Gastrin Receptors on Isolated Canine Parietal Cells

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Abstract. The receptors in the fundic mucosa that mediate gastrin stimulation of acid secretion have been studied. Synthetic human gastrin-17-I (G17) with a leucine substitution in the 15th position ([Leu^{15}]-G17) was iodinated by chloramine T; high saturable binding was found to enzyme-dispersed canine fundic mucosal cells. ^{127}I-[Leu^{15}]-G17, but not ^{127}I-G17, retained binding potency and biological activity comparable with uniodinated G17. Fundic mucosal cells were separated by size by using an elutriator rotor, and specific ^{125}I-[Leu-^{15}]-G17 binding in the larger cell fractions was highly correlated with the distribution of parietal cells. There was, however, specific gastrin binding in the small cell fractions, not accounted for by parietal cells. Using sequential elutriation and stepwise density gradients, highly enriched parietal and chief cell fractions were prepared; ^{125}I-[Leu^{15}]-G17 binding correlated positively with the parietal cell (r = 0.98) and negatively with chief cell content (r = −0.96).

In fractions enriched to 45–65% parietal cells, specific ^{125}I-[Leu^{15}]-G17 binding was rapid, reaching a steady state at 37°C within 30 min. Dissociation was also rapid, with the rate similar after 100-fold dilution or dilution plus excess pentagastrin. At a tracer concentration from 10 to 30 pM, saturable binding was 7.8±0.8% per 10^6 cells (mean±SE) and binding in the presence of excess pentagastrin accounted for 11% of total binding. G17 and carboxyl terminal octapeptide of cholecystokinin (26–33) were equipotent in displacing tracer binding and in stimulating parietal cell function ([^{14}C]aminopyrine accumulation), whereas the tetrapeptide of gastrin (14–17) had a much lower potency. Proglumide inhibited gastrin binding and selectively inhibited gastrin stimulation of parietal cell function.

Canine parietal cells have specific receptors for gastrin that mediate stimulation of parietal cell function. Gastrin receptors were undetectable on chief cells, and yet present on another smaller mucosal cell(s).

Introduction

In 1905, Edkins (1) first hypothesized the existence of an antral stimulant of acid secretion. With the discovery of histamine in the gastric mucosa and the recognition of its properties as a potent stimulant of acid secretion (2, 3), the existence of an antral "gastrin" hormone became the subject of a controversy that continued for 60 yr. In 1964, Gregory and Tracy (4) established beyond doubt the existence of the hormone gastrin in the antral mucosa. Subsequent studies established that gastrin is a potent stimulant of acid secretion; it has a major physiological role in mediating the acid secretory response to a protein meal (5, 6). Gastrin also has several other actions, including contraction of antral smooth muscle (5) and a trophic effect on the fundic mucosa (7).

Despite the evidence that gastrin is a major endogenous stimulant of acid secretion, uncertainty persists in regard to the locus of the receptor(s) that mediates the acid secretory actions of gastrin. Previous evidence showed that gastrin stimulates the function of parietal cells isolated from canine fundic mucosa (8, 9) and that these effects of gastrin are potentiated by interaction with histamine (10, 11). However, direct gastrin effects on parietal cells remain controversial. In the rabbit, gastrin has been reported to stimulate histamine release from gastric glands (12) and to cause only minimal, if any, direct stimulation of parietal cell function (13, 14), which suggests that the rabbit gastrin receptors may be present on the histamine cell as well as, or rather than, on the parietal cell. To further complicate the histamine-gastrin relationship, the cell types storing histamine vary between species. In the rat fundic mucosa, histamine is present in an enterochromaffin-like cell (15–17), whereas in canine (18) and human (19) fundic mucosa, current evidence

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indicates the presence of histamine only in mast cells. An additional difference is that gastrin stimulates the formation of histamine in the rat but not in canine fundic mucosa (15, 16). These divergent findings underline the uncertainty regarding the cellular locus of receptors of gastrin in the fundic mucosa.

Biologically active radiolabeled ligands provide a powerful tool for the localization and characterization of receptors for chemical transmitters. Recently, Takeuchi et al. (20) have demonstrated a receptor for gastrin in a particulate fraction from homogenates of rat fundic mucosa, although the cellular locus of this receptor and its role in mediating a direct biological response to gastrin were not identified. These workers found that native heptadecapeptide gastrin iodinated by chloramine T did not retain biological activity (20). This finding was at odds with a previous study that indicated that gastrin could be iodinated with preservation of biological activity (21). This difference probably indicates that iodination of gastrin, unless carefully controlled, risks oxidative damage. However, Takeuchi et al. (20) found that when there was a leucine substitution for methionine in the 15th position of gastrin-17 (G17),[1] iodination then yielded a biologically active product that demonstrated specific binding to rat gastric mucosa.

The present studies, using ^{125}I-synthetic human G17 with a leucine substitution in the 15th position (^{125}I-[Leu^{15}]-G17) as a probe for the gastrin receptor, were aimed at localizing the gastrin receptor(s) on cells isolated from canine fundic mucosa and correlating receptor binding to a biological response to gastrin.

Methods

Cell dispersion and separation. Cells were dispersed from adult canine fundic mucosa by using techniques adapted from those previously described (8). In brief, the fundic mucosa from dogs killed with a pentobarbital infusion was bluntly separated from the submucosa and then sequentially incubated in crude collagenase (0.25 mg/ml) and EDTA (1 mM). Cell separation was performed by using a elutriator rotor (Beckman Instruments, Inc., Fullerton, CA) (8, 22). This technique separates cells on the basis of varying sedimentation velocity, which is proportional to the square of the cell radius. Fractions were collected according to the protocol outlined previously (22).

Stepwise density gradients were formed by using a heavy solution of 24% bovine serum albumin (BSA) (Boehringer Mannheim, Indianapolis, IN) plus 9% Ficoll 400 (Pharmacia Fine Chemicals, Piscataway, NJ) dissolved in Hanks' balanced salt solution (Irvine Scientific, Irvine, CA) that had been diluted with water to 64% of the normal salt concentration. The light solution was composed of 12% BSA in Hanks' solution diluted with water to 87% of normal salt concentration. pH was adjusted to 7.4 with 2 N NaOH (higher concentrations of NaOH-precipitated protein). These solutions were modified from those used previously for linear gradient separations of canine fundic mucosal mast cells (18).[2] Gradients were formed in 50-ml polypropylene test tubes with a peristaltic pump. The respective densities of the heavy and light solutions were ρ = 1.066 and ρ = 1.037. These solutions were mixed in ratios of 25% heavy/75% light for the upper gradient step and 65% heavy/35% light for the bottom step. Gradients were centrifuged in a Sorvall RC-5 at 430 *g* for 60 min at room temperature; a rate controller was used for acceleration and deceleration. Cell bands formed at the interfaces were removed by using a peristaltic pump and a capillary tube.

Studies of gastrin binding. For these studies [Leu^{15}]-G17, G17, and pentagastrin were purchased from Research Plus (Bayonne, NJ). The carboxyl terminal octapeptide (26–33) of cholecystokinin CCK8 was purchased from BacChem (Torrance, CA), and the carboxyl terminal tetrapeptide common to gastrin and cholecystokinin (G14-17) was purchased from Peninsula Laboratories (San Carlos, CA). Proglumide was the gift of A. H. Robins Co. (Richmond, VA). Iodination of gastrin was kindly performed for our studies by Dr. John Walsh and Peter Chew, using a 20:1 molar excess of chloramine T over [Leu^{15}]-G17, rather than the 3:1 ratio previously described (21). Monoiodo-G17 was separated from free iodine and the diiodo forms by using DEAE chromatography. G17 and [Leu^{15}]-G17 were also iodinated with ^{127}I. The specific activity of the monoiodo ^{125}I-[Leu^{15}]-G17 prepared by these techniques was ~1.8 μCi/pmol. The chemical purity of the G17 and CCK8 peptides was verified by high pressure liquid chromatography, which was performed by Drs. J. Reeve and D. Abrahm using a Vydac C-4 reverse-phase column and eluting with increasing concentrations of acetonitrile, by starting from a 0.1% trifluoroacetic acid solution. For both G17 and CCK8 used in these studies, >95% of the absorbance at 220 and 280 nm was associated with a single, sharp peak containing the immunoreactive peptide. For both G17 and CCK8, amino-acid analysis was consistent with the composition expected for the pure peptides and the quantities were within 85% of the values predicted from the amount of peptide analyzed.

For the study of gastrin binding, cells were suspended in Hanks' balanced salt solution, with 0.1% BSA, and 25 mM Hepes (Calbiochem-Behring Corp., San Diego, CA) added. Binding studies were performed in 12 × 75-polystyrene tubes, with 50 μl of the tracer and 50 μl of unlabeled hormones or buffer added while the tubes were on ice. Cells (1 to 2 × 10^6/ml in 0.9 ml) were then added and the tubes were incubated at 37°C for 30 min in a shaker bath at 100 oscillations per minute, unless otherwise noted. At the end of the incubation period, triplicate 0.25-ml samples were layered over 0.9 ml of Hanks' solution (with 0.1% BSA) in 1.5-ml polypropylene microfuge tubes and then centrifuged at 8,700 *g* for 1 min in a Beckman Instruments, Inc., microfuge B. The supernatant was aspirated; the tips were excised; and the cell pellets were counted in a gamma counter (Tracer, Chicago, IL) with an 88% efficiency rate. Cell suspensions were also counted to determine the total

1. *Abbreviations used in this paper:* AP, [^{14}C]aminopyrine; APR, AP accumulation ratio; CCK, cholecystokinin; CCK8, the carboxyl terminal octapeptide (26–33) of cholecystokinin; G14-17, the carboxyl terminal tetrapeptide common to gastrin and cholecystokinin; G17, synthetic human gastrin-17 I; [Leu^{15}]-G17, synthetic human gastrin-17 I with a leucine substitution in the 15th position; K_i, the dissociation constant for an inhibitor.

2. It is important to check osmolality and density of the solutions used for the density separations. We determined osmolality of the Ficoll and BSA solutions by a vapor pressure osmometer and adjusted to 290 mosM by altering the concentrations of the Hanks' salts added. The osmolality achieved with different batches of albumin had varied, possibly due to differences in the amount of contaminating salts; and the formula for the density gradient solutions was thus modified from our previous report (18).

^{125}I-[Leu^{15}]-G17 present. Background radioactivity was routinely determined by counting tips excised from tubes that contained the tracer, but no cells; this value ranged from 60 to 110 cpm and was subtracted from the data. In each study the total binding of 10–30 pM ^{125}I-[Leu^{15}]-G17 and the binding of this tracer in the presence of 100 nM G17 or 1 μM pentagastrin (nonspecific or nonsaturable binding) was determined. Saturable binding, the difference between total and nonsaturable binding, was corrected for cell count as was determined by using an Electro Zone Celloscope (Particle Data, Inc., Elmhurst, IL) (8). The ^{125}I-[Leu^{15}]-G17 label was always used within 4 wk of preparation and significant deterioration of specific binding was not found during this time.

In preliminary studies, the binding of ^{125}I-[Leu^{15}]-G17 was compared by using parietal cell-enriched fractions that were suspended in Earles balanced salt solutions and in the Hanks' medium described above. In three experiments, the specific ^{125}I-[Leu^{15}]-G17 binding was 7.23±1.72% (mean±SE) per 10^6 cells in Hanks' and 5.14±0.55% per 10^6 cells in the Earle's medium. Hanks' was chosen for further studies since its use did not require a CO_2 gas phase. The effects of different washing steps on the binding were also studied. Using a cushion of 1.0 ml of 0.1% BSA produced backgrounds that were as low as those found with 10% BSA. Further washing steps after the first aspiration did not reduce the nonspecific binding enough to warrant the time required.

Aminopyrine accumulation. The accumulation of [^{14}C]aminopyrine (AP) was used as an index of parietal cell response to stimulation. AP accumulation ratio (APR) provides a quantitative index of parietal cell response to stimulation, which reflects the quantity of sequestered acid, but not the actual rate of acid secretion (9). This technique has been adapted for study of isolated canine parietal cells (9) from the original method developed by Berglindh (23) for rabbit gastric glands. In brief, 0.1 μCi of [^{14}C]aminopyrine and the stimulants and inhibitors to be tested were added to fractions of enriched parietal cells suspended in 2 ml of Earle's or Hanks' balanced salt solution with 0.1% BSA and 10 mM Hepes added.[3] Cells were incubated for 20 min and separated by centrifugation in a microfuge B (Beckman Instruments, Inc.), and radioactivity in the cell pellet was determined in a liquid scintillation counter. APR were calculated as the counts per minute per parietal cell volume divided by the concentration of radioactivity in the suspension, as previously described (9). The AP data have been expressed as the percent maximal response over basal to the agents indicated in the legends. For the dose-response studies illustrated in Fig. 9, the response to gastrin was studied with a background of histamine and data were expressed as the maximal response to gastrin over this histamine background.

Although the binding of ^{125}I-[Leu^{15}]-G17 to parietal cell-enriched fractions was highly consistent in different cell preparations, the degree of gastrin responsiveness as determined by AP accumulation varied in magnitude. Stimulation of AP accumulation by gastrin was statistically significant, but often small in magnitude (9). We have observed frequent "clustering" over time of poor responses to gastrin (as well as to other stimulants). In some instances, an explanation, such as deterioration of the collagenase or an introduction of a deleterious reagent, could be identified. In other instances, the explanation appeared to rest with variability among animals. Careful conditioning of animal subjects for 7–10 d after transport seemed to considerably improve the consistency of the parietal cell response to stimulation. The emphasis in the present studies was evaluating dose-response relations, and we have excluded those preparations in which gastrin did not produce a twofold increase in AP accumulation above basal, and used all of the remaining data for analysis.

Data analysis. Tests of the statistical significance of differences were performed by using the *t* test; paired analyses were used when indicated. In studies of both gastrin binding and of AP accumulation, the means of triplicate determinations for one or two separate incubations for each treatment group have been used for subsequent analysis, with *n* equal to the number of separate cell preparations. Where mean values for data are given they are followed by ±SE of the mean (*n* = number of separate cell preparations).

Receptor-binding models (defined where subsequently used) were fit separately to the data from each dog studied, by using a nonlinear, least-squares computer program (BMDPAR, 24). Means and standard errors of the estimated dissociation constants were computed (*n* = number of dogs).

Results

Biological activity of iodinated gastrin. Initial studies used iodinated native G17, since this ligand had been shown to be biologically active (21). Preliminary studies with some preparations of this label found significant saturable binding to fundic mucosal cells (25); however, other preparations of tracer showed no specific binding (Fig. 1 *B*). In contrast, iodination of [Leu^{15}]-G17 produced a ligand that had reproducibly high saturable binding to fractions enriched in canine parietal cells (Fig. 1 *A*).

Iodination of G17 and [Leu^{15}]-G17 with ^{127}I was performed

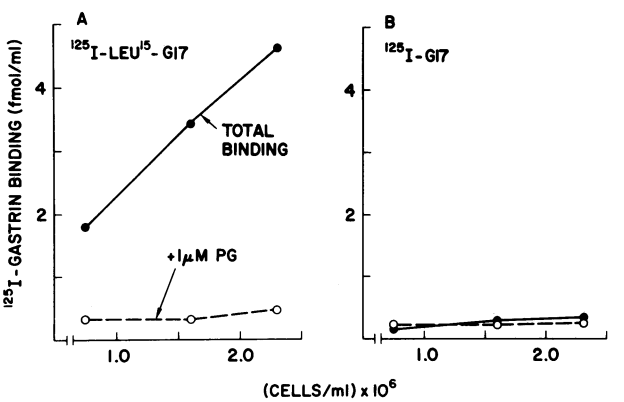


Figure 1. The effect of iodination on binding [Leu^{15}]-G17 and G17. Total binding and binding in the presence of 1 μM pentagastrin of ^{125}I-[Leu^{15}]-G17 and ^{125}I-G17 were determined on parietal cell-enriched fractions studied at the indicated cell concentrations and illustrated in *A* and *B*, respectively. Data are from a single preparation and have been expressed as femtomoles of gastrin bound per milliliter. In two other preparations, specific ^{125}I-[Leu^{15}]-G17 binding also correlated with cell concentrations between 0.6 and 3.0 × 10^6 cells/ml (r = 0.99 and 0.97, respectively, with five cell concentrations tested in each study.

3. In two cell preparations, parietal cell AP accumulation was tested by comparing cells suspended in both Earles' and Hanks' balanced salt solution; and their APR values to 10 μM histamine, 10 μM carbachol, and 0.1–10 nM G17 were found to vary <10% between these two conditions.

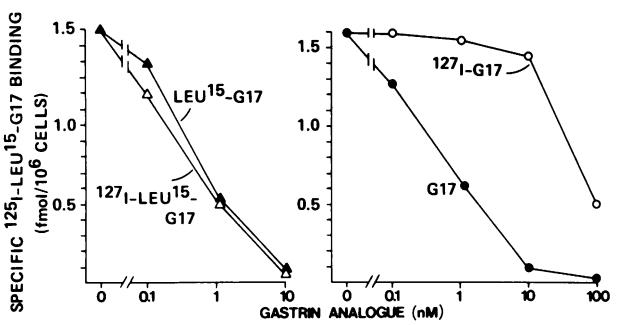
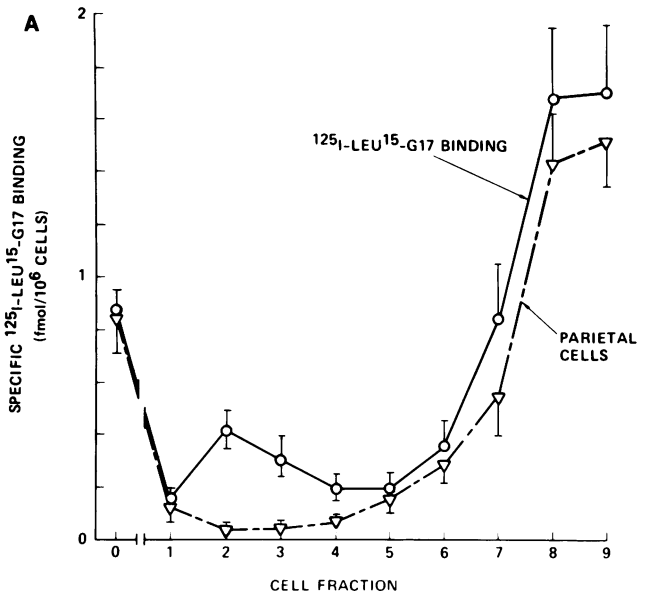


Figure 2. The effects of iodination with ^{127}I upon the inhibition of ^{125}I-[Leu^{15}]-G17 binding by [Leu^{15}]-G17 and G17. A parietal cell-enriched fraction was incubated in the presence of 20 pM of ^{125}I-[Leu^{15}]-G17 and the indicated concentrations of [Leu^{15}]-G17 and G17 and these two peptides after iodination with ^{127}I. These data are the mean of duplicate incubations in two separate preparations of cells.

to investigate the effects of iodination on binding properties and biological activity. Unlabeled [Leu^{15}]-G17 and G17 and ^{127}I-[Leu^{15}]-G17 produced comparable inhibition of the binding of ^{125}I-[Leu^{15}]-G17 (Fig. 2). In contrast, the ^{127}I-G17 competed poorly with ^{125}I-[Leu^{15}]-G17.

AP accumulation was used as an index of parietal cell response to stimulation in order to compare the biological activity of these gastrin analogues. Tested in two cell preparations, [Leu^{15}]-G17 and ^{127}I-[Leu^{15}]-G17 produced responses that were 96 and 98%, respectively, of the maximal response to uniodinated G17, whereas ^{127}I-G17 produced 18% of this maximal response. No differences in potency were found in these two preparations comparing [Leu^{15}]-G17, ^{127}I-[Leu^{15}]-G17, and uniodinated G17. Studies of somatostatin release by cultured canine fundic mucosal cells have yielded similar findings regarding the effect of iodination on the receptor binding and biological action of G17 and [Leu^{15}]-G17 (A. H. Soll, D. A. Amirian, J. Park, J. Elashoff, and T. Yamada, submitted for publication).

Cellular localization of the gastrin receptor. Cell separation studies were performed using the elutriator rotor. In our application of this technique, we collected nine fractions separated by cell size (Fig. 3). In this gradient, fractions 2 and 3 were enriched in mast cells, somatostatin cells and other endocrine cells; fractions 4 and 5 were enriched in mucous cells; fractions 6 and 7 were enriched in chief cells; and fractions 8 and 9 were



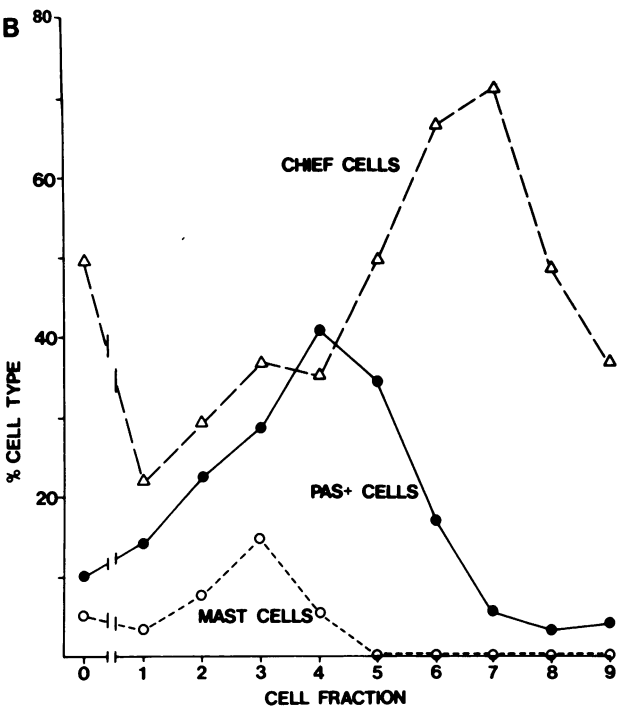


Figure 3. Gastrin binding to cell fractions separated by elutriation. Canine fundic mucosal cells were separated by elutriation into nine fractions by using a protocol previously described (22). Fraction 0 denotes the mucosal cells before elutriation. Parietal cells were counted on periodic acid Schiff (PAS)-stained slides; however, only mucous cells show magenta staining with PAS, i.e., PAS+ cells. Gastrin binding, expressed in femtomoles of ^{125}I-[Leu^{15}]-G17 specifically bound per 10^6 cells, is illustrated for these cell fractions. Nonsaturable binding was low in all fractions but somewhat greater in the fractions with larger cells, ranging from 0.41% of total binding in fraction 1 to 0.95% of total binding in fraction 9. These data are the mean from four preparations.

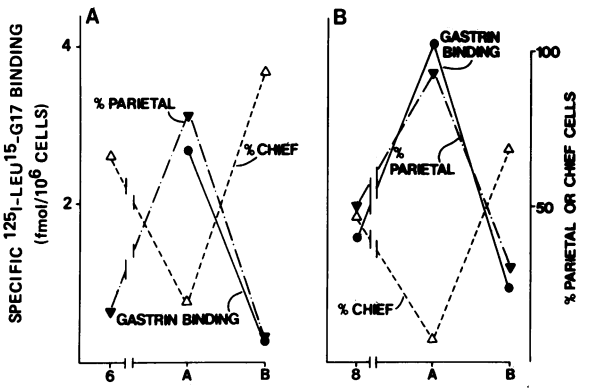


Figure 4. Stepwise density-gradient separation of enriched parietal and chief cell elutriator functions. Gradients were formed by varying the proportions of heavy and light solutions that were described in Methods. A 10-ml step of a 65% heavy solution was loaded first, followed by a similar volume step to a 25% heavy solution, with cells loaded in 30 ml of a 12% heavy solution. Stepwise density gradients were initially performed starting with elutriator fractions 6 and 7 (*A*) of fraction 8 (*B*). Two fractions were collected: fraction A banding above the 65% heavy step and fraction B that pelleted through this 65% heavy solution. The parietal cell and chief cell contents were determined counting slides prepared on a cytocentrifuge and stained with periodic acid Schiff (22). Illustrated in this figure are the percentage of parietal and chief cells in the fractions and the binding of ^{125}I-[Leu^{15}]-G17 expressed in femtomoles per 10^6 cells. Data are from a single preparation in which the correlation coefficients between gastrin binding and the distribution of parietal and chief cells were 0.98 and −0.96, respectively. Two other gradients were also performed using a similar protocol with the parietal cells enriched to 85 and 88% and the chief cells enriched to 76 and 77%, respectively. In these other two gradients, correlation coefficients between gastrin binding and parietal cells content were 0.98 and 0.99 and the correlation coefficients for the relationship between gastrin binding and the chief cell distribution were both −0.99 (n = 6 for each pair of density-gradient separations).

enriched in parietal cells. As indicated in Fig. 3, this technique enriches but does not purify these cell types. Although overlap exists between the distribution of each cell type, the patterns are highly reproducible among gradients. The specific binding of ^{125}I-[Leu^{15}]-G17 to these nine fractions were studied in four preparations and a highly significant correlation of binding was found with the content of parietal cells (r = 0.99, Fig. 3). However, in each of the four gradients, specific ^{125}I-[Leu^{15}]-G17 binding in fractions 2 and 3 was higher than in either fractions 1 or 4, and this binding was not accounted for by parietal cells present in those fractions. Thus, these data indicate that gastrin receptors are also present on a cell type(s) other than the parietal cell.

To further distinguish between gastrin binding to parietal cell and chief cells, stepwise density-gradient separations were performed on the chief cell-enriched fractions (fractions 6 and 7) and the parietal cell-enriched fraction (fraction 8). In the first three gradients performed with this technique, chief cells were enriched to 76–92%; and parietal cells, from 85 to 92%, respectively, in the more dense and less dense of the two steps collected (Fig. 4). In more recent studies, by adjusting the osmolality of the solutions and refining the density steps, it has been possible to use a starting fraction that includes fractions 6–9 from the elutriator separation and to achieve enrichment of chief cells between 80 and 90% and parietal cells to >95%. In these stepwise density gradients there is a striking positive correlation between the parietal cell content of the fraction studied and the saturable ^{125}I-[Leu^{15}]-G17 binding (r = 0.98), and an equally negative correlation between gastrin binding and the distribution of chief cells (r = −0.96). These findings indicate that parietal cells account for the saturable ^{125}I-[Leu^{15}]-G17 binding that was detected by the present techniques in the large cell fractions.

Our remaining studies have been directed at characterizing the gastrin receptor on parietal cells and examining the correlation between gastrin binding and stimulation of parietal cell function. Parietal cell-enriched fractions (8 and 9) that contain from 45 to 65% parietal cells have been used for these studies.

Tracer and cell concentration. The total and saturable binding of ^{125}I-[Leu^{15}]-G17 to parietal cells was studied as a function of the concentration of the label between 1 and 100 pM (Fig. 5). The quantity of bound ^{125}I-[Leu^{15}]-G17 increased proportionately with ligand concentrations < 50 pM. Because of the radioactivity involved and the high level of nonsaturable binding at high tracer concentrations, the concentration of ^{125}I-[Leu^{15}]-G17 gastrin was not increased to a range where complete sat-

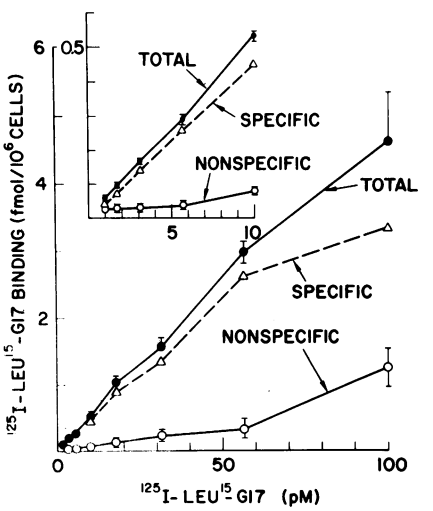


Figure 5. Gastrin binding as a function of the concentration of the ^{125}I-[Leu^{15}]-G17 label. The binding of the indicated concentrations of ^{125}I-[Leu^{15}]-G17 to an enriched fraction of parietal cells is illustrated. The data are the mean±SE for three preparations of cells. Data are illustrated for total binding and binding in the presence of 1 μM pentagastrin. Specific binding, the difference, is also shown.

uration occurred. The dissociation constant for gastrin was determined by displacement studies, as subsequently discussed.

The percentage of saturably bound ^{125}I-[Leu^{15}]-G17 did not vary between tracer concentrations of 10 to 30 pM, so we have used this range for our subsequent studies. In 20 consecutive experiments, we found that the specific binding of 20 pM ^{125}I-[Leu^{15}]-G17 to the elutriator-enriched parietal cell fraction was 7.84±0.84% per 10^6 cells (mean±SE, 15.7 fmol/10^6 cells). In the presence of 100 nM G17, 1.00±0.11% (mean±SE, 2 fmol/10^6 cells) of the tracer was bound; thus, nonspecific binding accounted for 11% of total binding in this system.

The binding of ^{125}I-[Leu^{15}]-G17 varied with cell concentration in a range of cell concentrations between 0.5 and 2.5 × 10^6 cells/ml (Fig. 1). Our studies were done with cell concentrations between 1 and 2 × 10^6 cells/ml, with binding corrected for cell concentration.

Time and temperature. At 37°C, saturable ^{125}I-[Leu^{15}]-G17 binding was detected after 1 min of incubation, with steady state binding achieved after ~30 min (Fig. 6 *A*). Binding was subsequently stable over the next 90 min. Saturable binding was also found with incubation at 25° and 15°C, but not at 4°C (Fig. 6 *B*). Nonsaturable binding accounted for a progressively larger component of total binding at lower temperatures (see legend to Fig. 6). All of our other studies of gastrin binding were thus performed using a 30-min incubation at 37°C.

Dissociation. After 30 min of incubation with 30 pM ^{125}I-[Leu^{15}]-G17, parietal cells were diluted 100-fold in the presence or absence of excess unlabeled pentagastrin and the residual

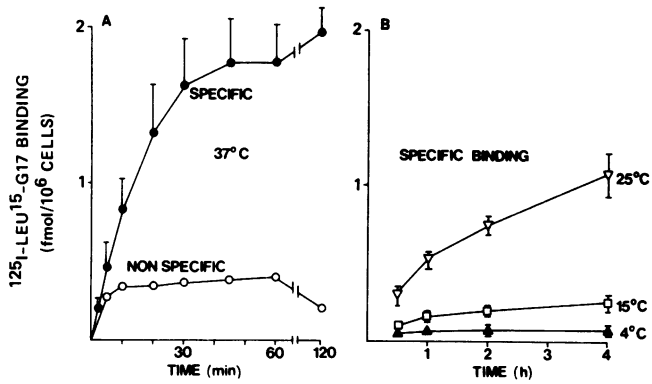


Figure 6. Time course for ^{125}I-[Leu^{15}]-G17 binding as a function of temperature. (*A*) Specific and nonspecific binding (plus 1 μM pentagastrin) are illustrated for incubation of an enriched fraction of canine parietal cells at 37°C for the indicated time periods. Data are the mean±SE from three preparations of cells. (*B*) Specific binding for matched incubations at 25°, 15°, and 4°C are illustrated, with the data also the mean of three preparations of cells. Expressed as femtomoles per 10^6 cells, nonspecific binding ranged from 0.21 to 0.26 at 25°C, 0.18 to 0.22 at 15°C and 0.05 to 0.17 at 4°C in the three experiments performed. Thus, nonspecific binding accounted for <15% of total binding at 37°C, 20 to 50% of binding at 25°C, and >50% of total binding at the lower two temperatures.

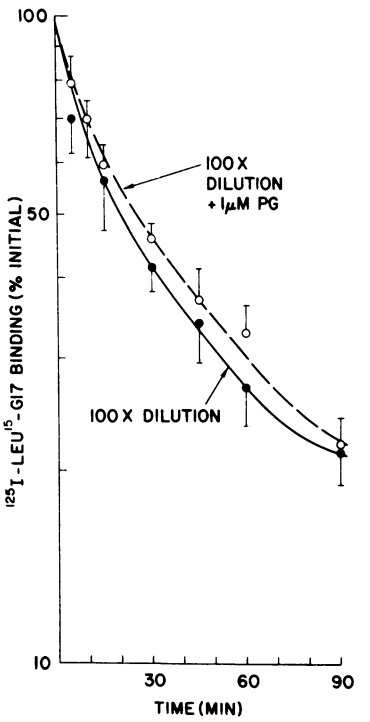


Figure 7. Dissociation of the ^{125}I-[Leu^{15}]-G17 from parietal cells. Cells were incubated in the presence of 20 pM ^{125}I-[Leu^{15}]-G17 for 30 min at a cell concentration of 3 × 10^6 per ml. A 1.5-ml sample of the cell suspension was then diluted into a total volume of 150 ml of medium, and triplicate 5.0-ml samples taken immediately and at the indicated times of incubation. Cells were centrifuged at 1,000 *g* for 2 min, the medium was aspirated, and the pellet radioactivity was determined. Data are the mean±SE from three preparations and have been expressed as the percentage of the initial binding, determined immediately after dilution. This protocol for studying dissociation did not include a wash step to remove tracer from the medium before the 100-fold dilution. We chose this protocol because it avoided the possibility of dissociation occurring during the wash step. Rebinding of tracer from the diluted medium may occur; however, this possibility seems unlikely since we did not detect binding to fresh cells with the tracer at a 100-fold dilution (data not illustrated). Also, such rebinding would be reduced when excess unlabeled hormone was added and we detected no differences with initiating dissociation by dilution and dilution plus excess unlabeled pentagastrin.

^{125}I-[Leu^{15}]-G17 binding to the cells was determined. At 37°C dissociation was rapid, with 50% of the label dissociating within 30 min (Fig. 7). After 90 min of incubation, 20% of the initial counts remained bound to the cells. The addition of excess pentagastrin (1 μM) did not accelerate the rate of dissociation beyond that found with dilution alone (Fig. 7). Reducing the temperature of incubation to 30°C did not reveal any difference in the dissociation rate found with dilution or dilution plus the addition of pentagastrin at concentrations of 0.32 nM, 1 nM, and 1 μM (n = 2, data not shown).

Hormone degradation. The degradation of ^{125}I-[Leu^{15}]-G17 during a 30-min incubation with an enriched fraction of parietal cells was determined by assessing the percentage of label in the supernatant that was capable of subsequent binding to fresh parietal cells. In four experiments, the amount of saturable binding to parietal cells decreased by 11% during a 30-min incubation, while nonsaturable binding roughly doubled (Table I). The retention of specific binding provides a sensitive index of hormone degradation (26), and the present data indicate a very low level of gastrin degradation by parietal cells. This finding

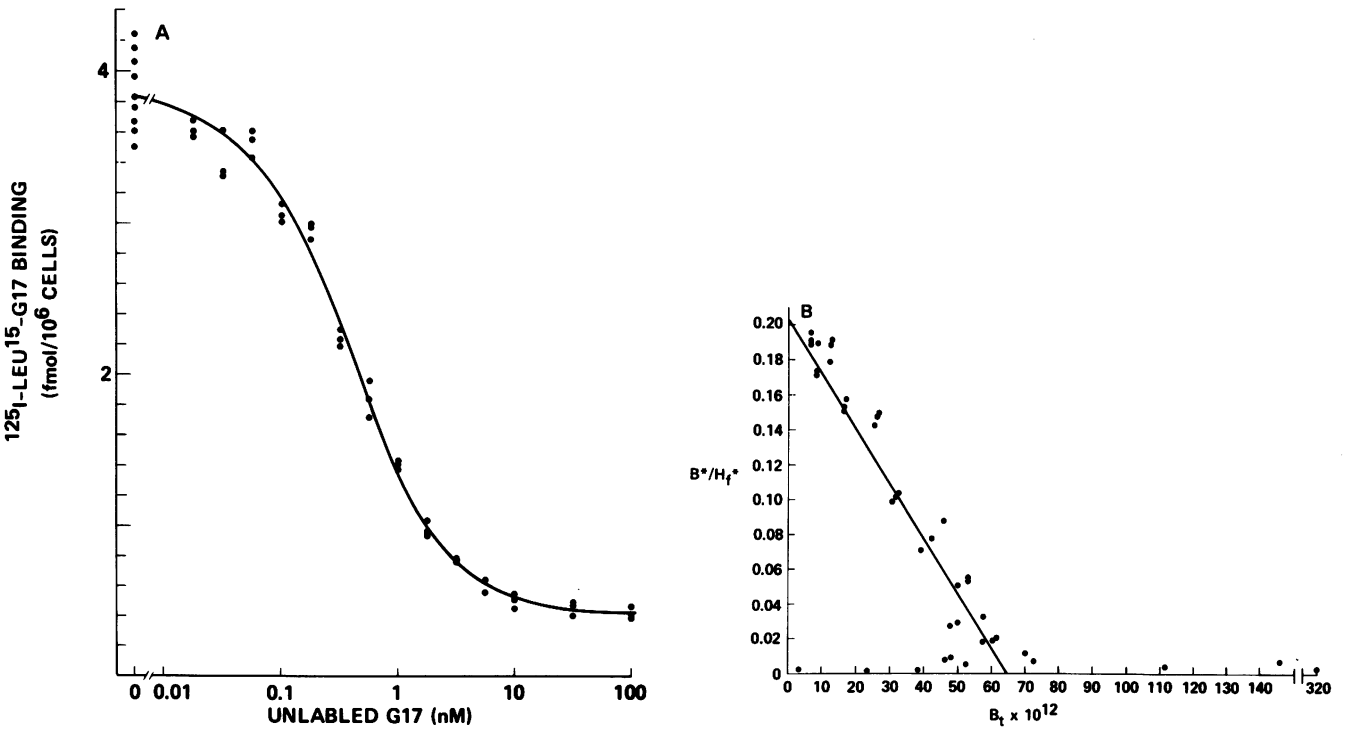


Figure 8. Single site equilibrium model for binding data. ^{125}I-[Leu^{15}]-G17 binding was determined to an enriched parietal cell fraction, with variable concentrations of unlabeled G17 added. The individual triplicates from a single cell preparation are shown, with the data analyzed by two methods. (*A*) Untransformed data for total binding are plotted in femtomoles of ^{125}I-[Leu^{15}]-G17 bound as a function of the unlabeled G17 concentration. The curve was fit by a nonlinear, least-square computer program, as described in the text. Similar analyses of dose-response curves from 11 other cell preparations were obtained. (*B*) The same data were analyzed by using the Scatchard linearization. The ratio of specifically bound hormone to free hormone (B*/H*$_f$) is plotted as a function of estimated specific bound hormone (B_t). In this plot the nonsaturable binding as determined by the curve fitted in *A* was subtracted. Note the wide scatter of the points, particularly at low values of B*/H*$_f$. This line was drawn using the parameter estimates for eq. 1 determined by the computer fit in *A*. When all of the data in *B* were fit by least-squares regression, the estimated K_d and receptor number per cell were, respectively, 0.2×10^{-10} M and 64,000 sites per cell. When the outlying points were excluded (by dropping data for 32 and 100 nM G17), then a K_d of 3.2×10^{-10} M and receptor number of 26,000 sites per cell were obtained. The necessity to consider excluding data from the Scatchard plot greatly compromises the statistical validity of this approach.

Table I. Degradation of ^{125}I-[Leu^{15}]-G17 by Canine Parietal Cells

	^{125}I-[Leu^{15}]-G17 binding (fmol/10^6 cells)	
	Saturable	Nonsaturable
No preincubation*	1.89±0.20	0.19±0.03
30-min preincubation‡	1.67±0.16	0.36±0.07

* Cells were incubated in the presence of 20 pM gastrin for 30 min and binding of ^{125}I-[Leu^{15}]-G17 was determined in the presence or absence of 1 μM pentagastrin, as noted.

‡ After 30 min of incubation with a parietal cell enriched fraction (1.5×10^6 cells/ml), the cell suspension was centrifuged. This supernatant was then used to resuspend a fresh cell pellet, and with this suspension then incubated for another 30 min. The binding of ^{125}I-[Leu^{15}]-G17 to these fresh parietal cells was determined and expressed in femtomoles of tracer bound, which was corrected for cell number. The data for both groups are the mean±SE and are from four separate cell preparations.

is consistent with the stable time course found even at 37°C for ^{125}I-[Leu^{15}]-G17 binding to parietal cells (Fig. 6 *A*).

Affinity and specificity of ^{125}I-[Leu^{15}]-G17 binding to parietal cells. The ability of gastrin analogues to interact with parietal cell receptors was tested by using the data for displacement of ^{125}I-[Leu^{15}]-G17 by unlabeled G17. Fig. 8 shows data from one of 12 similar studies on separate cell preparations; the quantity of ^{125}I-[Leu^{15}]-G17 bound in femtomoles per 10^6 cells is plotted against the concentration of unlabeled G17. The equilibrium model for displacement of binding from a single receptor becomes:

$$\frac{B^*}{H^*} = \frac{K_d + R + H_t - [(K_d + R + H_t)^2 - 4R \cdot H_t]^{1/2}}{2H_t} + NS, \quad (1)$$

where B*/H* is the ratio of bound to total labeled hormone, H_t is the concentration of labeled plus unlabeled hormone, R is the receptor concentration, K_d is the dissociation constant, and NS is the nonsaturable binding (see Appendix for derivation).

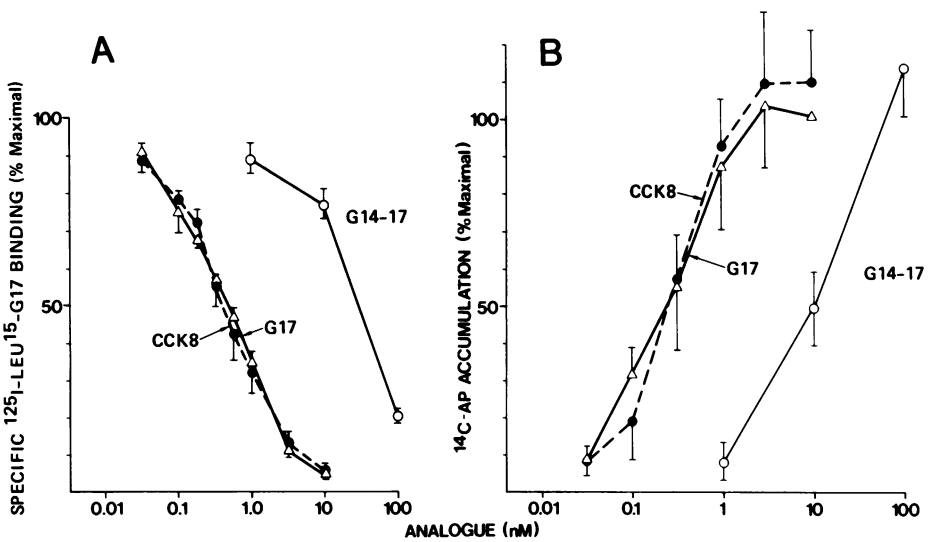


Figure 9. Inhibition of ^{125}I-[Leu^{15}]-G17 binding and stimulation of AP accumulation by related peptides. (*A*) The binding of ^{125}I-[Leu^{15}]-G17 to parietal cells was determined in the presence of the indicated concentrations of G17, CCK8, and G14-17. The data for CCK8 and G17 are the mean±SE from five preparations of cells, with the binding and function studies performed on the same day under identical conditions (Hanks' balanced salt solution, 0.1% BSA, and 25 mM Hepes and using a 37°C, 30-min incubation). Data have been expressed as specific tracer binding in femtomoles per 10^6 cells at a tracer concentration of 20 pM. The data from G14-17 are from a different set of six experiments and plotted for comparison. (*B*) The effects of these same peptides on AP accumulation has been determined in the presence of a histamine (10 μM) background. The data for CCK8 and G17 are the mean±SE for the same five preparations illustrated in *A*. The data have been expressed as the percentage of the response above the 10 μM histamine background produced by 10 nM gastrin. In these studies the APR for 10 μM histamine was 13.1±3.5 and the response to 10 nM G17 plus 10 μM histamine was 27.1±6.8. The data for G14-17 are from four separate cell preparations.

This model assumes that the K_d for labeled and unlabeled hormone is the same, an assumption that is supported by the finding that ^{127}I-[Leu^{15}]-G17, uniodinated [Leu^{15}]-G17, and G17 were equipotent in inhibiting ^{125}I-[Leu^{15}]-G17 binding (Fig. 2). The data were fit by using a nonlinear, least-squares procedure that has statistical advantages compared with the Scatchard linearization (27) of this equation.[4]

The K_d calculated for G17 was 4.64±0.71 × 10^{-10} M (mean±SE, n = 12). This analysis indicated 24,100±3,100 (mean±SE, n = 12) gastrin receptor sites per cell in the parietal cell-enriched fraction. Assuming that parietal cells account for the detectable binding (Fig. 4), this approach estimates 43,700±5,000 sites per parietal cell (mean±SE, n = 12). Displacement of ^{125}I-[Leu^{15}]-G17 binding by CCK8, G17, and G14-17 was compared (Fig. 9 *A*). CCK8 had a K_d of 2.71±0.50 × 10^{-10} M (mean±SE, n = 12), and, thus, was slightly more potent than the G17, although this difference was not statistically significant. G14-17 had a K_d of 4.1±0.9 × 10^{-8} M (mean±SE, n = 6), which reflected a potency much less than that for CCK8 or G17.

In parallel studies, stimulation of parietal cell AP accumulation was studied (Fig. 9 *B*). To determine the K_d, we assumed the response, as reflected in the APR, to be directly proportional to receptor occupancy, and used this Michaelis-Menton equation:

$$APR = \frac{APR_{max} \cdot H_t}{H_t + K_d}, \quad (2)$$

in which H_t is the concentration of stimulant and K_d is the dissociation constant for the stimulant. This equation was separately fit to data from each dog. In a given cell preparation, the APR_{max} was constrained to be the same for all stimuli. G17

4. The data in Fig. 8 have been fit to a one-receptor site equilibrium model for displacement binding by both nonlinear curve fitting of equation (1) with a computer program for least-square estimation and by a Scatchard plot (27). The Scatchard linearization has been derived in the Appendix. Although eq. 1 is algebraically equivalent to the Scatchard transformation, nonlinear curve fitting has advantages from a statistical point of view (28). In a least-squares curve fit, the x variable is assumed to be measured (or fixed) without error. When points are not weighted, errors in measurement of y are assumed to be the same magnitude at each value of x, which is a reasonable condition for the present data (see Fig. 8 *A*). In the Scatchard plot, errors appear in both x and y and the errors in estimating the total bound hormone are often very large when a low fraction of tracer is bound at high hormone concentrations (Fig. 8 *B*). Such errors at low values of the bound to free ratio, B^*/H_f^*, can give the impression of a curvilinear or two-component plot (Fig. 8 *B*). Secondly, nonsaturable binding is one of the parameters estimated by the nonlinear curve fitting, whereas the Scatchard plot requires that nonsaturable binding be subtracted from the data before plotting. Underestimation of nonsaturable binding also results in a curvilinear appearance to the Scatchard plot. By using the nonsaturable binding parameter determined by the nonlinear fit, the Scatchard plot for the present data yielded a straight line. However, with the scatter of the data, estimation of K_d and receptor number from the slope and x intercept, respectively, are subject to considerable error (Fig. 8 *B*). With the present availability of computers, nonlinear curve fitting with nontransformed data offers an attractive alternative to linearization (28).

*Table II. Specificity of ^{125}I-[Leu^{15}]-G17 Binding**

Agent	Concentration	Total binding
	M	*fmol/10^6 cells*
None	—	1.74±0.36
Pentagastrin	10^{-6}	0.16±0.04‡
Secretin	10^{-10}	1.64±0.30
Secretin	10^{-9}	1.68±0.35
Secretin	10^{-8}	1.62±0.28
Secretin	10^{-7}	1.60±0.32
Secretin	10^{-6}	1.61±0.34
Cimetidine	10^{-4}	1.67±0.28
Histamine	10^{-4}	1.64±0.35
Carbachol	10^{-4}	1.86±0.32
Atropine	10^{-5}	1.66±0.29
Dibutyryl cyclic AMP	10^{-3}	1.62±0.36
Insulin	10^{-7}	1.72±0.34
Somatostatin	10^{-6}	1.66±0.36

* Enriched parietal cells were incubated with ^{125}I-[Leu^{15}]-G17 (20 pM) for 30 min at 37°C in the presence and absence of the indicated agents. Total binding was determined as noted Methods; the data represent the mean±SE for four cell preparations.
‡ $P < 0.05$ compared with binding with tracer only.

and CCK8 had comparable K_d values of 2.0±0.3 × 10^{-10} M and 2.8±0.5 × 10^{-10} M, respectively, for stimulation of AP accumulation (mean±SE, n = 13). In five cell preparations (Fig. 9), CCK8 and G17 were compared for effects on binding and parietal cell function under identical conditions (Fig. 9). In these latter studies the K_d for G17 and CCK8 for inhibition of ^{125}I-[Leu^{15}]-G17 binding were 3.6±0.8 × 10^{-10} and 3.6±1.0 × 10^{-10}, respectively. K_d estimates for stimulation of AP accumulation were 2.1±0.3 × 10^{-10} and 3.7±0.8 × 10^{-10}, respectively (mean±SE, n = 5). These values were much lower than the K_d for G14-17 of 1.2±0.6 × 10^{-8} M (mean±SE, n = 4).

We did not find inhibition of ^{125}I-[Leu^{15}]-G17 binding with histamine, cholinomimetics or their antagonists, or with several unrelated peptide hormones (Table II). In contrast to the findings with gastrin receptors in rat gastric mucosa (20), we did not find displacement of ^{125}I-[Leu^{15}]-G17 binding with secretin in concentrations between 0.1 and 100 nM (Table II).

Gastrin receptor antagonist. Proglumide, a glutaramic acid derivative that antagonizes cholecystokinin (CCK) binding to pancreatic acinar cells (29), inhibited the binding of ^{125}I-[Leu^{15}]-G17 to parietal cells (Fig. 10 *A*). High concentrations of proglumide are required; 50% inhibition of the specific binding of the 20 pM ^{125}I-[Leu^{15}]-G17 tracer occurred at a proglumide concentration of ~1 mM. Proglumide at a concentration of 1 mM produced a surmountable rightward shift of the gastrin binding curve, although the effects of 3.2 mM proglumide were not clearly fully surmountable in the experiments performed (Fig. 10 *A*).

Proglumide also inhibited gastrin stimulation of parietal cell AP accumulation (Fig. 10 *B*). Proglumide at a concentration of 1 mM produced a rightward displacement of the dose response for gastrin stimulation of AP accumulation. Proglumide at a

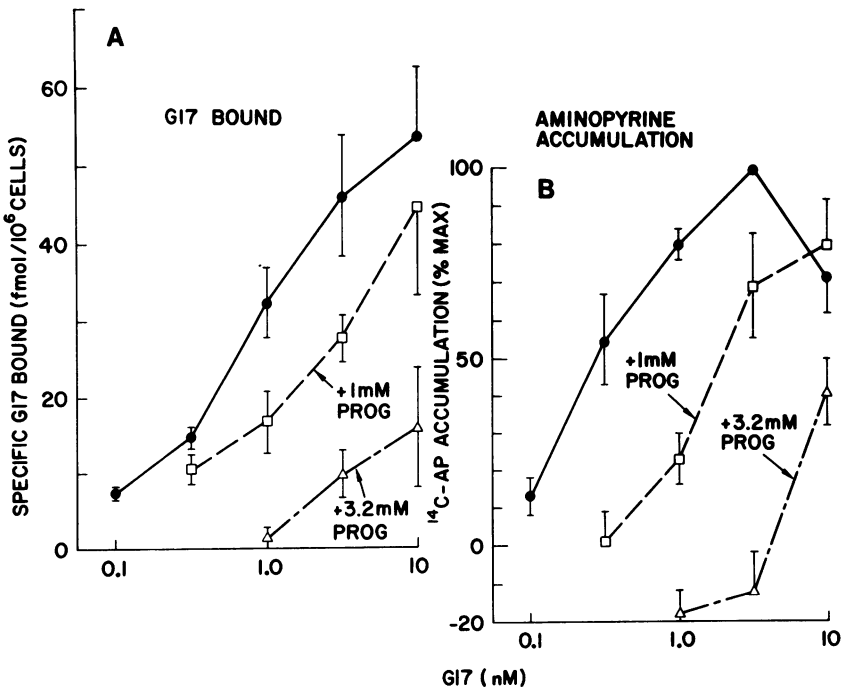


Figure 10. Proglumide (PROG) inhibition of gastrin binding and function. (*A*) The effects of the indicated concentrations of proglumide on ^{125}I-[Leu^{15}]-G17 binding are ilustrated. The data are the mean±SE from four preparations and are expressed as the specific G17 bound/10^6 cells. (*B*) The effects of the proglumide on AP accumulation stimulated by gastrin are illustrated, with these studies matched to those in (*A*). The AP accumulation has been expressed as the percentage of the response over basal found with 1 nM gastrin, which produced an AP accumulation ratio (9) in these four preparations of 15.5±3.5.

concentration of 3.2 mM, however, appeared to inhibit basal AP accumulation, although this effect was surmounted at higher gastrin concentrations (Fig. 10 *B*). The dissociation constant for proglumide inhibition of ^{125}I-[Leu^{15}]-G17 binding and G17 stimulation of AP accumulation were estimated by using nonlinear curve fitting, assuming equilibrium mass action at a single receptor site. For this analysis the following formula was used:

$$APR = \frac{R \cdot H_t}{K_d + H_t + I(K_d/K_i)}, \quad (3)$$

in which R is the total receptor concentration, K_d and K_i and H_t and I are respectively the dissociation constants for stimulant and inhibitor and the total concentrations of stimulant and inhibitor (see Appendix for derivation). In seven preparations, the dissociation constant for proglumide inhibition of ^{125}I-[Leu^{15}]-G17 binding was found to be $5.0\pm0.2 \times 10^{-4}$ M. For inhibition of gastrin-stimulated AP accumulation, the dissociation constant for proglumide was $1.4\pm0.2 \times 10^{-4}$ M.

Proglumide specificity for the gastrin receptor was tested by examining the effects of proglumide on stimulation of parietal cell AP accumulation by histamine, dibutyryl cyclic AMP, and carbachol (Fig. 11). Proglumide, in concentrations from 0.32 to 3.2 mM did not significantly decrease the response to histamine or dibutyryl cyclic AMP.[5] However, some inhibition of carbachol stimulation was found at the higher concentrations of proglumide (Fig. 11). Proglumide inhibition of carbachol stimulation has been previously described in studies of pancreatic acinar cells (28).

Discussion

The present studies provide evidence that localizes ^{125}I-[Leu^{15}]-G17 receptors to canine parietal cells. The binding of ^{125}I-[Leu^{15}]-G17 to these receptors displayed typical patterns for peptide hormone receptors; binding was rapid, reversible, and specific. After a 30-min, 37°C incubation with ^{125}I-[Leu^{15}]-G17, 80% of the bound tracer dissociated within 90 min after 100-fold dilution; this finding makes it unlikely that the tracer-receptor complex is rapidly internalized into parietal cells. We found no evidence for negative cooperativity between gastrin receptor binding sites. In the presence of these receptor site-site interactions, receptor affinity will vary inversely with the fraction of receptors occupied; thus dissociation rate will increase as a function of the unlabeled hormone concentration (30). In our present studies, adding pentagastrin to partially or completely saturated receptors did not accelerate the dissociation rate of tracer beyond

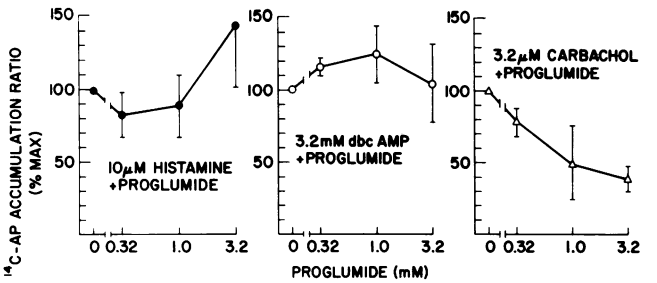


Figure 11. The effects of proglumide on stimulation of AP accumulation by histamine, dibutyryl cyclic (dbc) AMP, and carbachol. The effects of proglumide on AP accumulation in response to the indicated concentrations of stimulants was tested. The data have been expressed as the percentage of the response to the stimulants alone and represent the mean±SE from six preparations. In these experiments the AP accumulation ratios produced by histamine, dibutyryl cAMP, and carbachol were, respectively, 23.7±8.0, 66.4±11.5, and 58.0±20.4.

that produced by dilution in the absence of added hormone. The parietal cell gastrin receptor demonstrated specificity for the gastrin/CCK family of peptides and binding was not inhibited by secretin, histamine, H_2-receptor antagonists, cholinergic or anticholinergic agents, or unrelated peptides. By using a nonlinear, least squares computer program, data for ^{125}I-[Leu^{15}]-G17 binding to parietal cells were well fit by a mass action, equilibrium model for competition at a single receptor site. From these data, we estimated the K_d of the gastrin receptor to be 4.6×10^{-10} M, with ~40,000 receptor sites per parietal cell. Scatchard analysis was also compatible with interaction at a single receptor site; amplification of scatter in the data by the linearization impaired estimation of the K_d and receptor number, compared with that obtained with the computer least-squares curve fitting.[4]

The relationship between gastrin binding and gastrin stimulation of parietal cell function has been examined in the present studies from several standpoints. The K_d for gastrin displacement of ^{125}I-[Leu^{15}]-G17 was twofold greater than for stimulation of aminopyrine accumulation. These findings suggest that under these conditions, occupancy of part of the receptor population is sufficient to produce a maximal cell response. The data in Fig. 9 indicate that the maximal response to G17 is found at a concentration of ~3.2 nM. At higher concentrations, although a higher fraction of gastrin receptors are occupied, no further stimulation of AP accumulation occurs. In previous studies (9–11), we have not found that G17 at concentrations of 0.1 and 1 μM produced responses that were consistently higher (or lower) than those found at 10 nM. CCK8 and G17 were essentially equally potent in displacing ^{125}I-[Leu^{15}]-G17 binding and stimulating parietal cell function. Our present data support the conclusion that the interaction of gastrin with a surface receptor and activation of parietal cell function by gastrin are associated events.

5. Proglumide at 3.2 mM appeared to potentiate the effects of 10 μM histamine, although this effect was not statistically significant in the preparations tested. Proglumide may be a partial agonist and activate the parietal cell under certain conditions, such as the presence of the potentiating stimulant, histamine. A similar phenomenon has been noted with high concentrations of cimetidine (9).

Further evidence that gastrin binding and functional responses are related in this system comes from studies with proglumide and with the carboxyl terminal tetrapeptide common to CCK and gastrin, G14-17. G14-17 displayed a proportional decrease in potency compared with G17 for both displacement of ^{125}I-[Leu^{15}]-G17 binding and stimulation of parietal cell function. Proglumide, a derivative of glutaramic acid, inhibits acid secretion in vivo (31). We found that proglumide inhibited gastrin stimulation of parietal cell AP accumulation and inhibited ^{125}I-[Leu^{15}]-G17 binding to isolated canine parietal cells, with K_i values for these effects in the same order of magnitude (1.4×10^{-4} and 5.0×10^{-4} M, respectively).

Since the octapeptide of cholecystokinin and G17 were equally potent, the present data raise the question of whether the gastrin receptor on the isolated canine parietal cell is a CCK or a gastrin receptor. Furthermore, proglumide, which we found to interact specifically with the gastrin receptors on canine parietal cells, also interacts with the CCK receptor on pancreatic acinar cells (29). The present results add further support to the view that, just as there is a family of CCK-gastrin peptides, there is also a family of related cell surface receptors for CCK and gastrin. This family of receptors includes a receptor on the pancreatic acinar cell that has a much higher affinity for CCK than G17 (32, 33), a receptor in the brain that has a moderate preference for CCK over G17 (34, 35), and the canine parietal cell receptor that has an equal affinity for CCK8 and G17. Antral smooth muscle cell responses to gastrin and CCK also indicate the presence of a receptor with equal affinity for gastrin and CCK (36).

There have been several previous radioligand studies of gastrin receptor binding with antral smooth muscle homogenates, particulate and plasma membrane-enriched fractions of rat fundic mucosa, and isolated rat and rabbit fundic mucosal cells (20, 37–40). Two of these studies have established that the ^{125}I-gastrin tracer used was biologically active, by interacting with a specific receptor (20, 40). Our present work extends these previous studies by localizing the gastrin receptor to a given cell type and by providing a correlation between a biological response and the receptor interaction. Relatively high nonspecific binding was encountered in these previous studies. Proglumide was previously found to inhibit the binding of ^{125}I-gastrin to rat gastric homogenates (41). However, the label used in these studies was commercially iodinated native gastrin, thus leaving open the question of oxidative damage of the methionine in the 15th position, which would destroy biological activity (20, present study).

The localization of gastrin receptors within the fundic mucosa is a complex problem. Our present data indicate that specific gastrin receptors exist on canine parietal cells and are not detectable by present techniques on isolated canine chief cells. These observations agree well with our previous findings that gastrin does not stimulate pepsinogen release by canine chief cells in short-term culture (42). In vivo, pentagastrin is found to stimulate pepsin secretion, although the effect is modest compared with cholinergic stimulation (43, 44). Furthermore, pentagastrin stimulation of pepsin output may only occur in innervated mucosa (44) and is totally blocked by anticholinergic agents (43), raising the question as to whether pentagastrin acts directly on chief cell receptors. In contrast to these findings with pentagastrin, CCK stimulates pepsin secretion in both rabbit gastric glands (45) and canine chief cells (42). These contrasting data suggest the possibility that chief cells have a receptor with a much higher affinity for CCK than for gastrin, similar to the CCK receptor on pancreatic acinar cells. Our present studies with a G17 tracer do not adequately test this hypothesis.

An important question pertains to the other cellular loci of gastrin receptors in the fundic mucosa. Our present cell separation studies using the elutriator rotor indicate that gastrin receptors are present on a population of small mucosal cells, in addition to the much larger parietal cell. Cells containing somatostatin-like immunoreactivity are present in these small cell fractions and by studying short-term cultures of these fractions we have found that gastrin stimulates somatostatin release (Soll, A. H., J. Park, L. P. Thomas, and T. Yamada, submitted for publication). Thus, we conclude that at least one of the additional gastrin receptors in the canine fundic mucosa is present on the somatostatin cell, although the physiological significance of these receptors remains uncertain. Whether gastrin receptors also exist on any additional cells also remains an open question.

Although the present data indicate that the parietal cell accounts for the major proportion of gastrin receptors in the canine fundic mucosa, it may not be possible to generalize this conclusion to all other species. As noted in the introduction, gastrin has been demonstrated to increase histamine formation in the rat, a species in which fundic mucosal histamine is stored in endocrine-like cells, but not in the dog, a species in which fundic mucosal histamine is stored in mast cells. With these differences in cell type and function, differences in gastrin receptor localization are also possible. Further studies will be necessary to determine the presence and physiological relevance of the gastrin receptor on different cell types in different species.

The present reductionistic approach examines the individual pieces in order to learn about functioning of the whole and may at times appear only to add to the apparent complexity of the subject. Our present finding that CCK8 stimulates the isolated canine parietal cell with a potency and efficacy equal to that of G17 agrees with the observation that CCK is a full agonist of gastric acid secretion in vivo in the cat (46). However, in the dog, CCK weakly stimulates acid secretion (47). Thus, either the isolated canine parietal cell has very limited usefulness as a model for exploring physiologic mechanisms, or there are cellular elements involved in the response of the fundic mucosa to gastrin and CCK in addition to the parietal cell itself. Studies of the regulation of somatostatin cell function support the latter conclusion since CCK8 was found to be a much more effective stimulant of somatostatin release than was gastrin (48). Thus, despite being equally potent to G17 in stimulating parietal cell

function, CCK8 may not be a full agonist of acid secretion in vivo in the dog because of potent activation of inhibitory mechanisms mediated by somatostatin. This explanation for the contradictory actions of CCK in vitro of parietal cells and in vivo on acid secretion remains speculative. These mechanisms require a great deal of study, but we suggest that two concepts may be useful: (*a*) receptors for regulatory peptides, such as gastrin, will be present on more than one target cell in the fundic mucosa; and (*b*) differences in the specificity of receptor subtypes for such peptides may be an important factor in integrating the overall functional response.

Appendix

Derivation of the mass action relationship for nonlinear curve fitting.[6] By conservation of mass, we have:

$$H^* = H_f^* + B^*; \tag{A1}$$

$$H = H_f + B; \tag{A2}$$

$$R = R_f + B + B^*. \tag{A3}$$

and defining B_t as total bound hormone and H_t as total hormone, we have $B_t = B + B^*$; $H_t = H + H^*$. By the mass action law, we have:

$$\frac{H_f^*[R_f]}{B^*} = K_d = \frac{H_f[R_f]}{B}. \tag{A4}$$

Experimentally, B^*/H^* is determined and assumed to be equal to B/H. Rearranging eq. A4,

$$B^* = \frac{1}{K_d}[H_f^*][R_f].$$

$$B = \frac{1}{K_d}[H_f][R_f]. \tag{A5}$$

Using these expressions, eq. A3 can be solved for R_f,

$$R = R_f\left(1 + \frac{1}{K_d}[H_f] + \frac{1}{K_d}[H_f^*]\right);$$

$$R_f = R/\left(1 + \frac{1}{K_d}[H_f] + \frac{1}{K_d}[H_f^*]\right). \tag{A6}$$

Substituting eq. A6 into eq. A5 we have

$$B^* = \frac{[H_f^*][R]}{K_d + H_f + H_f^*} = \frac{R}{[K_d]/H_f^* + H_f/H_f^* + 1}. \tag{A7}$$

Because of the assumption that unlabeled and labeled hormone bind proportionately, $H_f^*/H_f = H^*/H$ and substituting into eq. A7 we have

$$B^* = \frac{R}{[K_d]/H_f^* + H/H^* + 1}. \tag{A8}$$

6. *Abbreviations used in appendix:* B and B*, unlabeled and labeled bound hormone; B_I, bound competitive inhibitor; H and H_f, total and free unlabeled hormone; H* and H_f^*, total and free labeled hormone; I and I_f, total and free unlabeled inhibitor; R and R_f, total and free receptor concentration.

Replacing H_f^* by $(H^* - B^*)$,

$$B^* = \frac{R}{[K_d]/(H^* - B^*) + (H/H^* + 1)}. \tag{A9}$$

Multiplying by the denominator on the right and rearranging,

$$B^*\left(\frac{[K_d]}{(H^* - B^*)} + H/H^* + 1\right) - R = 0;$$

$$\frac{B^*[K_d]}{(H^* - B^*)} + B^*[H/H^*] + B^* - R = 0;$$

$$B^*[K_d] + B^*[H/H^*](H^* - B^*) + B^*(H^* - B^*) - R(H^* - B^*) = 0;$$

$$-B^*[K_d] - B^*[H] + B^{*2}[H/H^*] - B^*[H^*] + B^{*2} + R[H^*] - R[B^*] = 0;$$

$$B^{*2}[(H + H^*)/H^*] - B^*[K_d + H + H^* + R] + R[H^*] = 0. \tag{A10}$$

Substituting H_t for $(H + H^*)$ and dividing by H^* we have:

$$(B^*/H^*)^2[H_t] - [B^*/H^*][K_d + H_t + R] + R = 0 \tag{A11}$$

Solving for the root of a quadratic and using the negative coefficient of the square root term, we have

$$B^*/H^* = \frac{K_d + R + H_t - \{(K_d + R + H_t)^2 - 4R[H_t]\}^{1/2}}{2H_t}. \tag{A12}$$

By adding a term for nonsaturable binding, this expression becomes eq. 1 in the text.

The Scatchard equation is obtained from eq. A8. Multiplying both sides by the denominator of the right, $B^*([K_d]/H_f^* + H/H^* + 1) = R$; $K_d[B^*/H_f^*] + [H/H^*]B^* + B^* = R$. Since we assume $B/H = B^*/H^*$, then the center term on the left side can be replaced, $K_d[B^*/H_f^*] + B + B^* = R$. Rearranging and replacing $(B + B^*)$ with B_t we have:

$$[K_d]\left(\frac{B^*}{H_f^*}\right) = (R - B_t);$$

$$\frac{B^*}{H_f^*} = \frac{1}{K_d}(R - B_t). \tag{A13}$$

To derive the expression for nonlinear determination of the K_i for a competitive receptor antagonist, eq. A4 and the following two expressions are solved for K_i:

$$K_i = \frac{I_f \cdot R_f}{B_I};$$

$$R = R_f + B_I + B. \tag{A14}$$

Substituting for B_I in eq. A14, we have $K_i(R - R_f - B) = I_f \cdot R_f$; $K_i(R - B) = R_f(I_f + K_i)$; $[K_i(R - B)]/[I_f + K_i] = R_f = (R - B)/(I_f/K_i + I)$. Substituting into eq. A4, we have $K_d = [H_f(R - B)]/[B(I_f/K_i + 1)]$. Solving for B, we have

$$B = \frac{H_f \cdot R}{H_f + K_d + I_f(K_d/K_i)} \tag{A15}$$

Assuming that the parietal cell response, APR, is proportional to the bound hormone, B, and assuming that H_f and I_f can be approximated respectively by H_t and I, eq. A15 becomes eq. 3. Approximating free

hormone by total hormone introduces only a small risk of error since only ~7% of total hormone is bound.

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Note added in proof. Methods for density gradients have been modified to allow separation of parietal and chief cells from elutriator fractions 6–9 (see Fig. 3) in a single step gradient. Using a peristaltic pump and a 100-μl capillary tube extending to the bottom of a 50-ml centrifuge tube, 30 ml of a 20% heavy solution containing the cells is loaded first, followed by a 10-ml step of a 60% heavy solution and then a 10-ml step of a 75% heavy solution. Parietal cells band above the 60% heavy step and chief cells pellet through the 75% heavy step.

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