PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 521–523 (1978)

Effect of Secretin on Colonic DNA Synthesis[1] (40238)

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The trophic effects of gastrointestinal hormones on the mucosa of the proximal digestive tract and on the exocrine pancreas are well known (1). Gastrin produces a pleiotypic response in the mucosa of the oxyntic gland area of the stomach and the entire small intestine. This trophic effect of gastrin is inhibited by secretin (2). Inhibition by secretin does not depend on its ability to inhibit gastric acid secretion for other secretory antagonists such as prostaglandin E_2 and metiamide do not block the trophic response to gastrin (2, 3). Gastrin (4) and cholecystokinin (5) stimulate growth of the exocrine pancreas when given chronically to rats. Whether or not chronic injections of secretin alter pancreatic growth has not been reported.

The cells of the colonic mucosa turn over rapidly. Therefore, a substance having any effect on DNA synthesis of this system would be expected to exert a profound influence on the growth of the entire mucosa. Gastrin has recently been shown to be a potent stimulator of colonic mucosal DNA synthesis leading to a rapid increase in mucosal DNA content and, hence, cell number (6). Whether secretin inhibits this action of gastrin in the colon, as it does in the duodenal and oxyntic gland mucosa, is unknown. Two structural analogues of secretin possess opposite effects on the colonic mucosa. Vasoactive intestinal peptide (VIP) did not stimulate colonic DNA synthesis when given alone and inhibited the trophic effect of pentagastrin when administered simultaneously (6). Glucagon, which has 14 amino acid identities with secretin, on the other hand, stimulated colonic DNA synthesis by itself and did not inhibit the trophic action of pentagastrin when administered concurrently (6).

The current experiments present evidence that secretin affects colonic mucosa in a manner similar to VIP. The actions of secretin on colonic mucosal growth appear to be identical to its effects on intestinal and oxyntic gland mucosa.

Methods. Eighteen male Sprague–Dawley rats, weighing between 100 and 120 g, were fasted for 48 hours in cages with wide mesh bottoms. During this time they had free access to water. They were randomly divided into 6 groups of three animals and during the 48 hr received six intraperitoneal injections of 0(NaCl), 31, 62.5, 125, 250, or 500 μg pentagastrin per kg. Pentagastrin was the kind gift of Dr. J. S. Morley of Imperial Chemical Industries Limited and was administered at 8-hr intervals. This experiment was repeated twice, so $n = 9$ for each group.

In the second study, 18 rats were again divided into six groups of three and subjected to the same fasting and injection schedule. Three animals were injected with saline. The other five groups were all injected with 250 μg pentagastrin per kg. Four of these groups were concurrently injected with secretin in doses of 9.0, 18.75, 37.5 and 75 U/kg. Secretin was synthesized by the Squibb Co. and made available through the NIH gastrointestinal hormone and peptide resource (1 U secretin = 0.25 μg). This study was repeated so that $n = 6$ for each group.

In the third study 12 rats were divided into four groups, fasted and injected as before. The first group received saline; the second pentagastrin (250 μg/kg), the third secretin (75 unit/kg); and the fourth 250 μg/kg pentagastrin plus 75 unit/kg secretin. This experiment was repeated twice, so that $n = 9$ (in each group).

All animals were killed 8 hr after the last injection and the colon was quickly removed, opened, washed and placed in a glass plate over ice. The mucosa from the second (from the ileal-cecal junction) 5 cm was blotted, rapidly scraped off with a glass slide and weighed. The mucosal scrapings were then dispersed in 2 ml of tissue culture medium 199 containing 2 μCi/ml [^{3}H]thymidine (5

[1] Supported by NIH Research Grant No. AM 16505.

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0037-9727/78/1584-0521$01.00/0

Ci/mmol, Amersham, England) and incubated at 37° for 30 min. The reaction was stopped with 0.4 *N* perchloric acid containing carrier thymidine at 5 m*M* concentration.

Tissue samples were then washed twice in cold 0.2 *N* perchloric acid and recentrifuged. The pellet was solubilized in 0.3 *N* KOH incubated at 37° for 90 min. DNA and protein were reprecipitated with 2 ml of perchloric acid. After standing for 10 min in ice, the tubes were centrifuged and the supernatants containing RNA removed. The pellets were washed with 4 ml 0.2 *N* perchloric acid and centrifuged. The DNA-containing pellet was dissolved in 4 ml of 10% perchloric acid by heating in a boiling water bath for 10 min. Denatured protein was removed by centrifugation for 20 min and filtration through Whatman No. 50 filter paper. Using calf thymus DNA as a standard, the DNA content of the samples was determined by the Burton (7) diphenylamine procedure as modified by Giles and Myers (8). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0.5 ml of the DNA-containing filtrate in a Beckman liquid scintillation counting system.

Results were calculated as disintegrations per minute per mg wet wt. DNA content was expressed as μg per mg mucosa. The difference between means was evaluated using the *t* test for unpaired data and was considered significant if $P < 0.05$.

Results. Figure 1 illustrates that the maximal trophic response in colonic mucosa to pentagastrin occurs with a dose of 250 μg/kg.

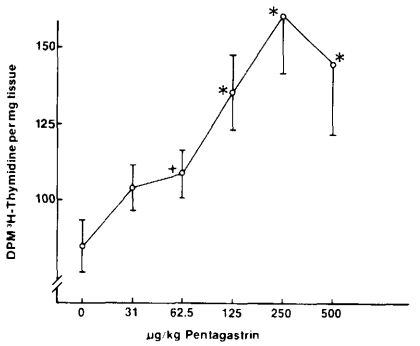

FIG. 1. The effect of increasing doses of pentagastrin on DNA synthesis in rat colonic mucosa. Each point is depicted as the mean ± SE of the mean of determinations in nine rats. + = $P < 0.05$, * = $P < 0.01$ as compared to saline injected controls (0 dose of pentagastrin).

Significant increases in DNA synthesis were seen at doses of 62.5, 125, 250, and 500 μg pentagastrin per kg. On the basis of these data the dose of 250 μg/kg was used for the experiments involving secretin.

In the studies shown in Fig. 2 pentagastrin increased the rate of colonic mucosal DNA synthesis from 165 dpm [^{3}H]thymidine per mg tissue to 330. Increasing amounts of secretin given with pentagastrin inhibited this stimulation in a dose related manner. Significant inhibition occurred with as little as 9 U/kg secretin. At doses of 37.5 and 75 U/kg secretin completely inhibited the response to pentagastrin. At these points the effect of pentagastrin plus secretin did not differ statistically from the NaCl controls.

Secretin (75 U/kg) given by itself did not significantly change either DNA synthesis or the DNA content of colonic mucosa (Fig. 3). In this particular series of studies pentagastrin increased DNA synthesis 115% and the DNA content of colonic mucosa 76%. Secretin in combination with pentagastrin completely prevented the increases in both DNA synthesis and content.

Discussion. Earlier experiments have shown that the trophic effects of gastrointestinal hormones can be accurately and rapidly assessed by examining changes in DNA synthesis (2, 3, 9). The determination of tissue weight or DNA content (Fig. 3) should be made concurrently to check that increased

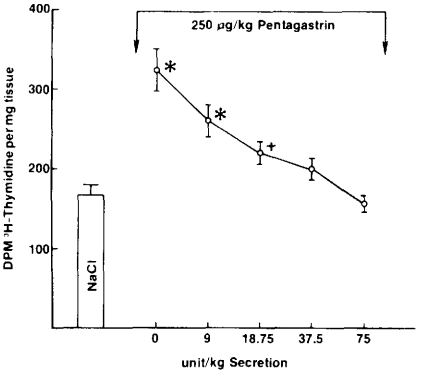

FIG. 2. The effect of increasing doses of secretin on colonic mucosal DNA synthesis stimulated by 250 μg/kg pentagastrin. The control level of DNA synthesis (no pentagastrin) is shown by the bar on the left (NaCl). Each point is the mean ± SE of the mean of determination of six rats. + = $P < 0.05$, * = $P < 0.01$ compared to NaCl.

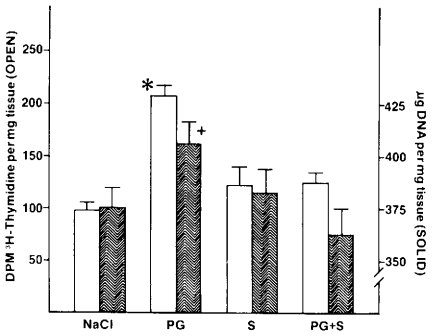

FIG. 3. DNA synthesis (open bars) and DNA content (shaded bars) in response to injections of NaCl, 250 µg/kg pentagastrin (PG), 75 U/kg secretin (S), and 250 µg/kg PG plus 75 U/kg S (PG + S). Each bar represents the mean ± SE of the mean of determinations in nine rats. + = $P < 0.05$, * = $P < 0.001$ compared to NaCl.

synthesis is not the result of increased DNA turnover and that it actually leads to growth. The trophic action of gastrin has been documented by numerous laboratory techniques, histology, autoradiography, protein synthesis; and the results have correlated well with measurements of DNA content and synthesis (1).

The purpose of the study depicted by the data in Fig. 1 was to find the dose of pentagastrin producing the maximal effect on colonic mucosal DNA synthesis. DNA synthesis increased in response to increasing doses of pentagastrin up to a dose of 250 µg/kg. A further doubling of the dose resulted in a slightly lower response. This particular pattern, including an optimal dose of 250 µg/kg, is identical to that described previously for mucosa of the oxyntic gland area (9) and the duodenum (6). The dose of 250 µg pentagastrin per kg causes maximal gastric acid secretion in the rat (10).

The inhibition of the trophic action of pentagastrin produced by secretin was nearly identical to that reported in the mucosa of the oxyntic gland, duodenum and ileum (2). Maximal inhibition was obtained with 75 U secretin per kg. In the rat this dose of secretin completely inhibits maximally stimulated acid secretion as well (11). Secretin had no trophic action on colonic mucosa when administered by itself; nor did it decrease DNA synthesis and content below the levels found in saline injected control animals (Fig. 3). Therefore, the effects of secretin on colonic mucosa are similar to those of its structural analogue, VIP, but different from those of glucagon, another structurally related peptide (6).

The physiological significance of the antitrophic action of secretin is as yet not established. Resection of the proximal gut, which contains the majority of secretin, results in hyperplasia of gastrointestinal mucosa including colonic (12). There is good evidence that this response depends, at least in part, on a humoral factor or factors (12). The removal and subsequent chronic absence of inhibitors of growth such as secretin and VIP could in part be responsible.

Summary. This study examined whether or not secretin inhibited pentagastrin stimulated growth of colonic mucosa in rats. Pentagastrin (250 µg/kg) caused an approximate doubling of mucosal DNA synthesis which was completely prevented by secretin in doses of 37.5 and 75 U/kg. The effect of secretin was dose dependent and occurred with as little as 9 U/kg. Secretin had no effect on either DNA synthesis or content when administered by itself. The dose of 75 U secretin per kg also completely prevented a 60% increase in DNA content stimulated by pentagastrin.

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Received March 27, 1978. P.S.E.B.M. 1978, Vol. 158.