Pentagastrin-induced stimulation of protein synthesis in the gastrointestinal tract

LEONARD R. JOHNSON, DOROTHEA AURES, AND LAWRENCE YUEN

Research Division, Veterans Administration Center, Los Angeles 90073; Psychopharmacology Research, Veterans Administration Hospital, Sepulveda 9143; and Department of Pharmacology, University of California at Irvine, Irvine, California 92664

In a recent symposium on gastric secretion Crean (3) reported that moderate duodenal stenosis in the rat caused gastric mucosal hyperplasia. During the discussion of his paper he suggested that this effect might be mediated through a chronic overproduction of gastrin. There is other circumstantial evidence from clinical work that antrectomy, and presumably the absence of gastrin, may lead to gastric atrophy (10). These suggestions indicate that gastrin may influence the growth of the gastric mucosa. If so, it would be expected to have an effect on protein synthesis. Snyder and Epps (14) have shown that the gastrin-stimulated increase in the histidine decarboxylase activity of gastric mucosa is dependent on an intact protein-synthesis mechanism. These findings have prompted us to see whether gastrin is capable of increasing the incorporation of amino acids into the protein of various tissues from the gastrointestinal tract.

METHODS

Male and female rats weighing between 120 and 200 g were fasted for 48 hr without restraint. The animals were randomly given intraperitoneal injections of either 250 μg/kg pentagastrin (ICI 50, 129) or 1.3 mg/kg histamine base. These doses were found by Barrett (1) to produce 70-85% of the maximal acid secretory response when given to rats as single intravenous injections. Rats in a control group received saline injections of corresponding volume.

The rats were killed by a blow on the head 1 hr after injection. The stomachs, the first 2 cm of duodenum, and, in some instances, a portion of the liver and a piece of skeletal muscle (hamstring) were quickly excised and placed in ice-cold saline. Duodenal mucosa and gastric mucosa from the oxyntic gland area were separated by scraping with a glass slide and were then homogenized. Liver and muscle samples were minced with scissors before homogenization. All samples were homogenized in 2 volumes (ml/g) of an ice-cold solution of medium M (19) (100 mM KCl, 10 mM MgCl₂, 40 mM NaCl, 20 mM Tris pH 7.6, 6 mM mercaptoethanol, and 250 mM sucrose). Samples were homogenized in conical glass homogenizers for 20 up-and-down strokes on a homogenizer with a motor-driven pestle.

A 0.10-ml aliquot (50 mg wet wt mucosa) of homogenate was added to a test tube containing 0.80 ml of medium M and 0.10 ml of a stock solution containing 25 mM ATP (final concn 2.5 mM), 1.0 mM GTP (final concn = 0.1 mM), and 0.5 μc L-leucine-³⁹C (12). Radioactive leucine was uniformly labeled (SA = 251 mC/m mole) and was obtained from New England Nuclear Corp. All solutions were kept in an ice-water bath until incubation.

After a 30-sec period for temperature equilibration, the tubes were incubated with gentle shaking at 37 C. Incubation was for 15 min unless otherwise specified. At the end of incubation the tubes were placed in an ice bath and their contents immediately mixed with 2 ml 5% TCA to precipitate protein (13). The TCA solution contained unlabeled leucine (14 mM) to remove unincorporated radioactive amino acid (12). At this point the samples were left overnight to insure complete precipitation.

Samples were then centrifuged, decanted and washed with 5% TCA containing carrier leucine. This procedure was then repeated to ensure complete removal of unincorporated L-leucine-³⁹C (2). The pellet of protein was re-suspended in 5% TCA and RNA extracted by maintaining the temperature at 90 C for 20 min (7). Lipids were then removed by two washes with 2 ml ethanol:ether (3:1, v/v).
A final centrifugation was performed and the precipitate was dried carefully over boiling water. The dried precipitate was then digested in 0.5 ml NCS (Nuclear-Chicago) for 1 hr at 60° C.

Using a toluene methanol-based scintillation fluid, the digested protein was quantitatively transferred to a counting vial and the radioactivity determined in a Packard liquid scintillation counter.

All extractions were carried out within the incubation flasks so there were no sample losses due to transfer. To insure uniformity of sample size, duplicate samples were run through the incubation and extraction procedures at regular intervals during each experiment. These samples were weighed after drying to a constant weight which did not vary significantly during an experiment or from day to day. The results were not corrected for quenching which was negligible for this procedure and counting efficiency did not vary significantly from 71%. For these reasons all the results are expressed as counts per minute per 50 mg (wet wt mucosa) sample.

RESULTS

Pentagastrin produced a stimulation of the incorporation leucine-14C into the TCA-precipitable (protein) fraction of gastric mucosa from the oxyntic gland area (Fig. 1). Stimulation was apparent after 2.5 min of incubation and increased to a peak at 15 min of incubation time. Incorporation in the saline-injected controls leveled off after only 5 min incubation. After 15 min the pentagastrin-treated rats showed 98% (P < 0.001) greater increase in incorporation than the corresponding saline controls.

Duodenal mucosa incorporated leucine-14C at a much lower rate than oxyntic glandular mucosa, and pentagastrin increased this incorporation by as much as 350% (P < 0.001) after incubation for 10 min (Fig. 2). The uptake of leucine-14C into protein of the duodenal mucosa in the saline-treated controls reached a plateau after 5 min of incubation, but incorporation continued to increase for another 5 min in the pentagastrin-injected animals.

Varying the dose of pentagastrin produced an S-shaped curve for the incorporation of leucine-14C into protein of various tissues (Fig. 3). Maximal stimulation of 90% above saline control occurred with a dose of 500 µg/kg. From Fig. 4 it can be seen that the effect of pentagastrin
on protein synthesis was confined to the oxyntic and duodenal mucosa and could not be reproduced by an equal stimulation of gastric secretion promoted by histamine. In addition, the injection of pentagastrin produced a significant \( P < 0.05 \) decrease in the rate of incorporation of labeled leucine into the protein of skeletal muscle.

**DISCUSSION**

A number of reports indicate that gastrin may influence the growth rate of upper gastrointestinal tissues. Recent studies demonstrated that during starvation the percentage weight loss of the small intestine is equal to nearly twice that of the whole body, the total population of intestinal cells is diminished, and there is a decrease in the RNA and protein content of individual cells (15). If gastrin stimulated protein synthesis as our results indicated, the decreased release of this hormone during starvation could account for some of the results of Steiner et al. (15).

Comparison of biopsy specimens from patients who have had either vagotomy or partial gastrectomy (antrectomy) for treatment of ulcer disease also indicates that gastrin may be a trophic hormone for the gastric mucosa. Lees and Grandjean (8) performed biopsies on 33 “healthy” post gastrectomy patients. They classified only one of these as normal and 22 had from moderate to complete gastric mucosal atrophy. On the other hand, Melrose et al. (11) found no instances of gastric mucosal atrophy in biopsy specimens from 41 patients 1–10 years postvagotomy. Although these studies are not paired, their results do indicate that, when gastric secretion is decreased by surgical intervention, retention of the gastrin-producing antrum helps prevent mucosal atrophy.

In patients with Zollinger-Ellison syndrome there is a chronic overproduction of gastrin by tumor tissue (7). Mucosal hyperplasia and an increased parietal cell count are characteristic of this disease (5).

Clinically, therefore, the overproduction of gastrin is associated with stimulation of gastric mucosal growth and the lack of it (antrectomy) is associated with atrophy.

Our results show that gastrin produces an unequivocal stimulation of protein synthesis in the gastric and duodenal mucosa. The dose of pentagastrin used was submaximal for stimulating protein synthesis as well as for stimulating gastric secretion. This means that chronic over- or under-production could have even a stronger effect on protein synthesis than was noted in these acute experiments. Since there was no difference between saline- and histamine-injected animals the effect of gastrin on amino acid incorporation cannot be explained by alterations in blood flow or the occurrence of gastric secretion. The fact that the experiments were done in vitro also precludes the occurrence of greater mucosal blood flow during gastric secretion as an explanation for the results. Other experiments (unpublished data), however, show that pentagastrin is capable of increasing the in vivo incorporation of amino acids into tissues of the gastrointestinal tract.

Since the incorporation of leucine into liver and skeletal muscle protein was not stimulated by gastrin, the effect appears to be specific for tissues of the gastrointestinal tract. In fact, gastrin produced a significant decrease in the incorporation of leucine into skeletal muscle.

It is difficult to assess the significance of the stimulatory effect of gastrin on protein synthesis or even to draw conclusions concerning the nature of the type of protein being synthesized. The severalfold stimulation of amino acid incorporation into protein of the duodenal mucosa, however, probably rules out increased peptinogen production as the sole factor. The magnitude of the stimulation in comparison to the control levels of incorporation, especially in the duodenum, indicates that it is highly unlikely that the effect represents increased production of the adaptive enzyme, histidine decarboxylase. In addition it has recently been shown that gastrin stimulated acid secretion by the isolated bull frog mucosa occurs independently of protein synthesis (4).

It has long been known that certain cells of the gastrointestinal tract, especially epithelial cells, possess rapid renewal rates (see review by Lipkin (9)). Some of the most potent releasers of gastrin are solutions of certain amino acids when placed in the gastric antrum (6). It seems strange that amino acids, the end products of protein digestion, should stimulate the release of a hormone which leads to increased digestion of protein. Perhaps this “late” release of gastrin serves to increase protein synthesis while the building blocks are readily available.

We conclude that gastrin stimulates the incorporation of amino acids into protein of the duodenal and oxyntic gland mucosa, and suggest that gastrin may be a trophic hormone for these tissues of the gastrointestinal tract.

The authors are grateful to Miss Sharon Laws for expert technical assistance and for the advice of Dr. Morton I. Grossman.

This study was supported by Veterans Administration Research Funds and by National Science Foundation Grant GB 7285X and by US Army Grant DA-HC19-07-G-004.

This work was done while L. R. Johnson held a Public Health Service Postdoctoral Fellowship 1F2, AM 20972. His present address is: Dept. of Physiology, University of Oklahoma Medical Center, Oklahoma City, Okla. 73104.

Received for publication 12 November 1968.

**REFERENCES**


6. **Elwin, C. E., and B. Uvnäs.** Distribution and local release of...


