

Calcium and Secretin as Provocative Stimuli in the Zollinger-Ellison Syndrome¹

A.A. Mihas, B.I. Hirschowitz and R.G. Gibson

Division of Gastroenterology, University of Alabama in Birmingham and Birmingham V.A. Hospital, Birmingham, Ala.

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Abstract. The effects of calcium and secretin were studied in 8 patients with the Zollinger-Ellison syndrome and 18 patients with duodenal ulcer disease. Intravenous infusion of calcium gluconate produced marked increases in serum gastrin levels in the patients with Zollinger-Ellison syndrome ($4,350 \pm 1,625$ pg/ml) and very slight increases in the patients with duodenal ulcer disease (140 ± 49 pg/ml). Secretin given as a single intravenous injection also induced marked elevations in serum gastrin in the group with the Zollinger-Ellison syndrome ($4,063 \pm 1,990$ pg/ml). By contrast, intravenous secretin resulted in a progressive fall in serum gastrin levels in the duodenal ulcer group (from 119 to 97 pg/ml). These results suggest that both stimuli are very useful diagnostic tools in discriminating between Zollinger-Ellison and non-Zollinger-Ellison patients. The secretin challenge test is felt to be superior to the calcium infusions because it is simpler, safer and very rarely produces false-negative or -positive results.

Introduction

In 1955, *Zollinger and Ellison* (28) reported 2 patients with a triad of: (a) recurrent ulceration of the upper gastrointestinal tract; (b) non- β -islet cell tumors of the pancreas; and (c) extremely high levels of gastric hydrochloric acid secretion. For several years the criteria for the diagnosis of the Zollinger-Ellison syndrome (ZES) depended largely upon the demonstration of greatly elevated rates of gastric acid secretion, particularly in the basal state (12, 15). With the

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development of gastrin radioimmunoassay (17, 27) it was soon recognized that many patients with ZES had remained undiagnosed because their symptoms and their gastric secretion pattern did not distinguish them from patients with ordinary peptic ulcer disease (11). Fasting serum gastrin levels were reported to be greatly elevated in patients with ZES, but when larger groups of patients were studied it was found that several of them had fasting gastrin values between 150 and 500 pg/ml (9, 23, 26). In addition, a certain number of patients with duodenal ulcer (DU), and without the ZES, had a fasting serum gastrin between 150 and 250 pg/ml (4, 11). Such values approach or overlap the lowest values for some patients with documented ZES and they fall into a category of questionable diagnosis which does not permit easy assignment as ZES or non-ZES.

Provocative stimulatory tests for release of gastrin have been used in an effort to distinguish between non-ZE ulcer patients and those with the ZES (1, 2, 10, 13, 19, 20, 25). It is the purpose of this paper to describe the effects of calcium and secretin administration on serum gastrin levels in 26 patients suspected on clinical grounds of having the ZES.

Patients, Materials and Methods

Patients. Serum gastrin responses to intravenous calcium and secretin administrations were studied in 26 patients, 18 males and 8 females, 21–63 years old. The following criteria for selection were applied: (a) history of peptic ulcer disease with a duodenal ulcer documented by both barium studies and fiberoptic endoscopy; (b) absence of previous surgery; and (c) no evidence of malignant disease.

Gastric secretion. After an overnight fast a Levin tube was placed in the stomach under fluoroscopic control. Intermittent mechanical suction producing a subatmospheric pressure of 150 mm Hg was used in the collection of the secretion. Histamine 0.04 mg/kg subcutaneous or pentagastrin (Peptavlon, Ayerst Laboratories) 0.1–5 $\mu\text{g}/\text{kg}\cdot\text{h}$ by intravenous infusion in a 'step-dose' fashion were used as stimulants. Gastric juice was collected at 15-min intervals and analyzed for acid and pepsin concentration.

Calcium infusions. Each of the subjects were given a constant, 3-hour intravenous infusion of calcium gluconate (5 mg $\text{Ca}^{++}/\text{kg}\cdot\text{h}$) in 0.9% NaCl. Blood was obtained from a separate intravenous polyethylene catheter without venous occlusion at 30-min intervals, beginning 60 min before the initiation of the infusion and extending 60 min after termination of the infusion. Serial electrocardiograms were routinely obtained during the calcium infusion.

Secretin injections. Secretin (G.I.H., Karolinska Institute, Stockholm) 2 IU/kg in 1.0 ml of 0.9% NaCl was injected intravenously during a 1-min period. Blood samples were obtained from an intravenous polyethylene catheter without occlusion at 0, 5, 10, 15, 30, 45 and 60 min.

Gastrin radioimmunoassay. Serum gastrin levels were determined by a radioimmunoassay (RIA) procedure as described previously (6). Briefly the procedure for the gastrin RIA is as follows: 100 μl of serum (at various dilutions) were incubated at 4 °C for 72 h in a mixture consisting of 900 μl veronal buffer (pH 8.4), 800 μl monoiodinated synthetic

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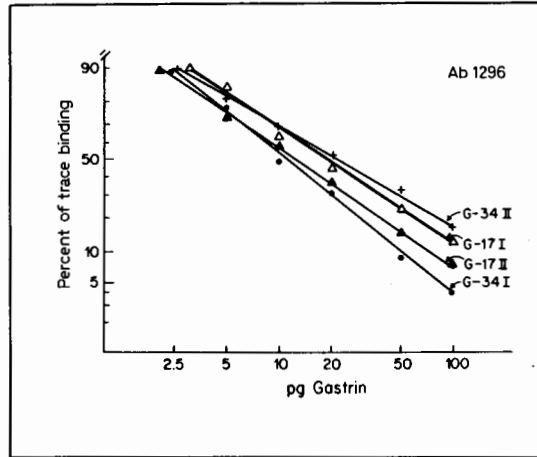


Fig. 1. The cross-reactivity of AB 1296 to the predominant circulating forms of gastrin in man.

human gastrin 17-1 in buffer (~ 2,500 cpm/tube), and 200 μ l of antibody (Ab 1296² 1:1,200,000 final dilution) specific for the C-terminal region of gastrin. The reactivity of this antibody to the predominant circulating molecular forms of gastrin are depicted in figure 1. At the end of 72 h free gastrin was absorbed on Amberlite IRP-58M resin and separated from the antibody-bound fraction by centrifugation and decantation. Both free and antibody-bound fractions were counted in a gamma scintillation counter and the results expressed as B/F ratio. The sensitivity of this assay was found to be 1 pg/ml. In addition, a series of 85 human sera covering a wide range (10–10,000 pg/ml) of values were assayed in this laboratory and a second reference laboratory using identical procedures yielding a correlation coefficient of $r = 0.99^7$.

Results

Patients. The clinical data of the patients with the ZES are shown in table I. All patients underwent exploratory laparotomy and histologic confirmation was sought. 3 patients were found to have small duodenal gastrinomas which were excised in combination with total gastrectomy. Postoperatively serum gastrin levels were found to be normal. 2 patients were found to have pancreatic gastrinomas, one with metastases to regional lymph nodes and liver. No tumor was found in the remaining 3 patients who underwent total gastrectomy. Serum gastrin levels continued being abnormal in 2 of them while the third patient was

² Antibody 1296 kindly supplied by Dr. John Walsh, Division of Gastroenterology, UCLA School of Medicine, Los Angeles, Calif.

Table I. Clinical data of patients with ZES

| Patients | Age | Sex | Fasting gastrin | Post-operative gastrin | Surgery | History |
|----------|-----|-----|-----------------|------------------------|---|---|
| A.S. | 24 | M | 410 | 40 | total gastrectomy | antral hyperplasia documented by IF and EM |
| S.C. | 51 | M | 425 | 30 | excision of gastrinoma + total gastrectomy | benign duodenal gastrinoma |
| A.L. | 58 | F | 1,250 | 2,300 | total gastrectomy | tumor in the pancreas; no biopsy taken |
| C.L. | 63 | M | 310 | 25 | total gastrectomy + excision of duodenal gastrinoma | benign duodenal gastrinoma, documented by IF and EM |
| J.H. | 56 | M | 725 | 600 | exploratory laparotomy | metastatic gastrinoma of the pancreas |
| B.W. | 36 | F | 400 | 900 | total gastrectomy | no tumor found |
| E.Y. | 43 | M | 425 | 32 | total gastrectomy + excision of duodenal gastrinoma | benign duodenal gastrinoma |
| R.S. | 67 | M | 1,100 | - | autopsy | malignant gastrinoma of the pancreas |

IF = Immunofluorescence; EM = electron microscopy.

found to have normal serum gastrin both at the fasting state and following a challenge with secretin as well. The histology of the antral mucosa in this case was suggestive of G-cell hyperplasia as evidenced by increased G-cell density by immunofluorescence using gastrin Ab 1296.

Gastric secretion. The mean value of BAO in the DU group was 9.7 mEq/h (range 0.4–29.6 mEq/h), whereas in the ZES group was 17.4 mEq/h (range 1.2–33.2 mEq/h). The respective mean values for MAO were 40.2 mEq/h for the DU group and 49.2 mEq/h for the ZES. The mean values of BAO/MAO ratio was 0.24 in the DU patients while the patients with ZES showed a mean value of 0.35. The difference is not statistically significant ($p > 0.1$).

Calcium. In each of the patients with the ZES infusion of calcium produced marked increases in serum gastrin concentrations. Augmented hypergastrinemia was maintained during constant calcium infusion, falling off only after discontinuation of calcium administration (fig. 2). The basal (fasting) serum gastrin levels in the ZES group ranged from 310 to 1,250 pg/ml whereas in the DU group they ranged from 50 to 118 pg/ml. Serum gastrin levels were increased during the calcium infusion in both groups but in the DU group the highest recorded value increased to slightly above the normal range (0–225 pg/ml). The peak serum gastrin value in both groups was observed 150 min following the initiation of the calcium infusion. Serum gastrin levels increased in parallel with calcium (highest value 14.2 mg/100 ml) and there was a significant positive correlation between serum gastrin and serum calcium levels ($r = 0.98$). The increases in serum calcium concentrations were accompanied by mild, transitory shortening by 0.02–0.08 sec of the Q-T interval on electrocardiogram.

Secretin. Following intravenous injection of secretin serum gastrin levels were found to be significantly increased in all patients with the ZES. In the DU group (fasting gastrin ranged from 50 to 230 pg/ml), on the other hand, serum gastrin concentrations not only were not increased but there was a progressive decline in serum gastrin for as long as 60 min following the secretin injection (fig. 3). In the ZES group the peak serum gastrin values were observed 5 min following the administration of secretin with a slow return to the basal values at 60 min.

Discussion

Zollinger and Ellison were the first to postulate that a hormone was responsible for the clinical manifestations of the ZES. It is now known that the ZES is due to the pathophysiological effects of excess circulating gastrin released from gastrin-rich tumors (gastrinomas). The clinical application of gastrin RIA has shown that an elevated serum gastrin is the *sine que non* in establishing the diagnosis of ZES. Patients with well-documented ZES usually have serum gastrin

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|------|---------------------|------|------|-------|-----|-------|------|--------|------|-------|--------|
| C.L. | (Ca ⁺⁺) | 5.6 | 79.2 | 0.071 | 31V | 730 | 4JUV | 10,000 | 4JUV | 9,270 | 1,278 |
| J.H. | (Sec) | 30.3 | 56.7 | 0.535 | 31V | 725 | 4JUV | 10,000 | 4JUV | 9,275 | +1,279 |
| J.H. | (Ca ⁺⁺) | 9.6 | 19.3 | 0.497 | 31V | 410 | 4JUV | 2,900 | 4JUV | 2,490 | +607 |
| A.S. | (Sec) | 4.7 | 42.8 | 0.110 | 31V | 425 | 4JUV | 2,500 | 4JUV | 2,075 | +606 |
| E.Y. | (Ca ⁺⁺) | 24.0 | 34.0 | 0.706 | 31V | 1,250 | 4JUV | 1,850 | 4JUV | 600 | +48 |
| A.L. | (Sec) | 33.2 | 47.2 | 0.703 | 31V | 400 | 4JUV | 1,500 | 4JUV | 1,100 | +275 |
| B.W. | (Ca ⁺⁺) | | | | 31V | 1,100 | 4JUV | 1,550 | 4JUV | 450 | +40 |
| R.S. | (Sec) | | | | 31V | | 4JUV | | 4JUV | | |

BAO = Basal acid output; MAO = maximal acid output; BG = basal (fasting) gastrin; PG = peak (stimulated) gastrin.

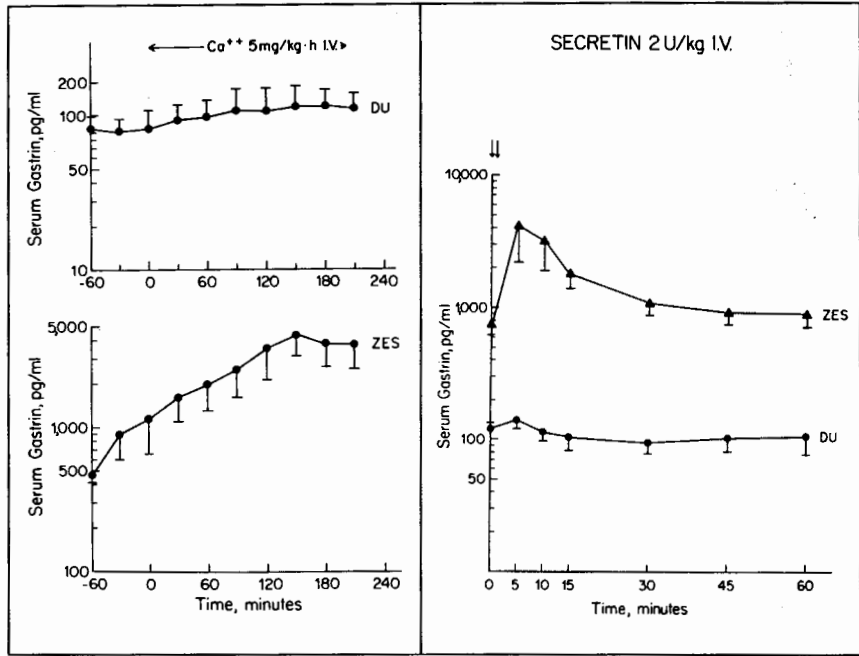


Fig. 2. The effect of calcium given as intravenous infusion at 0 min for 3 h on serum gastrin levels (mean ± SE) of patients with duodenal ulcer disease (DU) and with the Zollinger-Ellison syndrome (ZES).

Fig. 3. The effect of secretin given as a single intravenous injection at 0 min on serum gastrin levels (mean ± SE) of patients with duodenal ulcer disease (DU) and with the Zollinger-Ellison syndrome (ZES).

levels many times higher than the normal range, often greater than 1,000 pg/ml (16). However, several series have included patients with values between 150 and 600 pg/ml and several patients with DU in our study may have fasting serum gastrin levels between 150 and 250 pg/ml (11). This overlap indicates that basal (fasting) serum gastrin may lead to an incorrect diagnosis, particularly when the clinical picture and gastric secretion data are inconclusive (18). Furthermore, recent evidence suggests that a significant number of patients with a gastric secretion pattern typical of ZES have normal serum gastrin levels (3). The hypersecretory state in this group may be due to a hormone other than gastrin or alternatively to small fragments of gastrin not recognized by the gastrin antibody in the RIA system.

In our study 50% of the patients in the DU group had a basal acid output of greater than 10 mEq/h (10.4–29.6 mEq/h) and 4 of them greater than

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