor by somatostatin. Whereas EGF has been found to stimulate tyrosine kinase resulting in phosphorylation of tyrosine residues of the EGF receptor protein, somatostatin has been shown to cause protein dephosphorylation through induction of phosphoprotein phosphatase activity (21, 23). Such an antagonistic control of the putative message system could in part be responsible for the observed inhibition of the EGF-mediated stimulation of IEC cell proliferation by somatostatin. The finding that gastrin together with EGF produced a greater rise in both DNA synthesis and cell number than either of the peptides alone suggests for different postreceptor mechanisms for these peptides in IEC cells.

In conclusion, our current data demonstrate that both gastrin and EGF exert a direct proliferative effect on IEC cells. However, whereas somatostatin has been found to affect the action of EGF, no such interaction between somatostatin and gastrin could be demonstrated.

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