PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 396–402 (1987)

Gastrin Induction of Protein Synthesis in Isolated Gastric Mucosal Cells (42560)

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Abstract. Exposure of isolated rat gastric mucosal cells to 10^{-10} and 10^{-9} *M* gastrin (G-17-I) for 2 hr significantly stimulated [^{3}H]leucine incorporation (15 min pulse) into protein by 100 and 212%, respectively, when compared with the basal levels. Doses beyond 10^{-9} *M* lowered the maximal stimulatory effect of the hormone. Gastrin (10^{-9} *M*) specifically stimulated the synthesis of five proteins in isolated gastric mucosal cells with apparent molecular weights of 105, 76, 71, 63, and 54 kDa. Actinomycin-D (10 μg/ml) completely abolished the gastrin-mediated stimulation of protein synthesis in isolated gastric mucosal cells.

Gastrin is now considered to be a trophic hormone for mucosa of various regions of the gastrointestinal tract including the oxyntic gland area of the stomach (1, 2). One of the earliest indications of gastrin-mediated stimulation of gastrointestinal mucosal growth is the increment in the rate of protein synthesis. Johnson *et al.* (3, 4) have reported that in fasted rats, a single injection of pentagastrin or human synthetic gastrin (G-17) stimulates amino acid incorporation into gastric and duodenal mucosal proteins within 1–2 hr. We have further demonstrated that the capacity of gastric and colonic polyribosomes to synthesize protein in a cell-free system is greatly stimulated 1 hr after a single injection of pentagastrin or one of the circulating forms of gastrin (5, 6). However, little is known whether the isolated gastric mucosal cells would also respond to the protein stimulatory effect of gastrin. In the present investigation the changes in the rate of protein synthesis in isolated gastric mucosal cells were examined following exposure to gastrin. In addition, efforts were made to determine whether the gastrin-mediated stimulation in gastric mucosal protein synthesis affects all or some specific proteins.

Materials and Methods. *Materials.* L-[3,4,5-^{3}H]leucine (147 Ci/mmol) and L-[U-^{14}C]leucine were purchased from New England Nuclear Corp. (Boston, MA) and Amersham/Searle (Arlington Heights, IL), respectively. Collagenase (Type IA) and hyaluronidase were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (BSA), Fraction V, was a product of Miles Laboratory (Elkhart, IN) and human synthetic gastrin G-17-I was obtained from Peninsula Laboratories (Belmont, CA).

Isolation of gastric mucosal cells. Gastric mucosal cells were isolated with a slight modification of the procedure described by Magous and Bali (7). Briefly, the oxyntic gland area of the stomach from 16-h-fasted adult male Sprague–Dawley rats was pinned to a dental wax plate containing Hepes–Ringer (HR) buffer, pH 7.5 (8). Mucosa was stripped from the muscle layer, minced finely, and incubated for 15 min at 37°C in HR buffer containing 0.6% collagenase and 0.5% hyaluronidase, followed by a 10 min incubation in a fresh HR buffer containing 2 m*M* EDTA and then for another 60 min in the same buffer without EDTA. At the end of the incubation period, the tissue fragments were mechanically dispersed by suction through a wide pipet orifice and then strained through a nylon mesh (180 μm). Mucosal cells were collected by centrifugation at 200*g* for 3 min, washed four times in HR buffer, pH 7.5, and finally suspended in the same buffer without leucine. A small aliquot was mixed with an equal volume of 0.4% trypan blue in HR buffer, and exclusion of the dye by the cells, as an assessment of cellular viability, was monitored. Preparations containing over 95% viable cells were used.

Electron microscopy. An aliquot of the cell suspension was centrifuged and the pellet containing the cells was fixed in 4% glutaraldehyde in cocodylate buffer and postfixed in 1% osmium tetroxide. The specimen was dehydrated in graded concentrations of ethanol as described previously (9). One-micron-me-

0037-9727/87 $1.50

ter-thick sections were stained with toluidine blue to localize the area of interest (9), and examined and photographed in a Zeiss EM 109 electron microscope.

Incubation conditions and processing samples. In all experiments 1-ml aliquots of cell suspension were incubated at 37°C in the absence and presence of gastrin (G-17-I) as detailed in the legends to the figures. [^{3}H]Leucine (5 μCi/ml) was added 15 min prior to termination of the reaction by cycloheximide (1 m*M* final concentration). The cells were recovered by centrifugation at 250*g* for 5 min, homogenized in 1 ml of double distilled water in an Ultra-Turrax tissue homogenizer. To an aliquot (0.1 ml) of the homogenate 1 ml 10% tricholoroacetic acid (TCA) containing leucine (1 mg/ml) was added to precipitate protein.

After heating at 90°C for 20 min, protein precipitates were collected on a Whatman glass fiber (GF/C) filter. Each filter was washed with 15–20 ml of TCA–leucine solution and finally with 10 ml of ethanol–ether (1:1, v/v). Each filter was transferred into a scintillation vial, and 0.5 ml of NCS–isopropanol mixture (1:1, v/v) was added to each. After 60 min at room temperature, 0.025 ml of concentration of formic acid was added and then counted for radioactivity in 5 ml scintillation cocktail in a liquid scintillation spectrometer. Protein content was determined by the Coomasie blue method using Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA). In some experiments, ^{3}H-labeled proteins (single-label experiment) from control and gastrin-treated cells were electrophoresed on an SDS–polyacrylamide slab gel as detailed below.

To determine whether gastrin stimulates the

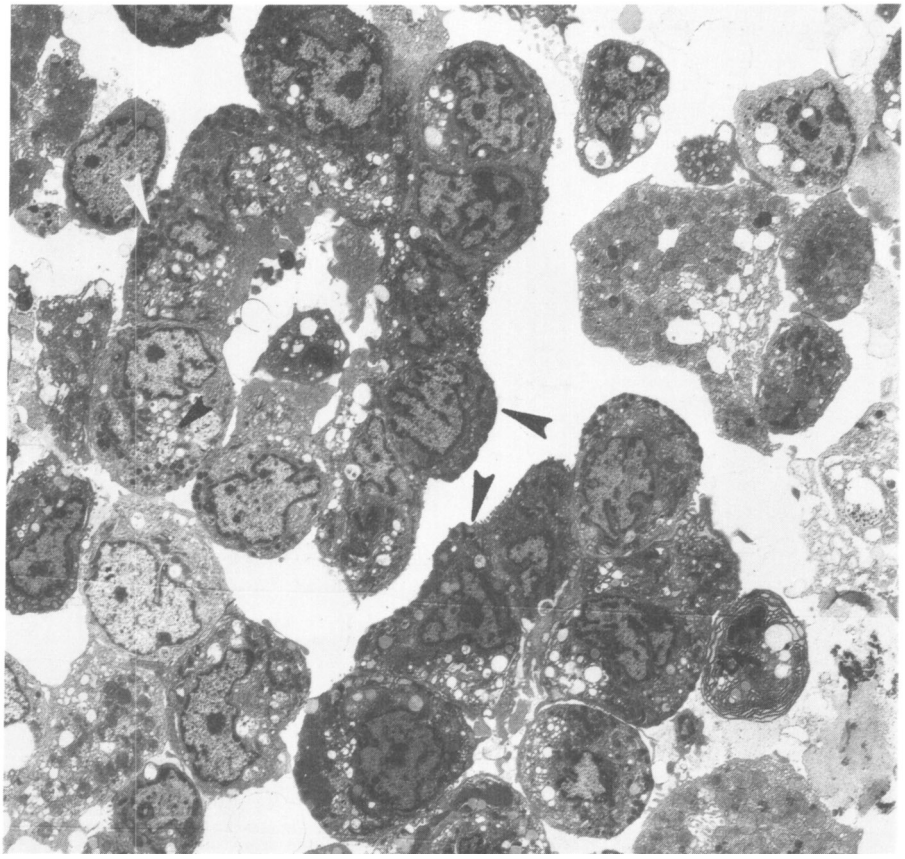

FIG. 1. Electron micrograph of isolated gastric mucosal cells. Photomicrograph shows abundant parietal cells (large black arrow). Cells containing zymogen granuales (white arrow) and mucin (small black arrow) are also shown. Final magnification was ×3680.

rate of synthesis of some specific proteins in the gastric mucosa, dual-labeling technique (10) was utilized. Experimental conditions were the same as described above for the single label study with the exception that the control incubations contained [^{14}C]leucine (2.5 μCi/ml) and those incubated with gastrin contained [^{3}H]leucine (10 μCi/ml). At the end of the incubation, cells were recovered by centrifugation at 1000g for 5 min, homogenized in water, and then sonicated. The suspension was centrifuged at 1000g for 10 min and the protein content of the supernatant was determined. Equal amounts of proteins labeled with [^{14}C]leucine (control incubation) were mixed with those labeled with [^{3}H]leucine (incubation in the presence of gastrin). The samples were mixed with the loading buffer (11), heated at 100°C for 3–4 min, and electrophoresed on a 10% polyacrylamide slab gel containing 0.1% SDS (12). After electrophoresis the gel was fixed for 1 hr in a fixing solution containing 250 ml methanol, 50 ml acetic acid, and 200 ml water and then cut into 1-mm slices. Each slice was dispersed in 0.5 ml of 30% hydrogen peroxide (overnight at 55°C) and dissolved in 0.25 ml NCS–isopropanol (1:1, v/v) solution by incubating at 55°C for 4–5 hr. The samples were counted for radioactivity in 5 ml scintillation cocktail containing 0.025 ml concentration of formic acid.

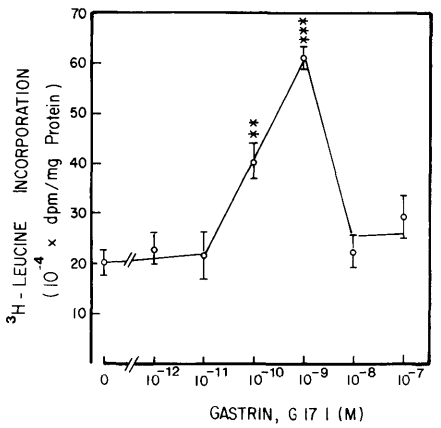

FIG. 2. Effect of increasing concentrations of gastrin on [^{3}H]leucine incorporation into protein in isolated gastric mucosal cells. Incubations at 37°C were performed for 120 min in the absence (basal) and presence of gastrin. [^{3}H]Leucine (5 μCi/ml) was added 15 min prior to termination of the incubation. Each value represents the mean + SEM of six observations. $^{**}P < 0.005$, $^{***}P < 0.001$, when compared with the basal controls.

TABLE I. EFFECTS OF GASTRIN AND ACTINOMYCIN-D, EITHER ALONE OR IN COMBINATION ON [^{3}H]-LEUCINE INCORPORATION INTO PROTEIN IN ISOLATED GASTRIC MUCOSAL CELLS

Incubation condition	[^{3}H]Leucine incorporation (10^{-3} × dpm/mg protein)
Basal	23.3 ± 1.5
Gastrin	45.4 ± 4.0[a]
Actinomycin-D (10 μg/ml)	34.5 ± 7.0
Gastrin and actinomycin-D	19.9 ± 2.0

Note. Incubations were performed at 37°C for 2 hr in the absence (basal) and presence of gastrin and actinomycin (alone or in combination). [^{3}H]Leucine (5 μCi/ml) was added 15 min prior to termination of the reaction by cycloheximide (1 mM). Each value represents the mean ± SEM of five experiments.

[a] $P < 0.025$ when compared with the basal level.

Results. Mucosal cells, isolated from the oxyntic gland area of the stomach predominantly contained parietal cells (Fig. 1). They retained their structural integrity, and the cellular membrane revealed no sign of damage (Fig. 1).

In the first series of experiments the effects of increasing concentrations of gastrin on the rate of [^{3}H]leucine incorporation into protein (will also be referred to as protein synthesis) were investigated. The results are shown in Fig. 2. Concentrations of gastrin up to 10^{-11} M produced no apparent change in [^{3}H]leucine incorporation into protein in isolated gastric mucosal cells. However, gastrin at a dose of 10^{-10} and 10^{-9} M increased protein synthesis by 100 and 212%, respectively, compared to the basal levels. Doses beyond 10^{-9} M were found to lower the maximal stimulatory effect of the hormone.

To determine whether ongoing RNA synthesis is required for gastrin-induced stimulation of protein synthesis in isolated gastric mucosal cells, incubations were performed in the absence and presence of actinomycin-D. Whereas in the absence of actinomycin-D gastrin caused a 93% stimulation in protein synthesis over the basal level, in the presence of this antibiotic the stimulatory effect of gastrin was totally abolished (Table I). Although the concentration of actinomycin-D (10 μg/

ml) used in the present study inhibited RNA synthesis in isolated gastric mucosal cells by about 80% (data not shown), the rate of [^{3}H]leucine incorporation into protein was found to be 48% higher in the presence of actinomycin-D alone than in the control (Table I).

To determine whether the rate of synthesis of different protein is affected by gastrin, equal amounts of protein ([^{3}H]leucine labeled protein, single label experiment) from basal and gastrin-treated cells were electrophoresed on an SDS–polyacrylamide slab gel. Radioactive profile from both samples revealed several peaks indicating *de novo* synthesis of various proteins. It was also observed that [^{3}H]leucine incorporation into most of the proteins from gastrin-treated cells was higher than in the corresponding control (Fig. 3).

To further determine whether gastrin might specifically stimulate the synthesis of certain protein(s), a dual labeling experiment was performed. In this experiment, [^{3}H]leucine was added to those cells which were incubated with gastrin (10^{-9} *M*), whereas the control incubations contained [^{14}C]leucine. Proteins, extracted from the control and gastrin-treated cells, were mixed in equal proportions and subsequently electrophoresed on an SDS–polyacrylamide gel. Measurement of ^{3}H/^{14}C ratios of gel has revealed five distinct peaks with apparent molecular weights of 105, 76, 71, 63, and 54 kDa (Fig. 4).

Discussion. Prolonged hypergastrinemia in man and in experimental animals has long been known to result in gastric mucosal hyperplasia (13–15). In short-term experiments, growth-promoting effects of gastrin were documented by stimulation in the rate of synthesis of DNA, RNA, and protein in the gastric mucosa following single or multiple injections of the hormone (3–6, 16–18). Little effort has

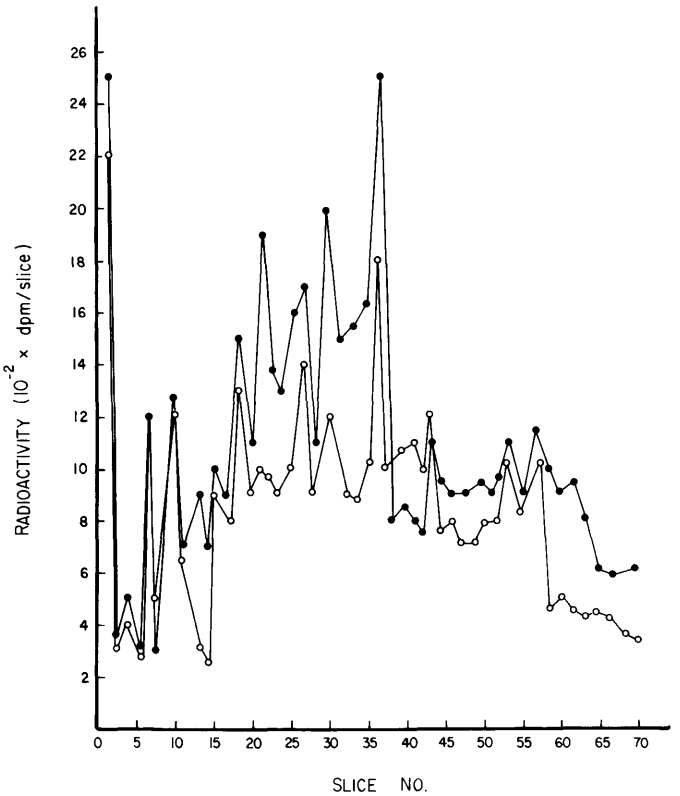

FIG. 3. Electrophoretic profile of proteins from isolated gastric mucosal cells, incubated in the absence (○) and presence (●) of 10^{-9} *M* gastrin. Equal amounts (50 μg) of proteins from both sets of samples were electrophoresed.

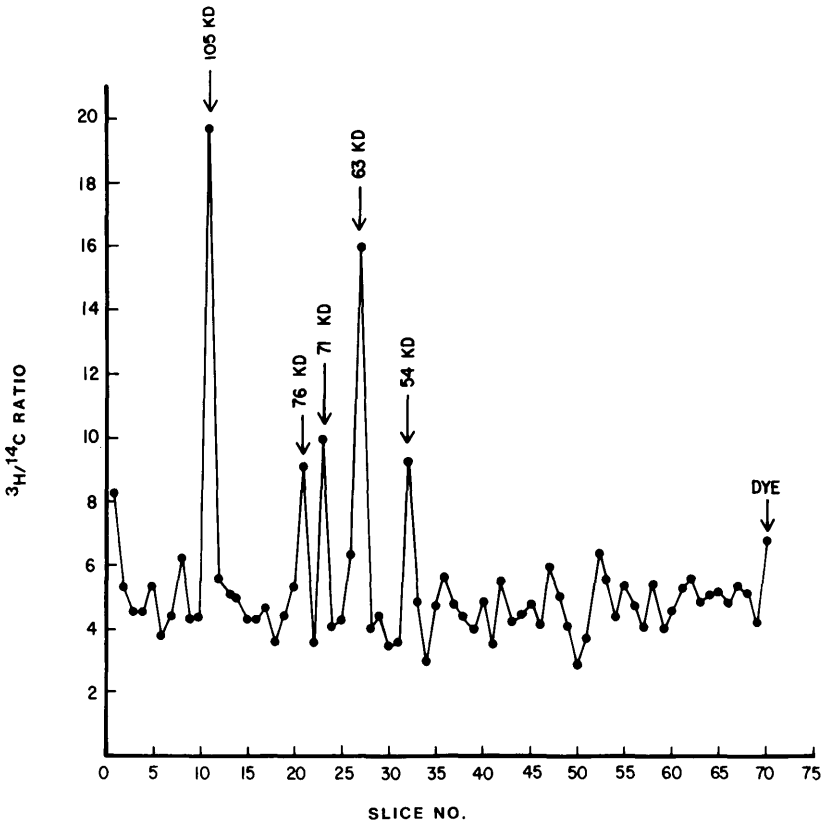

FIG. 4. $^3H/^{14}C$ ratios of the labeled proteins from isolated mucosal cells, incubated in the absence and presence of 10^{-9} M gastrin. Equal amounts of proteins from control (labeled with [^{14}C]leucine) and gastrin-treated cells (labeled with [^3H]leucine) were mixed and the mixture was electrophoresed. Each gel slice was counted for ^3H and ^{14}C, and $^3H/^{14}C$ ratio was calculated.

been made to determine whether gastrin exerts a direct growth-promoting effect on the gastric mucosa. Using organ cultures of oxyntic gland mucosa from rabbits, Sutton and Donaldson (19) demonstrated that although most secretagogues stimulated pepsinogen synthesis, only gastrin was capable of stimulating the synthesis of structural protein. In a similar organ culture system, Shield *et al.* (20) observed increased protein synthesis by pentagastrin in the stomach but not in the duodenum, jejunum, and colon of rabbits. However, since the organ cultures were maintained in mediums containing 10% fetal calf serum, a possibility of whether the observed increment in gastric mucosal protein synthesis by gastrin could in part be attributed to the interaction of gastrin with other component(s) of the serum could not be totally ruled out.

In the present investigation, however, isolated gastric mucosal cells were incubated (in the presence and absence of gastrin) in a physiological medium containing salts, amino acids, and bovine serum albumin. Our observation that the exposure of isolated gastric mucosal cells to gastrin for 2 hr results in an enhancement in protein synthesis suggests that gastrin exerts a direct protein stimulatory effect on the gastric mucosa. We have also observed that once the maximal response has been reached, further addition of increasing concentrations of gastrin decreased the stimulation of protein synthesis. This is similar to what has been observed for protein (3, 6, 21) and DNA (22) syntheses following administration of increasing doses of gastrin in rats.

The observed increment in protein synthesis by gastrin in isolated gastric mucosal cells ap-

pears to be under transcriptional control since in the presence of actinomycin-D, the gastrin-mediated stimulation of protein synthesis is completely abolished. Such a finding is in line with the observation that in intact fasted rats, administration of actinomycin-D prior to pentagastrin blocks the pentagastrin-mediated stimulation in gastric mucosal protein synthesis (15).

In the present investigation, actinomycin-D by itself caused a 48% increment in protein synthesis, when compared with the basal level. The reason for this is not fully understood. One plausible explanation for such a phenomenon could be that actinomycin-D enhances the stability of mRNA in isolated gastric mucosal cells, resulting in an increased amount of translatable mRNA in the cell. Addition of low doses of actinomycin-D to wheat germ and reticulocyte lysate cell-free system has been shown to stimulate exogenous mRNA-directed protein synthesis (23).

Although *in vivo* and *in vitro* experiments have demonstrated that gastrin stimulates gastric mucosal protein synthesis, little is known whether such a stimulation affects all or some specific proteins in the gastric mucosa. Our current data from the single-label experiment suggest that gastrin causes an overall stimulation in gastric mucosal protein synthesis. This interpretation comes from the observation that in the presence of gastrin, [^{3}H]leucine incorporation into most of the proteins (as evidenced by the gel electrophoretic profile) is higher than in the corresponding control (Fig. 3). To determine further whether gastrin might specifically stimulate the synthesis of certain protein(s), we performed a dual-labeling experiment where the control and gastrin-treated cells were pulse-labeled with [^{14}C]leucine and [^{3}H]leucine, respectively. Proteins, extracted from the two incubations, were mixed in equal proportions and then electrophoresed. It was hypothesized that a rise in ^{3}H/^{14}C ratio of a protein(s) would indicate an induction in the rate of synthesis of that protein. On the other hand, if the rate of synthesis of all proteins was equally stimulated by gastrin, ^{3}H/^{14}C ratios would remain unchanged. Our finding of five distinct peaks, as evidenced by ^{3}H/^{14}C ratio with apparent molecular weights of 105, 76, 71, 63, and 54 kDa, suggests a specific stimulation in synthesis of these proteins by gastrin in isolated gastric mucosal cells. Although the exact nature and role of these proteins remain to be elucidated, the results from the single and dual-labeling experiments indicate that gastrin action *in vitro* on protein synthesis in isolated gastric mucosal cells involves two mechanisms: (a) a general trophic effect that seems to affect most of the proteins, and (b) a specific stimulation of five proteins.

The work was supported by the Medical Research Service of the Veterans Administration.

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Received February 11, 1987. P.S.E.B.M. 1987, Vol. 185.
Accepted April 13, 1987.