Radioimmunoassay of gastrin: studies in pernicious anaemia

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SUMMARY Serum gastrin levels in patients with pernicious anaemia were measured by immunoassay in the fasting state, following gastric perfusion with 0·9% saline, 0·1N hydrochloric acid, and solutions of increasing acidity, and after the intravenous injection or infusion of secretin. The fasting serum gastrin level was measured in 21 patients with pernicious anaemia and found to be elevated at 1,036 ± 215 pg per ml. Gastric perfusion with saline (pH 4·7) caused a mean fall in serum gastrin of 30% in four patients; perfusion with hydrochloric acid caused a further slight fall. Perfusion with solutions of increasing acidity resulted in a sharp fall in serum gastrin levels when the acidity was changed from pH 6 to pH 4. A single intravenous injection of secretin produced a mean maximal fall of 44% in the serum gastrin level in four patients, whereas continuous infusion of secretin produced a fall of 35% in four other patients. These studies suggest that the gastrin-secreting cells of the stomach are not affected by the atrophic process in pernicious anaemia and remain subject to the regulating control of acid and secretin.

High gastrin levels in the serum of patients with pernicious anaemia have been reported recently by Hansky and Cain (1969), Yalow and Berson (1970), and McGuigan and Trudeau (1970). Yalow and Berson (1970) described a fall in serum gastrin levels in these patients following intragastric instillation of acid, but did not report whether this had been controlled by a prior or later instillation of water or saline. McGuigan and Trudeau (1970) studied postprandial serum gastrin levels and found no significant stimulation of gastrin release by food, in contrast to normal subjects and patients with duodenal ulcer (Hansky and Cain, 1969).

In an effort to explore the behaviour of this hypergastrinaemia in response to some normal physiological inhibitors, the effects of perfusion of the stomach with saline and acid and the effects of the intravenous injection or infusion of secretin have been studied and are the subject of this report.

Patients Studied

Twenty-one patients with Addisonian pernicious anaemia, seven males and 14 females, aged between 39 and 84 years, were studied. The average age of the males was 61 years and that of the females 66 years. The diagnosis of pernicious anaemia was confirmed in each patient by blood film, bone marrow aspiration, serum B12 and folate levels, radioactive B12 absorption with and without intrinsic factor, augmented histamine tests, and response to vitamin B12 therapy. No patient received antibiotics in the interval between measurement of absorption of radioactive B12 (dose alone) and absorption of radioactive B12 with intrinsic factor. The immunofluorescent test for parietal cell antibodies was positive in 19 of the 21 patients. The nature of the study was explained to each patient and they were all volunteers.

Methods

After an overnight fast, blood was drawn from each of the 21 patients to determine the fasting serum gastrin levels. Further procedures were then performed on 15 of the patients.

Seven patients had a 16F radiopaque tube inserted through the mouth into the stomach under radiographic control so that the tip of the tube lay in the antrum. A 19 gauge Court needle was inserted into a forearm vein and kept patent by flushing with a solution of 1,000 units heparin in 20 ml of 0·9% saline. Each patient received no more than 15 ml of this solution, over a minimum of one and a half hours. Serum gastrin levels are not affected by this

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small dose of heparin. Blood was drawn at 10 or 15 minute intervals throughout the test (Figs. 1, 2a, and 2b) and serum separated by centrifugation. With five patients, after a period of 15 minutes, 0.9% saline was infused into the stomach at the rate of 200 ml per hour for 30 minutes. After aspiration of the saline, 0.1N hydrochloric acid was infused at the same rate for 30 minutes. The tube was then removed without aspiration of the stomach and blood taken over a further 45 minutes.

With the other two patients, 100 ml of solution of pH 8 was introduced into the stomach, followed by infusion with the same solution at the rate of 200 ml per hour for 30 minutes. After aspiration and measurement of the pH of the aspirate with a glass electrode pH meter, the stomach was similarly infused with solutions of pH 6, pH 5 (one case only), pH 4, pH 2, and finally, pH 1. The solutions used were obtained by adjusting the pH of 0.9% saline with sodium bicarbonate or hydrochloric acid. The pH of the gastric contents remained steady over each 30-minute period. The tube was removed and blood taken over a further 30 minutes.

Four patients had an intravenous injection of 2 units per kg body weight secretin (Boots, Nottingham) given as a single injection, and after three basal readings serum gastrin was measured at 5, 10, 15, 20, 30, 45, and 60 minutes after injection.

A further four patients had a constant intravenous infusion of secretin at a dose of 2 units per kg body weight per hour, given by a Harvard constant infusion pump, and after three basal readings serum gastrin was measured at five, 10, 15, 20, 30, 45, and 60 minutes after the start of the infusion.

Serum gastrin was measured in duplicate in each blood sample by radioimmunoassay as previously described (Hansky and Cain, 1969).
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Results

The mean fasting serum gastrin level (± SEM) for the 21 patients was 1,036 ± 215 pg per ml. The normal laboratory range established from measurements on 60 normal subjects was 0-120 pg per ml. Only one patient had a fasting level within this range (25 pg per ml). The serum gastrin levels in the remaining patients ranged from 190 to 5,000 pg per ml. The patient with the normal serum gastrin was one of the two in the group with no detectable gastric parietal cell antibodies. The serum gastrin in the other patient without antibodies was 700 pg per ml.

RESPONSE TO SALINE AND ACID PERFUSION

The results of the studies in four patients are shown in Figure 1. The other subject had normal gastrin levels which fell to zero gastrin with acid perfusion. In the four patients, there was a mean fall of 30% during the control perfusion with saline and a further slight fall to 35% of basal levels during the acid perfusion.

RESPONSE TO INFUSIONS WITH SOLUTIONS OF INCREASING ACIDITY

The results of the studies in two patients are shown in Figures 2a and 2b. In one case a marked fall occurred when the acidity of the gastric perfusate was changed from pH 6 to pH 4. In the other there was a slight fall between pH 6 and pH 5 and a marked fall between pH 5 and pH 4.

RESPONSE TO SINGLE INTRAVENOUS INJECTION OF SECRETIN

The results of the four patients studied are shown in Table I. The average fall in serum gastrin (± SEM) was 44 ± 15% of basal levels. An example of the results obtained in an individual patient is shown in Figure 3.

Table I  Response to rapid intravenous injection of secretin (2 units per kg body weight)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum Gastrin (pg/ml)</th>
<th>Time of Lowest Serum Gastrin Level after Secretin (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Basal</td>
<td>Lowest Level after Secretin</td>
</tr>
<tr>
<td>8</td>
<td>1,393</td>
<td>560</td>
</tr>
<tr>
<td>9</td>
<td>1,273</td>
<td>1,000</td>
</tr>
<tr>
<td>10</td>
<td>1,177</td>
<td>1,000</td>
</tr>
<tr>
<td>11</td>
<td>633</td>
<td>140</td>
</tr>
</tbody>
</table>

Fig. 3  Serum gastrin response to the rapid intravenous injection of secretin (2 units per kg body weight) in a patient with pernicious anaemia.

Fig. 4  Serum gastrin response to the continuous infusion of secretin (2 units per kg body weight per hour) in a patient with pernicious anaemia.
RESPONSE TO CONTINUOUS INFUSION OF SECRETIN

The results of the studies of four patients are shown in Table II. The average fall in serum gastrin (± SEM) was 35 ± 4% of basal levels. The results obtained in an individual patient are shown in Figure 4.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum Gastrin (pg/ml)</th>
<th>Time of Lowest Serum</th>
<th>Patient</th>
<th>Serum Gastrin (pg/ml)</th>
<th>Time of Lowest Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Basal</td>
<td>Lowest Level</td>
<td>Gastrin Level</td>
<td>Infusion</td>
<td>during Secretin</td>
</tr>
<tr>
<td>12</td>
<td>740</td>
<td>460</td>
<td>30</td>
<td>13</td>
<td>805</td>
</tr>
</tbody>
</table>

Table II  Response to the continuous infusion of secretin (2 units per kg body weight per hour)

Discussion

In this study we have confirmed that most patients with pernicious anaemia have serum gastrin levels significantly greater than normal and often in the range found in the Zollinger-Ellison syndrome (Hansky and Cain, 1969).

Three factors must be considered in the genesis of this hypergastrinaemia.

OVERPRODUCTION OF GASTRIN

Recently, Rubin (1969) showed a proliferation of enterochromaffin cells in the gastric mucosa of patients with pernicious anaemia and postulated that these cells have an endocrine function. It is possible that overproduction of gastrin by an increased number of endocrine cells is a factor in determining the high serum gastrin levels found in pernicious anaemia. The high residual gastrin levels after 'inhibition' by acid or secretin may be further evidence of overproduction of gastrin, with consequent inability to suppress these levels to normal.

DECREASED UTILIZATION OF GASTRIN

A second factor to be considered is decreased utilization of gastrin associated with the loss of parietal cells. Gastrin levels may be raised because there are no parietal cells to stimulate, with a consequent accumulation of circulating gastrin. While we cannot exclude this possibility, the prompt fall in gastrin levels after acid instillation and after secretin indicates that the half life of gastrin is unlikely to be prolonged.

LACK OF INHIBITION OF GASTRIN RELEASE

We have considered the absence of normal autoregulation of gastrin by hydrochloric acid (Woodard, Lyon, Landor, and Dragstedt, 1954; Redford and Schofield, 1965; Nyhus, Chapman, DeVito, and Harkins, 1960) to be one of the main factors involved in the hypergastrinaemia. Yalow and Berson (1970) found that elevated gastrin levels were lowered by the intragastric instillation of hydrochloric acid in five patients with pernicious anaemia, suggesting that the gastrin-producing cells are still subject to normal autoregulation. The present study has explored the effect of both the gastric mechanism dependent on pH and one of the duodenal mechanisms inhibiting gastrin action, and possibly gastrin release, namely, secretin (Wormsley and Grossman, 1964; Wormsley, 1968; Stening, Johnson, and Grossman, 1969).

We were surprised to find that the 'control' instillation of 0.9% saline into the stomach produced a fall in serum gastrin of 30%. The fall could not be explained by the presence of an orogastric tube as the gastrin levels remained high while the tube was in situ without perfusion and conversely continued to fall after removal of the tube at the end of the acid perfusion. On finding that the pH of the saline was 4.7 units, consideration was given to the possibility that the response to saline was due to its relatively low pH. This was supported by studies in two patients which showed that the fall in serum gastrin levels occurred when pH was lowered from 6 units to 4 units; in one of the patients the principal fall occurred between pH 5 and pH 4.

In the dog acid secretion in response to gastrin release is inhibited by electrolyte solutions when the antral pH is reduced below 3 and maximal inhibition occurs at a pH below 2 (Andersson and Olbe, 1964; Posey and Franklin, 1967). This inhibitory mechanism is in part dependent on the stimulus used and occurs at a pH of between 3 and 5 in response to glycine (Elwin and Andersson, 1966). However, in our patients with pernicious anaemia, we are not concerned with exogenously stimulated gastrin but that due to endogenous factors. If these endogenous factors were similar to those acting in normal subjects, we would expect no activation of antral inhibitory mechanisms until the pH was reduced to 3.

The inhibition of gastrin release in pernicious anaemia at a pH of 4-5 units may be explained by the possibility that some of the solution instilled into the antrum passed into the duodenum and activated duodenal inhibitory mechanisms. It has been shown that the gastric acid response to sham feeding, which may depend on gastrin release from the antrum, is inhibited by a pH of 4 in the duodenal...
bulb in dogs (Nilsson, 1969). This may explain the relatively high intragastric pH at which gastrin is inhibited in pernicious anaemia but another possibility is that the inhibitory mechanisms are set at a higher pH level in pernicious anaemia because of the protracted achlorhydria.

We cannot explain the failure of complete inhibition of gastrin release by acid or exogenous secretin. Certainly perfusion of the antrum by acid was prolonged enough (over two hours at pH below 4) so that decreased time of contact cannot be the reason. It is of interest that in the one patient with normal fasting gastrin, we were able to inhibit release of gastrin completely with acid. This suggests that in the case of overproduction, the cells are only partially responsive to normal inhibitory mechanisms and some degree of autonomy must exist.

These studies indicate that the gastrin-secreting cells of the stomach are not involved by the atrophic process in pernicious anaemia and lead to an overproduction of gastrin. Gastrin release is significantly inhibited by acid and secretin and this suggests that failure of inhibition associated with protracted achlorhydria is another factor determining these high gastrin levels. Although decreased utilization of gastrin cannot be excluded, we consider overproduction and decreased inhibition to be the key factors in the genesis of hypergastrinaemia in pernicious anaemia.

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References


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