Binding of Gastrin\textsubscript{17} to Human Gastric Carcinoma Cell Lines

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ABSTRACT

The hormone gastrin stimulates acid secretion by gastric parietal cells and acts as a growth factor for the gastric mucosa. Gastrin receptors with dissociation constants of approximately 0.5 \text{nmol} have been detected on isolated gastric parietal cells, and on some cell lines derived from colon carcinomas. We now report that gastrin is also bound by five cell lines derived from human gastric carcinomas, but that the affinities of these lines for gastrin range from 0.2 to 1.3 \text{\mu M}. Cholecystokinin\textsubscript{8} binds to the cell line Okajima with an affinity similar to gastrin\textsubscript{17}, while shorter gastrin analogues bind with reduced affinity. Binding of gastrin is unaffected by acetylcholine, histamine, or a number of other hormones with the exception of insulin which inhibits binding with an IC\textsubscript{50} value of 0.5 \text{\mu M}. The ability to bind gastrin with affinities in the \text{\mu M} range appears to be a property widespread among other tumor cell lines.

INTRODUCTION

The role of gastrin as a stimulant of acid secretion was first recognized over 80 years ago (1). In the past decade there has been renewed interest in the hormone with the recognition that it also can act as a growth factor for the mucosa of the stomach (2–4). Trophic effects of gastrin on a number of gastric (5–9) and colonic (10, 11) carcinoma cell lines have also been reported. However in only one case, the mouse colonic carcinoma cell line MC26, have the affinity and number of the gastrin binding sites been fully defined (II, 12). As a first step in establishing a human system in which to study the mechanisms of gastrin’s trophic actions, we have screened a variety of human cell lines for the presence of gastrin receptors. This paper defines the gastrin-binding characteristics of five human gastric carcinoma cell lines and reports that the ability to bind gastrin is widespread among other established human cell lines.

MATERIALS AND METHODS

Cell Lines. Human gastric carcinoma cell lines were kindly supplied by Dr. T. Motoyama, First Department of Pathology, Faculty of Medicine, University of Niigata, Japan. Adherent lines of the MKN series were derived from either lymph node or liver metastases of gastric adenocarcinomas (13) while the suspension line Okajima was derived from pleural effusions of a gastric adenocarcinoma (14). Other cell lines were supplied by Drs. R. Whitehead and G. Morstyn of the Ludwig Institute for Cancer Research, Melbourne. All cell lines were grown in RPMI 1640 medium supplemented with 2.5 x 10^{-5} units/ml insulin, 1 \text{g/ml} hydrocortisone, 10 \text{\mu M} a-thioglycerol, and 10\% fetal bovine serum. Three cell lines derived from primary human gastric carcinomas, the suspension line Okajima and two adherent lines of the MKN series, were supplied by Drs. R. Whitehead and G. Morstyn of the Ludwig Institute for Cancer Research, Melbourne. All cell lines were grown in RPMI 1640 medium supplemented with 2.5 x 10^{-5} units/ml insulin, 1 \text{g/ml} hydrocortisone, 10 \text{\mu M} a-thioglycerol, and 10\% fetal bovine serum.

Hormones. The sources of gastrin and its analogues were as follows: [Met\textsubscript{15}]gastrin\textsubscript{17}, [Nle\textsubscript{15}]gastrin\textsubscript{17}, and cholecystokinin\textsubscript{8} (Research Plus, Bayonne, NJ); sulphanated [Met\textsubscript{15}]gastrin\textsubscript{17}, [Met\textsubscript{15}]gastrin\textsubscript{3} (Cambridge Research Biochemicals, Cambridge, England); tert-butyloxycarbonyl-gastrin\textsubscript{3} (ICI, Macclesfield, England); gastrin (Sigma Chemical Co., St. Louis, MO). [Met\textsubscript{15}]gastrin\textsubscript{17} and [Nle\textsubscript{15}]gastrin\textsubscript{17} were iodinated by the iodogen method and [mono-\textsuperscript{125}I]gastrin\textsubscript{17} (specific activity, approximately 2000 \text{dpm/fmol}) was separated by reversed-phase high-pressure liquid chromatography (15). This procedure does not affect the ability of [Met\textsubscript{15}]gastrin\textsubscript{17} to stimulate acid secretion in gastric fistula dogs (12).

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: PBS, phosphate buffered saline; IMX, isobutylmethylxanthine; buffer A, PBS containing 0.2% ovalbumin, 0.5 \text{mm}MgCl\textsubscript{2}, and 1 \text{mm}CaCl\textsubscript{2}.

RESULTS

Characteristics of Gastrin Binding. The binding of [mono-\textsuperscript{125}I]gastrin\textsubscript{17} to the human gastric carcinoma line Okajima increased linearly with cell number and was 80\% inhibited by simultaneous incubation with 10 \text{\mu M} unlabeled gastrin\textsubscript{17} (Fig. 1). Specific binding increased rapidly over 60 min at both 4 and 37\^\circC, and continued to increase more slowly over the next 120 min (Fig. 2). Cell viability was still 86\% after 3 h at 37\^\circC. Although progressive degradation of [\textsuperscript{125}I]gastrin\textsubscript{17} occurred over this period, amounting to 46\% after 30 min, 68\% after 1 h, and 70\% after 3 h (data not shown) the cold competition curves obtained at 30 min (Fig. 3) and 3 h (Fig. 4) were very similar, indicating that any fragments of [\textsuperscript{125}I]gastrin\textsubscript{17}, produced during this period bound to the cells with affinities similar to gastrin\textsubscript{17}. The analogue competition studies presented in Fig. 4 are consistent with this conclusion, since cholecystokinin\textsubscript{8} and gastrin\textsubscript{17} (which share a common C-terminus, but differ at their N-termini) bound to Okajima cells with equal affinity, implying that the N-terminus of gastrin did not contribute significantly to binding. Presumably the observed degradation is the result of proteolytic digestion within the N-terminal 9 residues of gastrin\textsubscript{17}, so that in this particular case the degradation did not affect the accuracy of the binding measurements.

Dissociation Constant for Gastrin. The levels of [\textsuperscript{125}I]gastrin\textsubscript{17} bound specifically to Okajima cells increased with increasing [\textsuperscript{125}I]gastrin\textsubscript{17} concentration (data not shown). Although the data could be fitted to a hyperbola, the data did not deviate...
GASTRIN BINDING SITES ON CARCINOMA CELLS

Fig. 1. Dependence of gastrin binding on cell number. The binding of [125I]-
gastrin, (6.35 x 10^5 cpm/ml, 0.29 nM) to various numbers of cells (viability, 81%) of the human gastric carcinoma line Okajima was determined as described in "Materials and Methods." •, iodinated gastrin bound; □, iodinated gastrin bound in the presence of 10 μM unlabeled gastrin, the difference between the above, or specifically bound gastrin. Points, mean of triplicate samples; bars, one standard deviation; straight lines, obtained by least squares fitting of the experimental data.

Fig. 2. Time course for gastrin binding at various temperatures. The specific binding of [125I]-
gastrin, (4.87 x 10^4 cpm/ml, 0.24 nM) to 5 x 10^5 cells (viability, 93%) of the human gastric carcinoma line Okajima was determined as described in "Materials and Methods" at various times and at the following temperatures: ○, 4°C; □, 37°C. Points, mean of triplicate samples; bars, one standard deviation.

significantly from a straight line at the maximum concentrations of [125I]gastrin, which could be used with safety. (Presumably the gastrin concentrations were well below the Kd value, since under these conditions the expression for the amount of gastrin bound, i.e. [gastrin]Bmax/(Kd + [gastrin]) reduces to [gastrin]Bmax/Kd, where Bmax and Kd are constants defined in Table 1, so that a plot of bound gastrin versus total gastrin is linear). A more accurate estimate of the dissociation constant for gastrin was obtained by measuring the levels of [125I]gastrin, bound in the presence of increasing concentrations of unlabeled gastrin, (Fig. 3). In this case a good fit of the data to a single

site model was obtained, with a Kd value of 0.23 μM and 8.1 x 10^5 binding sites per cell (Table 1). The mean (±SD) values obtained for Kd and the number of binding sites per cell in three separate experiments were 0.29 ± 0.13 μM and 8.5 ± 0.4 x 10^5, respectively. Similar values were obtained when the binding of [125I]-[Nle'5]gastrin, was competed with [Nle'5]gastrin, ( Fig. 3), Table 1). The values of Kd obtained for the binding of [125I]-[Met'5] and [125I]-[Nle'5]gastrin, to Okajima cells were considerably higher than the values of 9 and 0.45 nm reported for the binding of labeled gastrin to isolated rat (20) and canine (21) parietal cells, respectively. To determine whether this difference was genuine the levels of [125I]-[Met'5]- and [125I]-[Nle'5]gastrin, bound to isolated canine parietal cells in the presence of increasing concentrations of unlabeled gastrin, were measured (Fig. 3, Table 1). Kd values of 1.70 and 0.27 nM were obtained for [Met'5]-[Nle'5]gastrin, respectively. Thus, the higher Kd observed with Okajima cells was not the result of an inactive batch of gastrin.

Cholecystokinin, also competed for the gastrin binding sites

Fig. 3. Comparison of gastrin binding to canine parietal cells and Okajima cells. In A, the amount of [125I]-[Nle'5]gastrin, (○, ◇) or [125I]-[Met'5]gastrin, (●, ●) (approximately, 5 x 10^5 cpm/ml, 0.25 nM) bound to 10^5 canine parietal cells (○, ◇) or human Okajima cells (●, ●) in the presence of increasing concentrations of the appropriate unlabeled gastrin, was measured using the phthalate washing procedure described in "Materials and Methods." The data were constructed using the estimates obtained with the program LIGAND (19) and given in Table 1 for Kd, Bmax, and NS for [Nle'5]gastrin, ( ), and [Met'5]gastrin, ( ). The equation used was

\[
\%\text{Maximal binding} = \frac{\text{Pred} \times 100 - \text{Exp}}{\text{Exp}} \left(1 + \frac{[\text{Gastrin}]}{K_d + [\text{Gastrin}]}\right)
\]

where Pred, Exp, and NS are defined in the legend to Table 1, and [Gastrin] is the total concentration of labeled and unlabeled gastrin. The lines do not necessarily pass through 100% since the points with labeled gastrin only (i.e., Exp in Table 1) are fitted with the remainder of the experimental data.

933
on Okajima cells (Fig. 4). The IC50 value obtained (0.22 ± 0.11 
\mu M) was very similar to the Kd value for gastrin17 (0.43 
\mu M). The inhibitory potency of other gastrin analogues was 
approximately proportional to the length of their peptide chain (Fig. 4). No competition was observed with the secretagogues car-bachol or histamine, or with a number of unrelated hormones (Table 2), with the exception of insulin, which inhibited gastrin 
binding by 70% at a concentration of 1 \mu M. When the binding of 
[125I]gastrin17 was competed by increasing concentrations of 
insulin, an IC50 value of 0.49 ± 0.20 \mu M was obtained (Fig. 5).

Comparison of Okajima with Other Gastric Tumor Lines. A 
number of other gastric carcinoma lines were able to bind 
gastrin. The levels of [125I]gastrin17 bound specifically to the 
cells again increased with increasing concentrations of labeled 
gastrin17, and as with Okajima cells the data generally closely 
fitted a straight line (data not shown). When the levels of [125I]- 
gastrin17 bound in the presence of increasing concentrations of 
unlabeled gastrin17 were measured, good fits to a single site 
model were obtained in all cases (Fig. 6). The dissociation 
constants ranged from 0.50 to 1.28 \mu M and the number of 
binding sites per cell from 8.1 × 10^2 to 3.93 × 10^4 (Table 1).

Binding of Gastrin to Other Tumor Lines. Several tumor lines 
derived from tissues other than the stomach were then screened 
for gastrin binding. Tumor lines of both epithelial and nonepithelial 
origin were positive, with melanoma lines binding the 
greatest amount of gastrin (Table 3). The dissociation constant 
and number of receptors per cell determined for one of the 
positive cell lines (the colon carcinoma line, LIM1215) were 
similar to those observed with the gastric carcinoma lines (Table 1). In contrast the dissociation constant determined for the 
vulvar epidermoid carcinoma line A431 was approximately 10-
fold lower, and the number of binding sites 10-fold higher, than 
the values observed with the gastric carcinoma lines. In fact, 
values for the dissociation constant and number of receptors 
per A431 cell could not be assigned with confidence because at 
the highest accessible concentration of gastrin17 (10 \mu M) binding 
of [125I]gastrin17 was inhibited by only 44%.

### DISCUSSION

The dissociation constants for the binding of [mono-[125I]- 
gastrin17 to five gastric carcinoma cell lines vary between 0.2 
and 1.3 \mu M. These values are considerably higher than the 
dissociation constant of 0.5 nm reported by Singh et al. (12) 
for a mouse colon carcinoma cell line (MC-26). The binding 
sites on parietal cells which mediate the ability of gastrin to 
stimulate acid secretion also have a higher affinity than the 
binding sites on carcinoma cell lines described in the present 
article.

#### Table 1: Binding of gastrin to canine parietal cells and human carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Viability (%)</th>
<th>[125I]gastrin; added (nM)</th>
<th>Total [125I]gastrin17</th>
<th>Total bound [125I]gastrin17</th>
<th>Bmax ± SD (nM)</th>
<th>Kd ± SD (\mu M)</th>
<th>10^4 × gastrin binding sites cell ± SD</th>
<th>NS ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine parietal cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>95</td>
<td>0.152</td>
<td>10.11</td>
<td>9.31</td>
<td>4.17 ± 1.98 × 10^{-2}</td>
<td>1.70 ± 1.25 × 10^{-3}</td>
<td>5.01 ± 2.38 × 10^{-3}</td>
<td>58.3 ± 2.0</td>
</tr>
<tr>
<td>(b)</td>
<td>95</td>
<td>0.178</td>
<td>12.09</td>
<td>12.86</td>
<td>1.46 ± 1.26 × 10^{-2}</td>
<td>2.70 ± 1.18 × 10^{-4}</td>
<td>1.75 ± 0.31 × 10^{-3}</td>
<td>58.3 ± 3.3</td>
</tr>
</tbody>
</table>

| Gastric carcinoma lines | | | | | | | |  |
| Okajima | | | | | | | |  |
| (a) | 94 | 0.198 | 6.63 | 7.39 | 6.7 ± 1.1 | 0.23 ± 0.04 | 0.81 ± 0.11 | 24.2 ± 1.6 |
| (b) | 95 | 0.209 | 15.26 | 16.85 | 17.7 ± 9.2 | 0.24 ± 0.13 | 2.13 ± 1.11 | 14.4 ± 2.0 |
| MKN 1 | 89 | 0.187 | 3.80 | 3.91 | 14.4 ± 7.4 | 1.11 ± 0.53 | 1.73 ± 0.08 | 39.2 ± 19.7 |
| MKN 4 | 82 | 0.184 | 4.99 | 5.54 | 32.6 ± 2.14 | 0.28 ± 0.20 | 17.3 ± 0.14 |
| MKN 45 | 86 | 0.239 | 7.27 | 7.39 | 16.1 ± 0.7 | 0.64 ± 0.03 | 1.94 ± 0.08 | 23.1 ± 5.9 |
| MKN 74 | 91 | 0.141 | 8.44 | 8.11 | 23.8 ± 12.7 | 0.50 ± 0.28 | 2.87 ± 1.30 | 20.2 ± 3.1 |

| Other carcinoma lines | | | | | | | |  |
| A431 | 86 | 0.240 | 5.76 | 5.41 | 296 ± 60 | 12.5 ± 2.7 | 35.6 ± 3.08 | 0 |
| LIM1215 | 93 | 0.230 | 11.33 | 12.72 | 23.0 ± 3.1 | 0.42 ± 0.06 | 2.77 ± 0.32 | 7.8 ± 2.3 |

*Note: Values are means ± SD.*
GASTRIN BINDING SITES ON CARCINOMA CELLS

Table 2  Effects of secretagogues and hormones on binding of gastrin to human gastric carcinoma line Okajima

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Concentration</th>
<th>% Specific gastrin binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbachol</td>
<td>100</td>
<td>121</td>
</tr>
<tr>
<td>Histamine + IMX</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>Bombesin</td>
<td>1.0</td>
<td>102</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>1.0</td>
<td>97</td>
</tr>
<tr>
<td>Glucagon</td>
<td>1.0</td>
<td>117</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.25</td>
<td>88</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.0</td>
<td>27</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>1.0</td>
<td>124</td>
</tr>
<tr>
<td>Vasodilator intestinal peptide</td>
<td>0.1</td>
<td>114</td>
</tr>
</tbody>
</table>

In D, the results presented in A-C for Okajima (•), MKN 28 (•), and LIM 1215 (•). The lines in > were constructed as described in the Fig. 3 legend using estimates obtained with the program LIGAND (19) and given in Table 1 for Kd, Bmax, and NS for Okajima (A), MKN 28 (B), and LIM 1215 (C). In D, the results presented in A-C for Okajima (•), MKN 28 (•), and LIM 1215 (•) are presented as Scatchard plots.

**Fig. 5.** Inhibition of gastrin binding by insulin. The effect of various concentrations of insulin on the binding of [¹²⁵I]gastrin₇ (5.55 x 10⁴ m/ml, 0.26 nm) to 5 x 10⁵ cells (viability, 80%) of the human gastric carcinoma cell line Okajima was determined as described in "Materials and Methods." Incubation was for 60 min at 37°C. Points, mean of triplicate samples; bars, one standard deviation. The line was constructed with an IC₅₀ of 0.49 μM and a maximum binding of 108.9%. These values were obtained with the curve-fitting program EBD (22).

**Fig. 6.** Binding of gastrin to gastric carcinoma cell lines. The amount of [¹²⁵I]gastrin₇ (at the total concentrations given in Table 1, approximately 5 x 10⁴ cpm/ml) bound to 10⁵ cells of various human carcinoma cell lines in the presence of increasing concentrations of unlabeled gastrin₇ was measured using the phtalate washing procedure described in "Materials and Methods." Incubation was for 30 min (Okajima cells) or 60 min (all other cell lines) at 37°C. Points, mean of triplicate samples; bars, one standard deviation. The cell lines used were: A: Okajima (•), MKN 45 (□), MKN 74 (△); B: MKN 1 (○), MKN 28 (□), MKN 28 (□); C: A431 (□), LIM 1215 (▪). The lines in A-D were constructed as described in the Fig. 3 legend using estimates obtained with the program LIGAND (19) and given in Table 1 for Kd, Bmax, and NS for Okajima (A), MKN 28 (B), and LIM 1215 (C). In D, the results presented in A-C for Okajima (•), MKN 28 (•), and LIM 1215 (•) are presented as Scatchard plots.

Paper. Values in the nm range have been reported for the dissociation constants for the binding of labeled gastrin to isolated rat (20) and canine (21) parietal cells (9 and 0.45 nm, respectively), and for the ED₅₀ values for gastrin-stimulated aminopyrine uptake in isolated rabbit gastric glands (23) and canine parietal cells (21) (5 and 0.28 nm, respectively). The difference in affinity appears to be genuine since Kd values of 1.70 and 0.27 nm were obtained for the binding of [¹²⁵I][Met₉] and [¹²⁵I][Nle'⁵]gastrin₇, respectively, to isolated canine parietal cells.

Despite a difference of approximately 1000-fold in the dissociation constants of Okajima and parietal cells for gastrin, comparison of the relative affinities for gastrin₇, and gastrin analogues reveals some similarities between the two cell types. Thus, the nonsulphated forms of gastrin₇ and cholecystokinin₈ are equipotent as competitors for [¹²⁵I]gastrin₇ binding to both isolated canine parietal cells (21) and Okajima cells (Fig. 4). Moreover the C-terminal tetrapeptide gastrin₉ is at least 100-fold less potent in both cases. In contrast, the pancreatic receptor for cholecystokinin binds the nonsulphated form of cholecystokinin₈ 24-fold more tightly than gastrin₇ (24).

We have recently identified, by covalent cross-linking of labeled gastrin₉, a gastrin-binding glycoprotein of M₉ 78,000 in Triton extracts of porcine gastric mucosal membranes (15). A similar M₉ of 74,000 has been reported for a gastrin-binding protein on the surface of isolated canine parietal cells (25). The concentration of unlabeled gastrin required for 50% inhibition of cross-linking was 10 nm for cells or plasma membranes prepared therefrom, and 0.3 to 2 μM for membrane extracts, depending on the detergent used for extraction (15). There is thus some evidence that the presumptive gastrin receptor can exist in different conformational states with different affinities for gastrin. The observation that there are two classes of binding sites for the related hormone cholecystokinin on rat pancreatic acini, with Kd values of 64 pm and 21 nm (26, 27), is not inconsistent with this suggestion. However the relationship, if any, between the low affinity binding site on gastric carcinoma cell lines, and the high affinity binding site on gastric parietal cells, remains uncertain. Attempts to define the size of the low affinity binding protein on Okajima cells by covalent cross-linking are currently underway.

At present there is no evidence that occupation of the low affinity gastrin binding site on gastric and colonic carcinoma cell lines has any effect on cell proliferation in vitro. We have been unable to demonstrate any increase in cell number or [³H]thymidine uptake in response to gastrin₇ concentrations up to 1.5 and 15 μM, respectively, for any of the cell lines described in this paper or for the rat gastric carcinoma line BV9 (7). These experiments were performed in RPMI 1640 medium at concentrations of fetal calf serum ranging from 0 to 10%. The evidence currently available suggests that the trophic effects of gastrin are mediated via a high affinity receptor. Thus, the mouse colon carcinoma line MC-26, whose growth is stimulated by pentagastrin in vivo (10, 11), has gastrin receptors of high affinity (Kd = 0.5 nm) on its cell membranes (12). Similarly the human gastric carcinoma cell line TMK-1 shows a 20% increase in thymidine incorporation at 10 nm gastrin in vivo (9). However, demonstration of a trophic response of MC-26 cells in vitro to concentrations of gastrin in the nm range, or of high affinity gastrin receptors on TMK-1 cells, together with the demonstration that carcinoma cell lines with low affinity binding sites such as those described in this paper are unresponsive to gastrin in vivo as well as in vitro, are required to conclusively prove that only high affinity binding sites are
GASTRIN BINDING SITES ON CARCINOMA CELLS

Table 3 Binding of gastrin to human tumor cell lines and human erythrocytes

Specific binding of [125I]gastrin, to the indicated human tumor lines was determined as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Viability (%)</th>
<th>[125I]Gastrin concentration (pm)</th>
<th>Specifically bound gastrin (fmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIM1215</td>
<td>Colon carcinoma</td>
<td>90</td>
<td>240</td>
<td>0.45</td>
</tr>
<tr>
<td>Colo 397</td>
<td>Colon carcinoma</td>
<td>50</td>
<td>240</td>
<td>0.18</td>
</tr>
<tr>
<td>PMC42</td>
<td>Mammary carcinoma</td>
<td>85</td>
<td>370</td>
<td>1.63</td>
</tr>
<tr>
<td>T47D</td>
<td>Mammary carcinoma</td>
<td>85</td>
<td>240</td>
<td>0.76</td>
</tr>
<tr>
<td>MM96</td>
<td>Melanoma</td>
<td>93</td>
<td>240</td>
<td>1.56</td>
</tr>
<tr>
<td>MM200</td>
<td>Melanoma</td>
<td>91</td>
<td>370</td>
<td>1.92</td>
</tr>
<tr>
<td>Colo 239F</td>
<td>Melanoma</td>
<td>52</td>
<td>230</td>
<td>0.27</td>
</tr>
<tr>
<td>A431</td>
<td>Valvul epidermoid carcinoma</td>
<td>90</td>
<td>220</td>
<td>0.76</td>
</tr>
<tr>
<td>H146</td>
<td>Small cell lung cancer</td>
<td>84</td>
<td>370</td>
<td>0</td>
</tr>
<tr>
<td>H157/35</td>
<td>Large cell lung cancer</td>
<td>90</td>
<td>240</td>
<td>0</td>
</tr>
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<td>HL60</td>
<td>Promyeloecytic leukemia</td>
<td>90</td>
<td>230</td>
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<tr>
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<td>Myeloid leukemia</td>
<td>88</td>
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<td>T-cell lymphoma</td>
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<td>240</td>
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<td>230</td>
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<td>B-cell lymphoma</td>
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<tr>
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<td>B-cell lymphoma</td>
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<td>Erythrocytes</td>
<td>Bone marrow</td>
<td>100</td>
<td>230</td>
<td>0</td>
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<tr>
<td>Erythrocytes</td>
<td>Peripheral blood</td>
<td>100</td>
<td>230</td>
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</table>

capable of modulating the trophic effects of gastrin.

A novel aspect of the low affinity gastrin-binding site described in this paper is that binding is reduced in the presence of insulin. The failure to observe inhibition of [125I]gastrin binding to isolated canine parietal cells in the presence of 0.1 μM insulin (21) is consistent with the results presented in Fig. 5, where 10-fold higher insulin concentrations are required for significant inhibition. The fact that identical inhibition was observed with insulin from both Commonwealth Serum Laboratories and Novo suggests that inhibition is not due to a contaminating protein (data not shown). However, the IC50 value of 0.49 μM suggests that the inhibition is not a direct result of binding to the insulin receptor [IC50 value for insulin, 4–9 nm (28)], but may perhaps be mediated by the insulin-like growth factor I receptor [IC50 value for insulin, 0.3–1.0 μM (28, 29)]. Equally the absence of any obvious structural homology between gastrin and insulin suggests that insulin is not a direct competitor for the gastrin-binding site. A possibly analogous effect has been observed with the receptor for the related hormone cholecystokinin. The sensitivity to cholecystokinin of pancreatic acini from streptozotocin-induced diabetic rats is selectively reduced (30), although interestingly cholecystokinin receptor levels are increased 2-fold (31). Such an apparent selective reduction (30), although interestingly cholecystokinin receptor levels are increased 2-fold (31).

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GASTRIN BINDING SITES ON CARCINOMA CELLS


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