Pentagastrin-Stimulated Incorporation of ^{14}C-Orotic Acid into RNA of Gastric and Duodenal Mucosa (36727)

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Chronic administration of pharmacological doses of pentagastrin has been shown to increase the weight of the oxyntic gland area of the stomach, cause parietal cell hyperplasia (1), increase the pancreatic weight and RNA content, and the weight of the duodenum (2). Single injections of pentagastrin have been shown to stimulate the *in vitro* (3) and *in vivo* (4) incorporation of ^{14}C-leucine into protein of gastric and duodenal mucosa. This effect is apparently specific for tissues of the upper gastrointestinal tract, for pentagastrin did not increase leucine incorporation into liver or skeletal muscle (3). In addition, the trophic affects of pentagastrin were independent of the stimulation of acid secretion since comparable doses of histamine failed to influence incorporation (3). As a result of these studies we suggested that gastrin was a trophic hormone for certain tissues of the upper gastrointestinal tract (3, 4).

Most hormones which influence growth and protein synthesis also regulate RNA synthesis (5). The current study demonstrates that pentagastrin can stimulate the incorporation of ^{14}C-orotic acid into RNA of gastric and duodenal mucosa.

Materials and Methods. Rats of the Stanley-Gumbreck strain (6) weighing between 150 and 200 g were maintained on commercial chow and water until used. All animals were fasted for 24 hr prior to sacrifice. During this period solutions of pentagastrin, histamine di-HCl, or 0.9% saline were injected intraperitoneally in three separate injections at intervals of 24, 18 and 1 hr prior to sacrifice. Pentagastrin was injected at a dosage level of 250 μg/kg, and histamine HCl at a level of 2 mg/kg in a volume of 0.2 ml/injection. Saline controls received 0.2 ml of 0.9% saline/injection. Orotic acid-^{14}C (8.06 mCi/μmole, purchased from New England Nuclear) was injected intravenously via the jugular vein at a level of 25 μCi/kg 1 hr prior to sacrifice. Actinomycin D was injected intraperitoneally 25 hr before sacrifice at a level of 1.0 mg/kg.

The rats were sacrificed by decapitation and the stomachs and duodenal segments of the intestines were quickly removed, washed and placed in ice-cold saline. The organs were cut open and the mucosal cells were removed by scraping with a glass slide. The mucosal scrapings of three rats were pooled (stomach and duodenal fractions kept separate), weighed in tared microbeakers and homogenized in a small conical glass homogenizer in 3 ml of ice-cold 0.1 *M* sodium acetate buffer (pH 5.1), containing 0.5% sodium dodecyl sulfate and 2 μg/ml of polyvinyl sulfate. The viscous homogenate was transferred to a 50 ml glass homogenizer with enough homogenizing buffer to bring the final volume to 20 ml and was subjected to further homogenization. This latter step was necessary to ensure complete homogenization since the viscous mucoid material acted as a lubricant unless sufficiently diluted out. The homogenate was transferred to a 50 ml glass-stoppered Erylenmeyer flask along with an equal volume of 80% phenol. The flasks were shaken vigorously in a shaking water bath at 60° for 3 min and were rapidly cooled in ice water. The contents were transferred to 50 ml polyethylene centrifuge tubes. The phases were separated by centrifugation for two minutes at 20,000*g*. The upper aqueous layer and the thick interface layer were transferred to 50 ml Erlenmeyer flasks and reextracted at 60° with 80% phenol. Ad-

ditional acetate buffer was added, if necessary, to keep the volume of the aqueous layer near 20 ml. Following centrifugation the upper layer and interface were extracted a third time with phenol. The final aqueous layer was transferred to clean centrifuge tubes and the RNA was precipitated by the addition of 2 vol of 95% ethanol and 1 drop of 10% NaCl/2 ml of extract. The tubes were allowed to stand in the cold overnight and the precipitate was collected by centrifugation. The supernatant was decanted, the RNA was dissolved in acetate buffer (pH 5.1), and reprecipitated with ethanol. The final RNA precipitate was dissolved in 2 ml of acetate buffer. One milliliter was mixed with 14 ml of Bray's solution (7) and counted in a liquid scintillation counter. Another aliquot of the solution was diluted 1:30 with acetate buffer and the RNA content was calculated from readings taken at OD_{260} and OD_{232} according to the formula of Munro and Fleck (8). The final data were expressed in terms of cpm/mg RNA.

Results. Pentagastrin stimulated the incorporation of orotic acid into RNA from both gastric and duodenal mucosa but did not significantly alter the incorporation into RNA from the liver (Fig. 1). In the oxyntic gland area of the stomach, orotic acid incorporation was 349.3 ± 63.2% of that in the saline treated controls ($p < .02$ by t test for paired data). The level of incorporation into duodenal mucosal RNA was 219.6 ± 31.9% of that in the saline treated animals ($p < .001$). Incorporation of orotic acid into RNA from histamine injected animals was not significantly different from the values obtained from the saline controls.

The results of experiments designed to study the effects of the administration of actinomycin D on the pentagastrin stimulation of orotic acid incorporation are shown in Table I. Pentagastrin caused the expected increase in orotic acid incorporation when compared to the saline controls. The administration of actinomycin D failed to prevent the increase in incorporation of orotic acid into the stomach, and the administration of actinomycin D alone caused a marked increase in the incorporation of orotic acid in the duodenum.

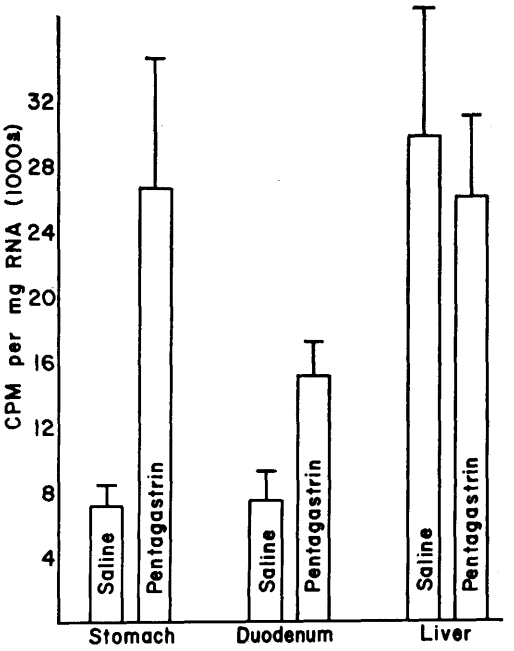

FIG. 1. ^{14}C-Orotic acid incorporation into the RNA from stomach, duodenum and liver of rats injected with saline or pentagastrin. Each bar represents the results from 5 separate experiments, each experiment involving the pooled tissues of 3 rats. The results are expressed in terms of means ± SEM. Significance was determined by t test for paired data. Stomach, $p < .02$; duodenum, $p < .001$; liver, ns.

Discussion. Injection of pharmacological amounts of pentagastrin (4 mg/day for 21 days) in rats resulted in significant increases in the weight of the oxyntic gland area, in the height and volume of the oxyntic mucosa and in the total parietal cell population (1). There was no significant difference between saline and histamine injected animals with regard to any of these parameters. Stanley *et al.* (9) have recently demonstrated a similar significant increase in the parietal cell population of rats injected 3 times/day with 250 μg/kg pentagastrin for a period of 2 wk. This dose of pentagastrin can be considered more physiological since it is close to the dose for maximal acid secretion in the rat (10).

Antrectomy and gastroduodenostomy performed on female rats resulted in gastric mucosal hypoplasia within 6 wk (11). The re-

TABLE I. Incorporation of ^{14}C-Orotic Acid into RNA of Gastric and Duodenal Mucosa from Rats Treated with Saline or Gastrin ± Actinomycin D.

	^{14}S-Orotic acid incorporation (cpm/mg RNA)[a]			
	Control		Actinomycin D[b]	
	Stomach	Duodenum	Stomach	Duodenum
Saline	2707 ± 521	3708 ± 533	2072 ± 179	13919 ± 4397
Pentagastrin[c]	3731 ± 451	5710 ± 973	3251 ± 521	9521 ± 2852
t[d]	3.49	2.80	2.55	1.62
p	<.01	<.025	<.05	ns

[a] Means and standard errors of means of 5 experiments using pooled mucosa of 3 rats in each of the 4 groups.

[b] 1.0 mg/kg injected intraperitoneally 25 hr before sacrifice.

[c] 250 μg/kg injected 3 times over 24 hr period.

[d] Values from paired analysis, $N = 5$.

sponse consisted of a significant decrease in the surface area of the oxyntic gland region, in the mucosal height, mucosal volume, and parietal cell density as well as total cells, chief cell density and total chief cells.

Chronic administration of pentagastrin also affects the size of the rat pancreas. Mayston and Barrowman (2) reported that injection of pentagastrin for a 2-wk period resulted in hypertrophy of the pancreatic acinar cells accompanied by a decrease in the specific activities of several pancreatic enzymes and a significant increase in the RNA/DNA ratio of the tissue. They concur with others (3, 4) who have suggested that gastrin may exert a trophic influence upon tissues of the upper gastrointestinal tract.

The increase in incorporation of orotic acid into RNA may be due to one of two possibilities: (a) the "induction" of an increased rate of synthesis of RNA by pentagastrin, or (b) the increased permeability of cell membranes to orotic acid resulting in an increased specific activity of nucleotide precursor pools. Actinomycin D is often used as a tool to determine whether or not an "induction" phenomenon has occurred as a result of a specific treatment since it is known to block new RNA synthesis. In the present case, however, the administration of actinomycin D alone caused a marked stimulation of orotic acid incorporation. This is most likely an example of the "superinduction" phenomenon as described by Tomkins *et al.* (12). This phenomenon has been observed to occur with plasma glycoprotein biosynthesis (13), with certain rat liver enzymes (14), with the alkaline phosphatase of mouse intestinal tract (15), and with tyrosine aminotransferase of rat liver hepatoma cells grown *in vitro,* and is probably a general phenomenon has made it difficult to interpret the effects of pentagastrin on orotic acid incorporation. Based upon prior observations of the effects of pentagastrin on protein biosynthesis (3, 4) and on tissue weights and thickness (1, 2) and also on the failure of pentagastrin to stimulate orotic acid incorporation into liver, it is most likely that pentagastrin stimulates RNA synthesis rather than increasing cell permeability. However, further experiments involving determinations of nucleotide precursor pool sizes and specific activities must be performed in order to clearly establish this point.

Summary. Fasted rats were injected 3 times over a 24 hr period with either pentagastrin, saline or histamine. ^{14}C-Orotic acid was injected 1 hr prior to sacrifice. The incorporation of orotic acid into phenol extracted RNA was determined for gastric and duodenal mucosa and the liver. Pentagastrin injected animals incorporated 3 times as much orotic acid into RNA of the gastric and twice as much into RNA of the duodenal mucosa when compared to saline or histamine injected controls. Incorporation of orotic acid into liver RNA was not affected by pentagastrin.

We conclude that pentagastrin stimulates the incorporation of orotic acid into mucosal RNA of the upper gastrointestinal tract and that is probably indicative of induction of RNA synthesis. These findings support the current evidence and hypothesis (3) that gastrin is a trophic hormone for gastric and duodenal mucosa.

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