Clearance and Acid-Stimulating Action of Human Big and Little Gastrins in Duodenal Ulcer Subjects

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Abstract Acid-stimulating action and clearance of pure natural human big gastrin (HG-34-I) and little gastrin (HG-17-I) were assessed in four male subjects with inactive duodenal ulcer (DU) disease. Disappearance half-times for HG-17-I after intravenous infusion (5.2 min) or rapid intravenous injection (6.4 min) were six to eight times shorter than those for HG-34-I (41.5 and 37.8 min, respectively).

Studies of clearance of synthetic human little gastrin (HG-17-I) were performed in three of these same four DU subjects, eight additional male DU subjects, and eleven normal male subjects. The disappearance half-time of synthetic HG-17-I averaged 6.2 min in both the DU subjects and the normal subjects. These data suggest that clearance of exogenous gastrin is not altered in patients with DU.

Acid secretion in response to rapid intravenous injection of HG-34-I reached a higher peak and lasted longer than in response to an equimolar dose of HG-17-I; the total response to HG-34-I was about three times that to HG-17-I. During constant intravenous infusion, acid responses to equimolar exogenous doses of the two peptides were similar but the increment in molar concentration of circulating gastrin was six to eight times greater with HG-34-I than with HG-17-I.

Chromatography of serum obtained during infusions of HG-34-I revealed no evidence of conversion to HG-17-I, nor was there any increase in circulating G-34 activity during infusions of HG-17-I.

The increment in serum gastrin concentration required to produce half-maximal stimulation of gastric acid secretion (Dw) was estimated in each subject for each gastrin from curves relating acid secretion to change in serum gastrin concentration produced by infusion of these peptides. After instilling peptone solution into the stomach, acid secretion was measured by intragastric titration, and increases in circulating G-17 and G-34 were determined by chromatography and radioimmunoassay of serum. Increases in circulating G-17 and G-34 in response to the peptone meal, taken together, were equivalent to 1.5 times the Dw determined from infusions of G-34 and G-17. Acid secretion during the same time period averaged 55% of maximal rates. Although G-34 comprised approximately three-fourths of the total molar concentration of circulating gastrin after stimulation, it was estimated to contribute less than half of the acid-stimulating activity.

Introduction Two major forms of gastrin, "big" and "little" gastrin, have been identified in human blood (1, 2). Two linear peptides that behave on molecular sieving like these circulating forms have been isolated from gastrin-secreting tumors and their amino acid sequences have been determined (3, 4). Human big gastrin (G-34) consists of 34 amino acid residues; the single tyrosine residue may be nonsulfated (G-34-I) or sulfated (G-34-II). Human little gastrin (G-17) contains 17 amino acid residues and likewise may be either nonsulfated (G-17-I) or sulfated (G-17-II). The COOH-terminal 17-amino-acid sequence of G-34 is identical with G-17. Digestion of G-34 with trypsin results in cleavage of a Lys-Gln bond with formation of G-17 (4).

We previously reported that human G-17-I, porcine G-17-I, and porcine G-17-II had similar acid-stimulating action and rates of elimination in the dog (5). Human G-34-I and G-34-II also had similar potency and elimination rates but differed from the G-17 peptides. G-17 peptides were metabolized approximately six times more.

Abbreviations used in this paper: DU, duodenal ulcer; G-17, little gastrin; G-34, big gastrin.
rapidly than G-34 peptides and approximately fivefold higher increments of molar concentrations in serum were required for G-34 than for G-17 peptides to produce equivalent acid-secretory responses.

The purpose of the present investigation was to compare acid-stimulating properties and metabolism of human G-17-I and G-34-I in patients with inactive duodenal ulcer (DU) disease. In addition, the relation between changes in immunoreactive serum gastrin and rates of acid secretion during infusion of these peptides and after a protein meal were compared. Finally, evidence was sought for conversion of G-34 or G-17 to other immunoreactive molecular forms during intravenous infusion of the pure peptides. Additional studies were done to compare the metabolism of synthetic human G-17-I in a larger number of DU and nonulcer subjects.

METHODS

Gastrins. Pure natural human gastrins, G-17-I and G-34-I, were the generous gifts of Prof. R. A. Gregory and Dr. Hilda Tracy, University of Liverpool. They were purified from a single large gastrin-secreting tumor (3, 4) and were free of contaminating peptides. Gastrin solutions were prepared as previously described (5). The number of studies performed with G-34-I was limited by the small amount of peptide available (1 mg). Synthetic human G-17-I was obtained from Imperial Chemical Industries, Ltd., Cheshire, England.

Subjects. Four male subjects (mean age 49 yr) with inactive DU were repeatedly studied with natural HG-17-I and HG-34-I. None was taking drugs known to affect gastric secretion. None had complications of peptic ulcer or previous upper gastrointestinal surgery. Clearance of synthetic G-17-I was studied in 3 of these subjects, 8 additional male subjects with inactive DU (mean age 48 yr) and 11 normal male subjects (mean age 32 yr).

All subjects gave voluntary informed consent. The study was approved by the Human Subject Protection Committees at UCLA and VA Wadsworth Hospital Center. Gastrin solutions were administered under Food and Drug Administration IND numbers 9598 and 10-872.

Infusions of human G-17-I and G-34-I. For studies of acid secretion and gastrin clearance, the doses of G-17-I and G-34-I were 6.25, 12.5, 25, 50, 100, and 200 pmol/kg-h. Gastrin solutions were diluted in 27 ml of 0.15 M NaCl and infused intravenously for 90 min by a Harvard syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.). Only one dose of gastrin solution was administered per day. On each study day a nasogastric tube was positioned under fluoroscopic control in the most dependent part of the stomach. Gastric juice was collected by automatic plus manual suction in consecutive 15-min periods during a 30-min basal period, during the 90-min infusion period, and for at least 105 min after discontinuing the infusion or until acid secretion had returned to basal level. Acid concentration was measured by titration of 0.2-ml samples to pH 7.0 with 0.2 N NaOH in an automatic titrator (Radiometer Co., Copenhagen, Denmark) (5). Gastric acid secretion was expressed as highest observed 30-min acid output during each test.

Blood samples were obtained at 15-min intervals from an arm vein through a 19-gauge scalp vein needle twice during the basal period and during each infusion. For determination of disappearance half-time, frequent blood samples were obtained after discontinuation of 200 pmol/kg-h infusions of natural human G-17-I and G-34-I and 540 pmol/kg-h infusions of synthetic human G-17-I. Blood samples were allowed to clot and centrifuged, and serum was removed and stored at -20°C.

Intravenous injection of human G-17-I and G-34-I. On separate days, each of the four DU subjects who received infusions of G-17-I and G-34-I was given 50 pmol/kg G-17-I and G-34-I by intravenous injection over 15 s. Gastric secretions and blood samples for gastrin determination were obtained as described above.

Intragastric titration. On a separate day, acid secretion and gastrin release in response to a protein meal were assessed in the four DU subjects tested with G-17-I and G-34-I. The meal consisted of 600 ml 10% wt/vol peptone (Bacto-peptone, Difco Laboratories, Detroit, Mich.) instilled into the stomach via a nasogastric tube. Acid secretion was measured by automatic intragastric titration (6, 7). Blood samples for serum gastrin were obtained during the 30-min basal period and 30 and 60 min after introduction of the meal.

Synthetic human G-17-I infusions. Additional studies of gastrin clearance were performed in 11 DU patients, including 3 of the 4 patients who received the natural gastrin peptides, and 11 normal subjects. Each subject received an intravenous infusion of synthetic G-17-I at a dose of 540 pmol/kg-h for 40 min. Blood samples were obtained for serum gastrin determinations as described above for natural G-17-I.

Gastrin radioimmunoassay. Serum gastrin concentrations were measured by radioimmunoassays as previously described (8, 9). Antibody 1296 was used at a final dilution of 1:300,000 with tracer amounts (0.5 fmol) of moniodinated [125I]natural human G-17-I. With this antibody, natural and synthetic G-17-I had equal immunopoten-
ty, while G-34-I was approximately 0.6 times as immunopotent on a molar basis. Apparent immunoreactive gastrin concentrations in whole serum were measured with G-17-I as standard and expressed as picogram HG-17-I equivalents per milliliter serum. Because serum contains a mixture of gastrins with different affinities for the antibody, total molar concentration of serum gastrin could not be determined without knowledge of the proportion of individual gastrin components in the specimen. Under two conditions molar concentrations of gastrin could be measured directly and expressed as femtomoles per milliliter of serum. During infusion of a single pure gastrin solution, changes in immunoreactive gastrin concentration over basal were due entirely to the type of gastrin being infused (see below). These changes were measured on a molar basis by standard curves prepared from the solution infused, either G-17-I or G-34-I. After fractionation of serum by column chromatography, G-34 and G-17 emerged in distinct peaks and gastrin concentration in each peak was measured by use of the appropriate standard curve.

Fractionation of molecular forms. Serum samples, obtained 85-90 min after initiation of gastrin infusions and before and 30 and 60 min after intragastric instillation of peptone, were chromatographed on 1x100 cm columns of G-50 superfine Sephadex (9). Columns were equilibrated and eluted with 0.02 M sodium barbital buffer, pH 8.4, containing 0.2 g/liter sodium azide, and run at 4°C. Samples (100 μl or 1 ml) were applied to the column with 0.5 pg moniodinated [125I]G-17-I as a marker. Serum protein and salt peaks were detected by absorbance at 280 nm and conductivity, respectively. Elution volume was expressed in
terms of the distance between the protein peak (0%) and the salt peak (100%). Fractions were obtained of 1 ml, and 0.5-ml aliquots were tested for gastrin activity by radioimmunoassay. Standard solutions of G-34-I added to normal serum emerged with the same elution volume (32–36%) as peaks of immunoreactivity obtained by chromatography of serum taken during infusions of G-34-I. The peak elution volumes of standard solutions of G-17-I and serum obtained during G-17-I infusion ranged between 63 and 69%. Gastrin activity in column eluates was measured by radioimmunoassay with a G-34-I standard for the peak emerging before 50% elution volume. The gastrin radioimmunoassay system was sufficiently sensitive to detect gastrin concentrations of 0.25 fmol/ml G-17-I in the final incubation or concentrations greater than 1 fmol/ml in column eluates tested.

Calculations. Disappearance half-time (t₁) was calculated for each subject after rapid intravenous injection of human G-17-I and G-34-I and after cessation of intravenous infusions (5). Basal gastrin values were subtracted and the regression of the natural logarithm of increment in serum gastrin vs. time was computed to yield the slope which, with change of sign, is equivalent to the disappearance constant (k). Disappearance half-time was calculated from the equation: t₁ = ln2/k.

The infusion period of 90 min was 10–20 times t₁ for G-17-I, so that during the latter part of the infusion period, serum gastrin concentrations reached equilibrium. However, equilibrium was not achieved during G-34-I infusions, since the infusion period was only 1.5–3 times t₁. To predict the serum concentration that would have been achieved if infusions had been prolonged sufficiently, equilibrium steady-state gastrin concentrations were calculated from the equation: Δ gastrin corrected = Δ gastrin observed/(1-0.5⁴); n = infusion time/t₁. Volumes of distribution were calculated as previously described (5), except that corrected steady-state blood concentration of G-34-I was calculated from the observed value at 90 min, as described above.

RESULTS

Serum immunoreactive gastrin during gastrin infusions. Increments in serum gastrin produced by infusions of human G-17-I and G-34-I were linearly related to dose, but equimolar doses of G-34-I produced higher concentrations than did G-17-I (Fig. 1). Equations for the regression lines (y = equilibrium change in serum gastrin in femtomoles per milliliter, x = dose in picomoles per kilogram-hour) were y = 1.15x – 1.3, r = 0.999 for G-17-I, and y = 8.34x – 24.9, r = 0.994 for G-34-I. The y intercept of the regression lines did not differ significantly from zero. The slope for G-34-I was 7.3 times greater than the slope for G-17-I, indicating slower clearance of G-34-I.

Gastrin half-time studies. Gastrin t₁ values are shown in Table 1. Mean t₁ calculated after infusions of natural G-17-I in the four DU subjects (5.2 min) did not differ significantly from the t₁ values obtained with synthetic G-17-I in the larger groups of 11 DU subjects (6.2 min) or 11 normal subjects (6.2 min). Mean t₁ of natural G-34-I after infusion was 41.5 min in the four DU subjects. Mean data for disappearance of

G-17-I and G-34-I after 50 pmol/kg rapid intravenous injections are shown in Fig. 2. After rapid intravenous injection, t₁ results in the four DU subjects were similar to those obtained after constant infusion (6.4 min for G-17-I and 37.8 min for G-34-I). The slight differences in mean half-times in the figure and table are due to calculation of mean half-time in the figure from mean gastrin concentrations of the four subjects at each time period, compared with averaging individual half-times in the table. In addition, after infusion of synthetic human G-17-I, the mean half-times in the four DU patients

![Graph showing mean plateau increments in immunoreactive gastrin (basal values subtracted) as a function of molar infusion rates of human G-17-I and G-34-I in four DU subjects. Plateau values for G-34-I were calculated from the 90-min value as described in the text; plateau values for G-17-I represent mean values for 60, 75, and 90-min points during infusion.](attachment:image)

**Table 1**

<table>
<thead>
<tr>
<th>Disappearance Half-Times of Gastrin Preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Subject</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>V. W.</td>
</tr>
<tr>
<td>J. I.</td>
</tr>
<tr>
<td>L. N.</td>
</tr>
<tr>
<td>E. M.</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SE</td>
</tr>
<tr>
<td>11 DU subjects* Mean</td>
</tr>
<tr>
<td>11 normal subjects Mean</td>
</tr>
</tbody>
</table>

* Including subjects V. W., L. N., and E. M. Subject J. I. was not tested (NT).
Mean values for intravenous FIGURE DU subjects. Basal ulcer and similar were body weight infusions after group spaces rapidly than G-34-I. These three were not measured during the serum infusions. These three serum were cleared reasonably regression of slopes calculated from serum gastrin on dose (7.3). These three independent estimates indicate that G-17-I is cleared from the circulation about six to eight times more rapidly than G-34-I.

Acid secretion rates vs. exogenous dose and change in serum gastrin. Highest observed 30-min acid outputs during the 90-min infusions of human G-17-I and G-34-I in the four DU subjects are shown in Fig. 3. Responses were not significantly different after equivalent molar doses.

Mean acid secretory responses, normalized as a percentage of the highest observed response during each infusion, in the four DU subjects, are shown in Fig. 4. The acid secretory responses to G-17-I reached a plateau within 60 min after starting the infusion. During infusion of G-34-I, acid secretion began promptly but had not reached a peak plateau 90 min after starting the infusion, and did not return to preinjection level 105 min after discontinuing the infusion. Acid secretory half-times, calculated from individual tests after G-17-I, ranged from 19.5 to 27.5 min (24.3±1.8 min), and after G-34-I from 71.0 to 96.2 min (84.1±6.2 min). Secretory half-times after low (12.5 pmol/kg-h) and high doses (200 pmol/kg-h) did not differ significantly. For G-17-I the secretory half-times were 24.2±3.3 and 26.7±4.2 min at low and high doses, respectively. The corresponding results for G-34-I were 87.5±13.7 and 96.2±11.0 min.

Mean acid secretory responses to rapid i.v. injection of 50 pmol/kg of G-17-I and G-34-I in the four DU subjects are shown in Fig. 5. The response to G-17-I reached a peak during the first two 15-min periods and fell to basal rates within 60 min. After G-34-I injection, secretion increased promptly but the peak rate occurred later, was greater than that achieved with G-17-I, and persisted above basal rates for 2h. Total secretory response to G-34-I was approximately three times greater than to G-17-I.

In contrast, there was a marked difference in potency of circulating human G-17-I and G-34-I as stimulants of acid secretion. To produce the same rate of acid secre-
tion, approximately four to eight times greater increment in the molar concentration of G-34-I than in G-17-I was required (Fig. 6). Exact ratios could not be calculated because the dose-response curves were not parallel.

**Chromatography of serum obtained during gastrin infusions.** In all four DU subjects, serum obtained 90 min after onset of G-34-I infusion yielded a single peak of immunoreactive gastrin, recovered with the characteristic elution volume of G-34-I. Less than 1% of gastrin immunoreactivity was recovered in the G-17-I region. Similarly, 92% (range 81–100%) of gastrin immunoreactivity was recovered in the G-17-I region during infusions of G-17-I. The remaining 8% eluting in the G-34-I region (16 fmol/ml) did not differ significantly from mean recovery in this region in basal serum (17 fmol/ml). Thus, no evidence was obtained for significant conversion of G-17-I or G-34-I into the other molecular form during infusion of either pure peptide.

**Response to peptone meals.** Acid secretion rates 30–60 min after introduction of peptone meals averaged 55% of peak G-17-I-stimulated secretory rates, compared with 6% in the basal period. Increases in total serum gastrin activity in unfraccionated serum at 30 min and 60 min after the meal were 60 and 57 pg equivalents G-17-I/ml, respectively (Table II). In serum obtained during the basal period and 30 and 60 min after the meal, specific molar increases in serum G-17 and G-34 were measured by column chromatography and radioimmunoassay of peaks with appropriate standards. Recovery of 100 fmol G-34-I added to 1 ml basal serum or 1 ml 60-min-postprandial serum was assessed by column chromatography. Recovery averaged 65±12% in basal and 66±9% in 60-min-postprandial serum. Recovery of 50 fmol G-17-I added to the same serum specimens averaged 97±8% and 87±6%. Mean increase in G-34 over basal was 44 fmol/ml at both 30 and 60 min, while mean increase in G-17 was 18 fmol/ml at 30 min and 14 fmol/ml at 60 min (Table II).

The relative contributions of G-17 and G-34 to acid secretion stimulated by the peptone meal were estimated from mean molar increments in G-17 and G-34 30 and 60 min after the meal, compared with molar increments in circulating G-17-I and G-34-I required to produce half-maximal secretory responses (Dw) during infusions of pure gastrins (Table III). Estimated mean total circulating activity represented by G-17 and G-34 were 0.88 and 0.67 × Dw, respectively, at 30 min, and 0.72 and 0.65 × Dw at 60 min. Mean acid secretion was 55% of peak acid output during the 30–60-min time period. At both 30 and 60 min, G-17 appeared to contribute the majority of acid-stimulating activity as estimated from multiples of Dw present in the serum. The fraction of acid-stimulating activity contributed by G-17 was estimated as 62% when calculated from individual subjects or as 55% when calculated from mean data.

**DISCUSSION**

The results of the present studies in DU subjects indicate that: (a) equimolar infusions of human G-17-I and G-34-I produced similar acid secretory responses; (b) circulating G-17-I was a more potent stimulant of gastric acid secretion than G-34-I; (c) the secretory and serum half-times of G-34-I were significantly longer than G-17-I; (d) serum half-time of G-17-I was not different in DU and controls; (e) there was no ap-
parent conversion of one molecular form of gastrin to the other; and (f) after a protein meal, G-17-I had a greater role in the acid secretory response than G-34-I.

The number of studies with G-34-I had to be limited because only 1 mg was available. This was the remaining G-34-I which had been extracted from a single gastrin-secreting tumor by Prof. R. A. Gregory for determining the amino acid sequence of human big gastrin (4) and for studies on the biological activity of this peptide in dogs (5). Additional pure natural human G-34 is not likely to be available in the near future, so it will not be possible to extend these studies until synthetic G-34-I has been prepared.

**Table II**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Basal</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-34</td>
<td>G-17</td>
<td>G-34</td>
</tr>
<tr>
<td>V. W.</td>
<td>30</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>J. I.</td>
<td>25</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>L. N.</td>
<td>50</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>E. M.</td>
<td>45</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>Mean</td>
<td>38</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>SE</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

* T, total serum gastrin activity in unfractionated serum, picograms G-17-I equivalents per milliliter.
† G-34, G-17, molar concentration of G-34 and G-17 per milliliter serum determined by Sephadex G-50-S fractionation and radioimmunoassay with G-34 or G-17 standard.

Serum disappearance half-times for human G-17-I and G-34-I were two to three times longer in the human subjects of this study than measurements obtained previously in dogs (5), but the relative rates of disappearance were similar. No difference in metabolism of synthetic G-17-I could be demonstrated between subjects with and without DU. The serum half-times of natural and synthetic G-17-I in the present study are in general agreement with those found by other workers who studied metabolism of synthetic G-17-I in man (10, 11). Volumes of distribution were similar to those found by Straus and Yalow in dogs (12), but were lower than the values of approximately 20% we obtained previously in dogs (5). These differences may be due in part to greater losses of immunoreactive gastrin during storage of dog serum.

There are at least three ways in which potency can be expressed: acid secretory response as a function of exogenous dose by i.v. injection; as a function of exogenous dose by continuous i.v. infusion; and as a function of change in circulating hormone concentration. In these experiments the potency of G-17-I relative to G-34-I was approximately one-half to one-third by the first criterion, equal by the second, and four to eight times greater by the third. The duration of response after rapid injection was measured until secretion rates returned to base line and therefore represents unequal time periods, whereas response during infusion was measured as a rate, that is response per unit time.

Since human G-34-I has a longer circulating half-time and lower circulating potency than G-17-I, it is reasonable to question whether conversion of G-34-I to G-17-I is necessary for biological action. Failure to detect generation of G-17-I by chromatographic analysis of serum specimens obtained during and after G-34-I infusion appears to exclude significant conversion in the

**Table III**

<table>
<thead>
<tr>
<th>Acid secretion</th>
<th>Gastrostom stimulation PAO</th>
<th>Peptone stimulation HG-34-I</th>
<th>HG-17-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>µg/min</td>
<td>% PAO</td>
<td>fmoi/ml</td>
</tr>
<tr>
<td>V. W.</td>
<td>847</td>
<td>62</td>
<td>350</td>
</tr>
<tr>
<td>J. I.</td>
<td>878</td>
<td>76</td>
<td>22</td>
</tr>
<tr>
<td>L. N.</td>
<td>1204</td>
<td>40</td>
<td>152</td>
</tr>
<tr>
<td>E. M.</td>
<td>610</td>
<td>41</td>
<td>58</td>
</tr>
<tr>
<td>Mean</td>
<td>884.7</td>
<td>55</td>
<td>146</td>
</tr>
<tr>
<td>SE</td>
<td>122</td>
<td>8.7</td>
<td>73</td>
</tr>
</tbody>
</table>

* Estimated from individual dose response curves, Δgastrin vs. acid secretions. D₅₀ represents increment in circulating gastrin concentration required to produce half-maximal acid secretion. PAO, peak rate of acid output achieved during gastrin infusions.
by protein meals, 

plays a role in stimulation of acid secretion by a protein meal, but does not exclude simultaneous stimulation by other factors, such as gastric distension.

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REFERENCES


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