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Evidence that Secretin Does not Have Direct Antitrophic Effects on the Rat Stomach (39527)

DAVID A. WISEMAN AND LEONARD R. JOHNSON

Department of Physiology, University of Texas Medical School, Houston, Texas 77030

The discovery that gastrin stimulates the growth of the pancreas and gastrointestinal mucosa opened up a whole new area of investigation in gastrointestinal physiology. Since that time the other two "classical" gastrointestinal hormones, cholecystokinin and secretin, have been tested to determine whether they, too, have trophic effects on the tissues of the digestive tract (1). There is now considerable evidence to suggest that cholecystokinin does, indeed, have trophic effects, at least on the exocrine pancreas. In the case of secretin the story is not as clear. One thing that is clear, however, is that secretin can block the trophic effect of pentagastrin on the rat stomach. This was first demonstrated by Stanley *et al.* in 1972 when they showed that pentagastrin-induced parietal cell hyperplasia in the rat can be prevented if secretin is given simultaneously with the pentagastrin (2). In a more recent study Johnson and Guthrie reported that pentagastrin-stimulated DNA synthesis *in vitro* in the rat stomach and small intestine can also be prevented if secretin is given simultaneously with the pentagastrin (3). The inhibition of pentagastrin-stimulated growth by secretin suggests that, instead of having a trophic effect, secretin may, if anything, have an antitrophic effect on the gastrointestinal tract of the rat. The purpose of the present study was to determine whether secretin, when given alone, inhibits the growth of the rat stomach. The answer to this question is not only important in terms of understanding the physiology of gastrointestinal hormones but takes on added significance in view of the considerable potential of secretin as an anti-ulcer drug.

Materials and methods. Twenty-four male Sprague–Dawley rats, weighing between 200 and 225 g, were divided into two equal groups. One group was injected subcutaneously every 8 hr with synthetic secretin (Schwarz/Mann Inc., Orangeburg, N.Y.) at a dose of 100 units/kg. This dose of secretin is identical to that used by Stanley *et al.* (2) to block pentagastrin-induced parietal cell hyperplasia and slightly larger than the dose used by Johnson and Guthrie (3) to block pentagastrin-stimulated DNA synthesis. The injected secretin was dissolved at a concentration of 10 μg/ml in a gelatin solution consisting of nine parts of calcitonin diluent B (Armour Pharmaceutical Co., Kankakee, Ill.) and one part of 0.01 *N* HCl. The second group of rats was injected with only the gelatin solution and served as controls. All rats were allowed free access to water and a stock diet of Lab-Blox (Allied Mills Inc., Chicago, Ill.).

After 14 days of treatment the rats were etherized and killed by exsanguination. Secretin had no effect on the final body weight of the rats used in this study. Blood samples were drawn from the heart of each rat and centrifuged at 3000*g* for 15 min. The resultant serum samples were stored at $-20°$ until assayed for gastrin content. The oxyntic glandular mucosa was isolated from each stomach by scraping it free from the underlying serosa using a glass microscope slide. Each mucosal scraping was homogenized in 3 ml of ice-cold water, and the resultant homogenates were centrifuged at 3000*g* for 15 min. The supernatant solutions were assayed for carbonic anhydrase activity as well as for pepsinogen and protein content. Nucleic acids and protein were extracted from the remaining pellet as previously described (4).

Samples assayed for pepsinogen content were adjusted to pH 8.0 with 0.01 *N* NaOH and allowed to stand on ice for 10 min. During this time, the pepsin initially present in the samples was permanently inactivated by the high pH. The samples were then adjusted to pH 2.0 with 0.01 *N* HCl, converting the pepsinogen present to pepsin. This newly activated pepsin was assayed by

the method of Anson (5). Carbonic anhydrase was assayed by the electrometric method of Wilbur and Anderson (6). DNA and protein were determined by the methods of Burton (7) and Lowry *et al.* (8), respectively.

Antral gastrin was extracted by homogenizing each antrum in 2 ml of ice-cold distilled water. One milliliter of homogenate was added to 4 ml of boiling water, and the mixture incubated at 100° for 20 min. The mixtures were then centrifuged at 3000*g* for 15 min, and the resultant supernatant solutions were filtered by vacuum through 0.45-μm Millipore filters. The filtrates were stored at −20° until assayed for gastrin content. Gastrin was assayed in the serum samples and the antral filtrates by radioimmunoassay as previously described (9).

Results and discussion. We examined the effect of multiple secretin injections on the weight of the stomach and its component tissues, on the biochemical characteristics of the oxyntic glandular mucosa, and on the levels of endogenous gastrin. Secretin had no effect on the weight of the stomach, oxyntic gland area of the stomach, antrum, or oxyntic glandular mucosa (Table I). The DNA, protein, and pepsinogen contents as well as the carbonic anhydrase activity of the oxyntic glandular mucosa were also unaffected by secretin (Table II). In addition, secretin had no effect on antral or serum gastrin levels (Fig. 1).

Each of the measurements made in this study is capable of detecting changes in the growth of the stomach. Any changes occurring in gastric growth would, of course, be reflected by a similar change in the weight of the stomach or one of its component tissues. Both DNA and protein content have been used previously to demonstrate the trophic effect of gastrin on the rat gastrointestinal mucosa and are thus proven indices of mucosal growth (10, 4). In the stomach, pepsinogen is found primarily in chief cells. Consequently, the pepsinogen content of the oxyntic glandular mucosa should be an indication of the number of chief cells present. Similarly, Davenport has found that an excellent correlation exists between the carbonic anhydrase content and the parietal cell mass of the stomach (11). Therefore, both pepsinogen content and carbonic anhydrase activity should provide information about changes occurring in the cell populations of the gastric mucosa. Endogenous gastrin levels are also an index of mucosal growth. For example, the low antral and

TABLE I. WEIGHT OF THE RAT STOMACH AND ITS COMPONENT TISSUES AFTER MULTIPLE SECRETIN INJECTIONS.[a]

Tissue	Secretin	Control
Stomach (mg/100 g body wt)	556 ± 12[b]	559 ± 12
Oxyntic gland area (mg/100 g body wt)	341 ± 9	338 ± 10
Antrum (mg/100 g body wt)	59.9 ± 2	57.6 ± 2
Oxyntic glandular mucosa (mg)	440 ± 37	386 ± 39

[a] 100 units/kg injected subcutaneously every 8 hr for 14 days.
[b] Standard error of the mean.

TABLE II. BIOCHEMICAL CHARACTERISTICS OF THE OXYNTIC GLANDULAR MUCOSA AFTER MULTIPLE SECRETIN INJECTIONS.[a]

Measurement	Secretin	Control
DNA (μg/g mucosa)	37.4 ± 3.0[b]	39.2 ± 4.7
Protein (mg/g mucosa)	7.88 ± 0.4	7.43 ± 0.4
Pepsinogen (μg pepsin/mg mucosa)[c]	2.04 ± 0.19	2.02 ± 0.12
Carbonic anhydrase activity (units/mg protein)	19.6 ± 1.3	19.6 ± 1.2

[a] 100 units/kg injected subcutaneously every 8 hr for 14 days.
[b] Standard error of the mean.
[c] Pepsinogen is expressed as equivalent micrograms of activated pepsin.

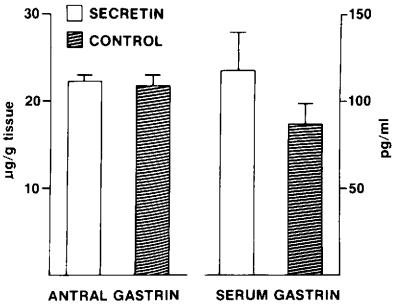

FIG. 1. Endogenous gastrin levels of rats after multiple secretin injections. Secretin was injected subcutaneously every 8 hr for 14 days at a dose of 100 units/kg. Each bar represents the mean ± SE of 12 rats.

serum gastrin levels observed in antrectomized rats (10), starved rats (9, 12), and rats maintained by total parenteral nutrition (4) are accompanied in each case by a reduction in the growth of the gastrointestinal mucosa. Furthermore, the high levels of endogenous gastrin observed in patients with Zollinger–Ellison syndrome are associated with an increase in mucosal growth (13, 14).

Although each measurement in this study is capable of detecting changes in stomach growth, not one measurement provided any evidence of an inhibitory role for secretin. Secretin did not decrease the carbonic anhydrase activity or, presumably, the parietal cell mass of the oxyntic glandular mucosa even though Stanley *et al.* have previously reported that secretin induces parietal cell hypoplasia in the rat stomach (2). There may not be a conflict in results, however, since the decrease in parietal cell mass reported by these workers was not significant in every experiment. Our findings agree with those of Johnson and Guthrie, who were unable to demonstrate an inhibitory effect of secretin on *in vitro* DNA synthesis in the rat stomach (3). We conclude that secretin itself does not have an antitrophic effect on the rat stomach and that the previously reported antitrophic effect of secretin on this tissue (2, 3) was due to the inhibition of pentagastrin.

The findings of this study do not, of course, rule out an antitrophic role for secretin in the other tissues of the digestive tract. Pansu *et al.* recently reported that secretin can prevent a nocturnal increase in cell proliferation in the rat jejunum (15). This nocturnal increase in cell proliferation is part of a circadian rhythm in mitosis that exists in this tissue. Thus, secretin may well have an antitrophic effect on the rat small intestine.

Summary. Rats were injected subcutaneously with synthetic secretin (100 units/kg) every 8 hr for 14 days. We examined the effect of secretin on the weight of the stomach and its component parts, on the biochemical characteristics of the oxyntic glandular mucosa, and on the levels of endogenous gastrin. Each of these measurements is capable of detecting changes in the growth of the stomach. Nevertheless, we found no evidence that secretin inhibits gastric growth. It is, therefore, concluded that secretin itself does not have an antitrophic effect on the rat stomach and that the previously reported antitrophic effect of secretin on this tissue was due to the inhibition of pentagastrin.

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