RELATIONSHIP BETWEEN SERUM GASTRIN LEVELS AND GASTRIC SECRETION IN HEIDENHAIN POUCH DOGS

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Abstract - Relationship between serum gastrin levels and gastric secretion was studied in Heidenhain pouch dogs. Bethanecol and tetramethylammonium increased gastric secretion without any significant change in the serum immunoreactive gastrin (IRG) level. Histamine increased gastric secretion but decreased the serum IRG level. Tetragastrin evoked gastric secretion concomitantly with an elevation of the serum IRG level, and the relationship was significant. Food-intake promptly increased the serum IRG level which correlated with the increased gastric secretion. Except for the first 15-min value after food-intake, a better correlation was obtained and was almost the same as that with tetragastrin-stimulation. Hexamethonium reduced the food-induced secretion rate in parallel with reduction of the serum IRG level. Correlation between the secretion rate and serum IRG level after dosing was almost the same as that of the control. Atropine and secretin induced a stronger inhibition on the secretion rate than on the serum IRG level. Prostaglandin E1 reduced the secretion rate, but produced no inhibitory effect on the serum IRG level. These results suggest that the food-induced gastric secretion in Heidenhain pouch dogs is due to the action of endogenous gastrin, and that hexamethonium and prostaglandin E1 affect respectively the gastrin and parietal cells. Atropine and secretin affect both parietal and gastrin cells.

Food-induced gastric secretion from denervated fundic (Heidenhain) pouch is considered to be produced by an action of gastrin (1). Involvement of cholinergic nerve pathway in the gastrin release has also been postulated (2). These studies, however, have been done using gastrin bioassay. Thus, changes in response of oxyntic glands to gastrin may be involved in the observed changes in gastrin release.

Recently developed radioimmunoassay of gastrin has made it possible to determine gastrin concentrations in the blood without the complexity mentioned above (3, 4, 5). Although at least three components have been identified in “immunoreactive gastrin (IRG)” (4, 5), the hypothesis concerning the gastrin mechanism should be re-examined using gastrin radioimmunoassay.

We investigated the relationship between secretion rate and serum IRG levels in Heidenhain pouch dogs challenged by several secretory stimulants and inhibitors.

MATERIALS AND METHODS

Male and female Beagle dogs, weighing 5-10 kg, were used. A Heidenhain pouch was prepared with the dog under pentobarbital anesthesia according to the conventional method (6). The pouch was drained by a stainless steel cannula. At least 4 weeks were allowed
for recovery from surgery before the start of experiments.

The dogs were deprived of all solid food but were given free access to water for 24 hr before each experiment. Tests were carried out once a week. The test animals were fed 160 g of canned beef or given s.c., histamine dihydrochloride (50 μg/kg), tetragastrin (8 μg/kg) or bethanecol chloride (30 μg/kg) every 15 min. Under these conditions, dogs secrete a submaximal amount of gastric juice (2-7 ml/15 min).

Gastric juice was collected continuously from the pouch in 15-min samples. Gastric juice volume was recorded, and acid concentration was determined by titration with 0.1 N NaOH up to pH 7 using an automatic titrator (Hiranuma, Japan).

Blood samples for the determination of serum gastrin were collected from the leg vein once before each test, and 15, 30, 45, 60, 90 and 120 min after feeding or dosing. Each sample was allowed to clot, centrifuged at 3,000 rpm at 4°C for 10 min, and the serum was aspirated and frozen at -20°C until assay.

Serum IRG level was determined by radioimmunoassay as described by Yalow and Berson (3). Antisera to gastrin were produced in rabbits by immunization with synthetic human gastrin I (SHG: 1-17) conjugated with bovine serum albumin. 125I-SHG (specific activity: approx. 600 mCi/mg) was obtained from Dainabot Radioisotope Lab., Japan. Antibody-bound 125I-SHG was separated with activated Amberlite IRG-58 (100-400 mesh, Organo, Japan). SHG was used as a standard gastrin. Serum samples were assayed twice.

The following drugs were used; atropine sulfate (E. Merck), hexamethonium bromide (Methobromin, Yamanouchi), synthetic human gastrin (SHG: 1-17, Imperial Chemical Industries), tetragastrin (Aoc-Try-Met-Asp-Phe-NH2, Protein Research Foundation, Mino), caerulein (Sigma Co.), cholecystokinin-pancreozymin (Eisai), secretin (Eisai), bethanecol chloride (Fujita Pharm.), tetramethylammonium chloride (Nakarai) and histamine dihydrochloride (Sigma). Prostaglandin E1 was a gift from Upjohn Research Labs., U.S.A.

RESULTS

Standard curve for immunoassay

Using 0-1000 pg/ml of SHG as the standard solutions, the percent of bound 125I-SHG in total 125I-SHG after incubation for 5 days was plotted against the concentration of SHG using various concentrations of antisera. The slope of the standard curve was steeper for the range of 0-500 pg/ml of SHG when 1:2000 final dilutions were used. Thus, 1:2000 dilution was used in the following study. The standard curve is shown in Fig. 1.

Specificity of antisera

Immunoreactivities of caerulein, cholecystokinin-pancreozymin (PZ-CCK), secretin and tetragastrin were compared (Fig. 2). Cross-reactivity was found when more than 100 pg/ml of caerulein, 1000 pg/ml of tetragastrin and 0.01 Harper U/ml of PZ-CCK were added. The cross-reactivities of caerulein and tetragastrin were respectively one-tenth and one-hundredth that of SHG. No significant cross-reactivity was found under 5 Harper U/ml of secretin.
FIG. 1. Standard curve for radioimmunoassay of gastrin. Vertical bars represent ranges of values in duplicate determinations.

FIG. 2. Cross-reactivity of some peptides with gastrin antisera

Relationship between secretion and acid output

There was a significant linear correlation between the secretion volume and acid output from Heidenhain pouch in dogs given food, tetragastrin, histamine, bethanecol or tetramethylammonium. The correlation was almost the same irrespective of the secretagogues used (Table 1). Based on these findings we refer hereinafter to the secretion volume in order to avoid complexity.

Effect of secretory stimulants on serum gastrin level and secretion

(i) Food

The mean serum IRG level of 43 pg/ml increased to a peak of 211 pg/ml at 15 min after
feeding, dropped slightly, leveled off between 30 and 45 min, and thereafter decreased gradually to 85 pg/ml at 120 min (Fig. 3).

TABLE 1. Relationship between secretion volume and acid output induced by various secretory stimulants

<table>
<thead>
<tr>
<th>Secretory stimulant</th>
<th>Y: Acid output (μEq/15 min)</th>
<th>X: Secretion volume (ml/15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y = a(0) + a(1)X</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>a(0) = -14.2, a(1) = 156.2</td>
<td></td>
</tr>
<tr>
<td>Tetragastrin</td>
<td>a(0) = -37.3, a(1) = 150.6</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>a(0) = -70.3, a(1) = 160.9</td>
<td></td>
</tr>
<tr>
<td>Bethaneol</td>
<td>a(0) = -52.6, a(1) = 159.8</td>
<td></td>
</tr>
<tr>
<td>Tetramethylammonium</td>
<td>a(0) = -62.1, a(1) = 140.2</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.996, 0.993, 0.997, 0.983, 0.965</td>
<td></td>
</tr>
</tbody>
</table>

The equations of regression were calculated according to the least square method.

Gastric secretion was insignificant when the animals were fasted. The secretion increased gradually after feeding and reached a maximum of 3.6 ml/15 min at 30-45 min and decreased gradually to 0.5 ml/15 min at 120 min.

(ii) Drugs

Subcutaneous injection of bethaneol (30 μg/kg) and tetramethylammonium (300 μg/kg/
kg) every 15 min provoked gastric secretion. The serum IRG level tended to increase during the first 30 min after the commencement of injection, and then decreased. (Fig. 4)

Gastric secretion was initiated by intermittent subcutaneous injections of tetragastrin and increased gradually to a plateau of 2.1–2.6 ml/15 min 60 min after the first injection (Fig. 5). Tetragastrin also prompted an increase in the serum IRG level and the time-course of increase paralleled that of secretion.

Gastric secretion commenced at the first 15 min after the subcutaneous injection of histamine and leveled off at about 7 ml/15 min 60 min after the first injection (Fig. 5). Serum IRG level decreased inversely with the increase in secretion, and fell to 22 pg/ml 120 min after the first injection.

(iii) Relationship between gastrin level and secretion

There was a significant relationship (r = 0.540) between the increased serum IRG level and secretion volume prompted by food-intake. A better correlation was obtained when the first 15 min value was excluded, the correlation coefficient being 0.927 and almost identical with that for tetragastrin stimulation (Fig. 6).

There was also a significant relationship between IRG level and secretion volume induced by histamine. However, the secretion volume was inversely correlated with the IRG level (r = −0.937), which was in marked contrast to the results with tetragastrin and food were used as stimulants.

No correlation was seen between gastric secretion induced by bethanecol and tetra-

![Fig. 5. Effect of tetragastrin and histamine on the serum gastrin level and gastric secretion in Heidenhain pouch dogs. See Fig. 4.](image)

![Fig. 6. Relationship between the serum gastrin level and gastric secretion stimulated by food, tetragastrin and histamine. The equations of regression were calculated according to the least square method. The value 15 min after feeding is excluded from the calculation.](image)
methylammonium and serum IRG level.

Effect of secretory inhibitors on food-induced serum gastrin level and secretion

(i) Drugs

Atropine (16 μg/kg) almost completely reduced gastric secretion induced by food (Fig. 7). Gastrin response, though depressed slightly, was still apparent even after administration of the drug. Hexamethonium (1 mg/kg) reduced both secretion and gastrin response to feeding, and the duration and degree of inhibition of the former paralleled those of the latter.

Secretin (8 U/kg) reduced the food-induced secretion by approximately 50% for the first 120 min after feeding (Fig. 8). The gastrin response was inhibited slightly immediately

Fig. 7. Effect of atropine and hexamethonium on food-induced serum gastrin level and gastric secretion. Solid lines represent the values without drug application. Broken lines and hatched areas represent the value with drug application. The arrow indicates the feeding time (160 g of canned beef). Drugs were given i.v. immediately before feeding. N = number of animals.

Fig. 8. Effect of secretin and prostaglandin E₁ on food-induced serum gastrin level and gastric secretion. See Fig. 7.
after dosing but was increased slightly at 60 min after dosing.

Prostaglandin E1 (8 μg/kg) reduced food-induced secretion as in the case with secretin (Fig. 8). Gastrin response was unchanged for the initial 45 min after dosing and thereafter increased slightly.

(ii) Relationship between gastrin level and secretion

The correlation between the serum IRG level and secretion after administration of 1 mg/kg of hexamethonium was almost the same as that in the animals not given the drug. The slope and intercept of the regression line with hexamethonium were approximately the same as those without the drug (Table 2).

There was a significant correlation between serum IRG and secretion after dosing with 8 μg/kg of prostaglandin E1. The slope of the regression line was approximately the same as that of the control, whereas the intercept was 5.6 times less than that of the control. These results indicate that the regression line in the control experiment shifted to the right after treatment with prostaglandin E1.

The secretion after injection of secretin (8 U/kg) did not correlate with the serum IRG level.

The correlation between the serum IRG level and secretion after administration of atropine 16 μg/kg differed greatly from that in the control.

**DISCUSSION**

The present study provides a reliable system for assay of the serum IRG level. The standard curve using 1:2000 final dilution of the antisera was fairly reproducible, and the steep slope of the standard curve allowed for a good determination of the serum IRG level in the physiological range of 0–500 pg/ml.

PZ-CCK showed a slight cross-reaction and secretin none at all. However, PZ-CCK appears to have no effect on gastrin radioimmunoassay because the serum levels of PZ-CCK are much lower (7). It is known that the C-terminal pentapeptide amide sequence of PZ-

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**TABLE 2. Effect of drugs on the relationship between serum gastrin level and secretion after feeding**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Y = a(0) + a(1)X</th>
<th>Y: Gastric secretion (ml/15 min)</th>
<th>X: Serum gastrin level (Pg/ml)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-0.51</td>
<td>0.020</td>
<td>0.927</td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>-0.076</td>
<td>0.0009</td>
<td>0.405</td>
<td></td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>-0.60</td>
<td>0.018</td>
<td>0.771</td>
<td></td>
</tr>
<tr>
<td>Prostaglandin E₁</td>
<td>-2.31</td>
<td>0.024</td>
<td>0.737</td>
<td></td>
</tr>
<tr>
<td>Secretin</td>
<td>-1.0</td>
<td>0.015</td>
<td>0.249</td>
<td></td>
</tr>
</tbody>
</table>

The equations of regression were calculated according to the least square method. The values of serum gastrin level and secretion at 15 min after feeding were excluded from the analysis.
CCK is identical with that of the gastrin molecule, whereas there is no similar sequence between secretin and gastrin (8). These results suggest that the C-terminal peptide amide sequence plays an important role in the binding of gastrin with its antisera. In fact, C-terminal tetra- and penta-peptide of gastrin, i.e. respectively tetragastrin and caerulein, show cross-reactivity with gastrin antisera. Similar results have been obtained by Yalow and Berson (3). All these results indicate that exogenously administered tetragastrin and caerulein result in an elevation of the serum IRG level.

The peak of secretion after feeding occurred later than that of the serum IRG level, except after tetragastrin stimulation when a significant correlation was obtained between the secretion and serum IRG level. McGuigan et al. (9) have reported a good parallel of secretion rate and serum IRG level in Heidenhain pouch dogs after gastrin infusion. On the other hand, a highly significant correlation was obtained between the serum IRG level and secretion rate when values obtained in the first 15 min after feeding were excluded from the calculation. The regression line thus obtained was almost identical with that by tetragastrin stimulation. These results suggest that the gastrin released immediately after feeding is active immunochemically but not physiologically. These findings also can be explained by the fact that a rapid increase in the serum IRG level does not stimulate, but rather depresses the parietal cells. Gregory and Tracy (10) reported that single intravenous injections of gastrin do not provoke acid secretion in dogs. Furthermore, a single large dose of gastrin is known to depress gastrin- and histamine-induced acid secretion (11).

The relationship between the secretion rate and serum IRG level in the control experiment was maintained even after an injection of hexamethonium indicating that hexamethonium reduced the secretion through a reduction of gastrin release. Such is in good agreement with the conclusion of Elwin and Uvnäs (12) who used gastrin bioassay. After administration of atropine, the food-induced gastric secretion was almost completely blocked, whereas the serum gastrin response was reduced partially. The stronger effect of atropine on the gastric secretion than on the gastrin release may be attributed to the fact that atropine exerts inhibition on parietal as well as on gastrin cells. Atropine has been shown to inhibit gastric secretion induced by exogenous gastrin (13). In turn, these results support the view that cholinergically mediated gastrin release plays an important role in the food induced gastric secretion in Heidenhain pouch dogs.

In contrast to the results with food or tetragastrin stimulation, neither bethanecol nor tetramethylammonium increased the serum IRG level in spite of their apparent gastric secretogogue effect. These drugs may either induce a more potent effect on parietal than on gastrin cells, or antral acidification may inhibit endogenous release of gastrin. The first explanation is favoured since there is a more dense cholinergic innervation in the parietal than in the antral region (14). Secretory stimulation in fasting dogs soon results in antral acidification which has been postulated to inhibit gastrin but not parietal cells (15). Endogenous gastrin release by bethanecol and tetramethylammonium is no doubt inhibited by antral acidification. In fact, the serum IRG level decreased with increase in acid secretion, though such tended to increase at the initiation of bethanecol or tetramethylammonium
injections. Furthermore, histamine stimulation decreased the serum IRG level, and this decrease correlated with increase in the secretion rate.

On the other hand, tetragastrin injection increased the serum IRG level, though the antral acidification also occurred in this case. The increase in serum IRG is considered to be due to the increase in serum levels of tetragastrin which was shown to cross-react with gastrin antisera.

The regression line representing the relationship between the food-induced serum IRG level and secretion rate showed a parallel shift to the right after treatment with prostaglandin E₁. These findings indicate that prostaglandin E₁ inhibits gastric secretion by reducing the response of the parietal cells to gastrin, and such is compatible with the report by Way and Durbin (16) that prostaglandin E₁ inhibits gastric secretion induced by exogenous gastrin.

There was practically no correlation between the secretion rate and serum IRG level after treatment with secretin, and such may be due to the inhibition of gastrin and parietal cells by secretin (17).

REFERENCES

15) MAGEE, D.F. AND NAKASHIMA, S.: The effects of antral acidification on the gastric secretion
