Novel Expression of Gastrin (Cholecystokinin-B) Receptors in Azaserine-induced Rat Pancreatic Carcinoma: Receptor Determination and Characterization

Weigong Zhou, Stephen P. Povoski, Daniel S. Longnecker, and Richard H. Bell, Jr.

Department of Surgery, University of Cincinnati College of Medicine, and Department of Veterans Affairs Medical Center, Cincinnati, Ohio 45267 [W. Z., S. P. P., R. H. B. J., and Department of Pathology, Dartmouth Medical School, Hanover, New Hampshire 03756 [D. S. L.]

ABSTRACT

Many reports have emphasized the role of gastrin as a growth factor for normal gastrointestinal mucosa and gastrointestinal cancers. Recent studies have pointed out that this model also acts as a growth factor for the pancreatic cancer cell line AR42J. This effect is mediated by gastrin [cholecystokinin (CCK)-B] receptors. In the present study, we investigated gastrin (CCK-B) receptor expression in the azaserine-induced rat pancreatic carcinoma DSL-6, comparing it to normal rat pancreas, and we also characterized CCK receptor subtypes in this tumor.

The results showed that there is extensive gastrin binding to the DSL-6 pancreatic carcinoma. No evidence of specific gastrin binding to normal pancreas was found. Analysis of the ability of gastrin-17-I to inhibit $^{125}$I-gastrin binding demonstrated that gastrin bound to a single class of receptors with a $K_d$ of 0.21 ± 0.04 nM and a binding capacity of 184 ± 29 fmol/mg protein. $^{125}$I-Gastrin-I binding was inhibited by the specific CCK-B receptor antagonist L365,260 approximately 40 times more effectively than by the specific CCK-A receptor antagonist L364,718. Analysis of the ability of cholecystokinin octapeptide (CCK-8) to inhibit $^{125}$I-Bolton-Hunter-CCK-8 binding revealed two CCK binding sites, i.e., a high affinity site and a low affinity site. The observed binding affinities of CCK-8 were then introduced into the computer analysis of the dose-inhibition curve of the ability of gastrin-17-I to inhibit binding of $^{125}$I-Bolton-Hunter-CCK-8, which was significantly better fit by a three-site model than by a two-site model. The three sites meet the criteria for CCK-B, high affinity CCK-A, and low affinity CCK-A receptors. The binding capacity of CCK-B receptors constitutes 34% of the total high affinity CCK binding sites.

This study demonstrated that DSL-6 pancreatic carcinoma expresses three subtypes of CCK receptors. Gastrin (CCK-B) receptors, which were not detected in normal rat pancreas, constitute about one third of the total high affinity CCK receptors. We suggest that novel expression of gastrin (CCK-B) receptors may be generated by gene mutation or amplification during carcinogenesis and may play an important role in promoting tumor growth.

INTRODUCTION

Adenocarcinoma of the pancreas has become the fifth most common cause of cancer death in the United States and remains nearly incurable, with an average survival from diagnosis until death of approximately 3 months (1). For this reason, there has been considerable interest in trying to identify factors involved in the growth of pancreatic cancer, particularly those factors which may be manipulated. In recent years, several experimental models of chemically induced carcinoma in the exocrine pancreas have been developed in rodents for studying pancreatic carcinogenesis (2), of which one well described example is azaserine-induced pancreatic carcinoma in the rat (3–5). In response to a single injection of the carcinogen azaserine (30 mg/kg body weight) at age 2 weeks, approximately 50% of Lewis rats develop acinar cell adenocarcinoma of the pancreas after a latency period of 18 months.

The peptide hormone gastrin, which was the first gastrointestinal hormone to be isolated and sequenced (6), has been shown to modulate the growth of normal gastrointestinal mucosa (7) and pancreas (8–10) through stimulation of protein and DNA synthesis. Recent studies have pointed out that gastrin may also act as a growth factor in gastrointestinal cancers. Gastrin stimulates the growth of colon carcinomas in vivo (11) and those transplanted into nude mice (12). In addition, it induces proliferation of colon and gastric cancer cell lines (13, 14). These effects are presumed to be receptor mediated. Indeed, in vitro studies have demonstrated the presence of gastrin receptors on gastric and colon cancer cells (15), suggesting that growth of some gastrointestinal cancers is hormonally regulated.

Pancreatic acinar cells from guinea pig have been shown to possess three classes of CCK receptors (16). The high affinity CCK-A receptor, having a high affinity for CCK and a low affinity for gastrin peptides, is involved in pancreatic enzyme secretion. By contrast, the CCK-B receptor does not discriminate between CCK and gastrin peptides. The third receptor is a low affinity CCK-A receptor that has a low affinity for CCK and gastrin. The biological events related to CCK-B and the third receptor subtype are still poorly documented.

It has been previously reported that the rat pancreatic carcinoma cell line AR42J possesses both CCK-A and CCK-B receptor subtypes (17). It has also been demonstrated that occupancy of CCK-B receptors by gastrin stimulates early events associated with cell proliferation, such as ornithine decarboxylase activity (18). $^{3}$Hthymidine incorporation (19), and the $Na^+/H^+$ antiporter (20).

We have previously demonstrated the overexpression of high affinity CCK receptors in the azaserine-induced rat pancreatic carcinoma DSL-6 (21). In the present study, we looked for the presence of gastrin binding sites (CCK-B receptors) by competitive binding assays. If, compared to normal pancreas, pancreatic tumors possess an enhanced ability to bind gastrin, this may imply that there is a gene mutation or amplification during carcinogenesis and that gastrin may play an important role in pancreatic tumor promotion and progression.

MATERIALS AND METHODS

Chemicals. MES and leupeptin were from Boehringer Mannheim GmbH (Penzburg, Germany). NaCl, KCl, MgCl$_2$, ethylene glycol bis(β-aminoethyl ether)-$N,N,N,N'$-tetraacetic acid, $N$-[2-hydroxyethyl]-piperazine-$N'$-[2-ethanesulfonic acid], bacitracin, chymostatin, and bovine albumin were from Sigma Chemical Company (St. Louis, MO). Gelatin was from Mathes. i Coleman & Bell Manufacturing Chemists (Norwood, OH). Azaserine was from Calbiochem-Behring (La Jolla, CA). The abbreviations used are: CCK, cholecystokinin; $^{125}$I-BH, $^{125}$I-Bolton-Hunter-labeled; $B_{max}$, maximal binding capacity; MES, 2-(N-morpholino)ethanesulfonic acid; CCK-8, cholecystokinin octapeptide.
were then placed in tubes for counting in a gamma counter. Binding of ~ 4

cance. All data in the experiments described below are presented as

reagent. by the method of Bradford (25) using the Bio-Rad protein assay

creas, the pH, time, and temperature dependence was deter-

bacitracin, 4 #g/ml leupeptin, 2 #g/ml chymostatin, 130 mM NaCl, 7.7 7

were preincubated in 50 mM MES buffer containing 0.5% albumin for

radiolabeled ligand in the absence of unlabeled ligand was taken to D O represent maximum binding. Binding of labeled ligand in the presence to 3

tumor-bearing animals was harvested via a midline laparotomy (n = 6). -

and the tumor was harvested. At the same time, normal pancreas from

Gastrin-I (2200 Ci/mmol) and 125I-BH-CCK-8 (2200 Ci/mmoll were I-- 6

Preparation of Tissue Sections for Binding Studies. DSL-6 was har-

Preparation of Tissue Sections for Binding Studies. DSL-6 was har-

tained from donor animals, minced, and injected s.c. in the interscap- o 4

Experimental Design. The pH dependence of binding of ~25I-gastrin-I to DSL-6 pancreatic carci-

mined for each tissue. For DSL-6 carcinoma, maximal binding

RESULTS

To determine the optimum conditions for binding of 125I-

t tumor sections. Sections were incubated at the indicated pH with 125I-gastrin-I alone (O) or with 10 #M

Analysis of Binding Data. Binding parameters (Kd and Bmax) were 0

lower at 37°C than at 22°C. When 10 #M gastrin-17-I was added

by 6% for sections of normal pancreas. Binding of 125I-gastrin-I to DSL-6 carcinoma time was temperature

To study the reversibility of binding of 125I-gastrin-I to

Gastrin-I to sections of DSL-6 carcinoma and normal rat pancre-

Binding of ~ 125I-Gastrin-I to Tissue Sections. Binding assays were performed using the method of von Schrenck et al. (22). Tissue sections were preincubated in 50 mM MES buffer containing 0.5% albumin for 20 min at pH 6.5 and 22°C. Sections were then incubated for 4 h at 22°C, pH 6.5, in 50 mM MES buffer containing 0.5% albumin, 0.025% bacitracin, 4 #g/ml leupeptin, 2 #g/ml chymostatin, 130 mM NaCl, 7.7 mM KCl, 5 mM MgCl2, 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, and 25 pm 125I-gastrin-I. Incubation volume was 3 ml for four slides. For competitive binding assays, the appropriate concentrations of unlabeled ligands were added to the incubation buffer. At the conclusion of the incubation, tissue sections were rinsed three times in 50 mM MES washing buffer (pH 6.5, 4°C) with 0.5% albumin and were then wiped from the slides with a filter paper. The filter papers were then placed in tubes for counting in a gamma counter. Binding of radiolabeled ligand in the absence of unlabeled ligand was taken to represent maximum binding. Binding of labeled ligand in the presence of an excess of unlabeled ligand (1.0 x 10-3 M) was considered to represent nonsaturable binding. Nonsaturable binding was <20% of total binding in all experiments reported below.

Binding of ~125I-BH-CCK-8 to Tissue Sections. Binding of ~125I-BH-

CCK-8 to tissue sections was measured by using the same procedure described above, except that the pH of the preincubation, incubation, and washing buffers was 6.0 instead of 6.5.

Analysis of Binding Data. Binding parameters (Kd and Bmax) were determined for each binding site by using a nonlinear model-fitting program (LIGAND) (23). The values of Kd were determined by the method of Cheng and Prusoff (24). The number of binding sites was determined by repetitive fits with increasing numbers of sites and was established by determining the smallest number of sites that statistically best fit the data, using the F test and P < 0.05 as the level of significance. All data in the experiments described below are presented as mean ± SD unless otherwise indicated.

Receptor binding capacity was normalized to protein by cutting additional 20-μm tissue sections, which were analyzed for protein content by the method of Bradford (25) using the Bio-Rad protein assay reagent.

RESULTS

To determine the optimum conditions for binding of 125I-

gastrin-I to sections of DSL-6 carcinoma and normal rat pancre-

CA). Gastrin-17-I and CCK-8 were from BACHEM, Inc. (Torrance, CA). L365,260 and L364,718 were gifts from Dr. William Friedinger, Merck Sharp & Dohme Research Laboratories (Rahway, NJ). 125I-

Gastrin-I (2200 Ci/mmol) and 125I-BH-CCK-8 (2200 Ci/mmoll were from DuPont NEN Research Products (Boston, MA). Bio-Rad protein assay reagent was from Bio-Rad Laboratories (Richmond, CA).

Preparation of Tissue Sections for Binding Studies. DSL-6 was harvested from donor animals, minced, and injected s.c. in the interscapular area of 1–2-month-old male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA). After the implanted tumor had grown to 1–2 cm in diameter, animals were decapitated under ether anesthesia and the tumor was harvested. At the same time, normal pancreas from tumor-bearing animals was harvested via a midline laparotomy (n = 6). All tissues were immediately frozen on dry ice and were then transferred to a −20°C cryostat microtome, mounted on gelatin-coated microscope slides, and dried for 18 h at −22°C.

Binding of ~125I-Gastrin-I to Tissue Sections. Binding assays were performed using the method of von Schrenck et al. (22). Tissue sections were preincubated in 50 mM MES buffer containing 0.5% albumin for 20 min at pH 6.5 and 22°C. Sections were then incubated for 4 h at 22°C, pH 6.5, in 50 mM MES buffer containing 0.5% albumin, 0.025% bacitracin, 4 #g/ml leupeptin, 2 #g/ml chymostatin, 130 mM NaCl, 7.7 mM KCl, 5 mM MgCl2, 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, and 25 pm 125I-gastrin-I. Incubation volume was 3 ml for four slides. For competitive binding assays, the appropriate concentrations of unlabeled ligands were added to the incubation buffer. At the conclusion of the incubation, tissue sections were rinsed three times in 50 mM MES washing buffer (pH 6.5, 4°C) with 0.5% albumin and were then wiped from the slides with a filter paper. The filter papers were then placed in tubes for counting in a gamma counter. Binding of radiolabeled ligand in the absence of unlabeled ligand was taken to represent maximum binding. Binding of labeled ligand in the presence of an excess of unlabeled ligand (1.0 x 10-3 M) was considered to represent nonsaturable binding. Nonsaturable binding was <20% of total binding in all experiments reported below.

Binding of ~125I-BH-CCK-8 to Tissue Sections. Binding of ~125I-BH-

CCK-8 to tissue sections was measured by using the same procedure described above, except that the pH of the preincubation, incubation, and washing buffers was 6.0 instead of 6.5.

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Receptor binding capacity was normalized to protein by cutting additional 20-μm tissue sections, which were analyzed for protein content by the method of Bradford (25) using the Bio-Rad protein assay reagent.

RESULTS

To determine the optimum conditions for binding of 125I-

gastrin-I to sections of DSL-6 carcinoma and normal rat pancre-

Fig. 1. pH dependence of binding of ~125I-gastrin-I to DSL-6 pancreatic carcinoma tissue sections (A) and to normal rat pancreas tissue sections (B). Sections were incubated at the indicated pH with ~125I-gastrin-I alone (O) or with 10 #M unlabeled gastrin-17-I (F) with either MES (pH 5.0–6.5) or N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (pH 7.0 or 7.5) buffer. Results are percentages of total radioactivity added to the incubation volume that bound to tissue sections. Results are means from at least three separate experiments. In each experiment, each value was determined in duplicate. Vertical bars, SE.
To determine the number of different binding sites on DSL-6 pancreatic carcinoma, we first used CCK-8 to inhibit binding of $^{125}$I-BH-CCK-8 (Fig. 6). The ability of CCK-8 to inhibit binding of $^{125}$I-BH-CCK-8 was analyzed by using a nonlinear model-fitting computer program (LIGAND). The dose-inhibition curve for CCK-8 was fit significantly better ($P = 0.02$) by a two-site model than by a one-site model (Fig. 7), and a
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Fig. 5. Ability of L365,260 and L364,718 to inhibit binding of 125I-gastrin-I to DSL-6 pancreatic carcinoma tissue sections. Tissue sections were incubated for 4 h at 22°C, pH 6.5, with 25 pM 125I-gastrin-I alone or with the indicated concentrations of L365,260 (○) or L364,718 (●). Saturable binding is expressed as a percentage of radioactivity bound in the absence of L365,260 or L364,718. Results are means from six separate experiments. In each experiment, each value was determined in duplicate. Vertical bars, SE.

Table 1. Affinities and binding capacities from dose-inhibition curves for the ability of L365,260 and L364,718 to inhibit 125I-gastrin-I binding in DSL-6 pancreatic carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L365,260</th>
<th>L364,718</th>
<th>Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki (nM)</td>
<td>0.02 ± 0.01</td>
<td>0.78 ± 0.15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>183.7 ± 19.8</td>
<td>51.0 ± 10.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Pa Student's t test.

Table 2. Scatchard plot of ability of CCK-8 to inhibit binding of 125I-BH-CCK-8 to DSL-6 pancreatic carcinoma tissue sections. Data from Fig. 6 were analyzed by using a nonlinear model-fitting program (LIGAND), and curves were best fit by a two-binding site model. Kd and Bmax for CCK-8 at these two sites are listed in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>125I-BH-CCK-8</th>
<th>125I-BH-CCK-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>324 ± 63</td>
<td>3899 ± 1497</td>
</tr>
<tr>
<td>Kd (nM)</td>
<td>0.20 ± 0.10</td>
<td>0.21 ± 0.04</td>
</tr>
</tbody>
</table>

Fig. 6. Ability of CCK-8 to inhibit binding of 125I-BH-CCK-8 to DSL-6 pancreatic carcinoma tissue sections. Sections were incubated at 22°C, pH 6.0, for 4 h with 25 pM 125I-BH-CCK-8. Saturable binding of 125I-BH-CCK-8 is expressed as a percentage of radioactivity bound in the absence of CCK-8. Results are means from six separate experiments. In each experiment, each value was determined in duplicate. Vertical bars, SE.

three-site model did not give a significantly better fit than a two-site model (P = 0.65). The high affinity site had a Kd of 0.51 ± 0.03 nM with a binding capacity of 324 ± 63 fmol/mg protein and the low affinity site had a Kd of 2753 ± 1153 nM with a binding capacity of 3899 ± 1497 fmol/mg protein (Table 2). A previous study (16) demonstrated that 125I-BH-CCK-8 binds to both high affinity CCK-A sites and CCK-B sites with almost equal high affinity; therefore, the high affinity binding capacity represents binding to both of these sites. In an attempt to further delineate the high affinity CCK binding sites on DSL-6 carcinoma, we used gastrin-17-I, which distinguishes binding to CCK-B receptors from that to high affinity CCK-A receptors, to inhibit binding of 125I-BH-CCK-8 (Fig. 8). Analysis of the dose-inhibition curve of the ability of gastrin-17-I to inhibit binding of 125I-BH-CCK-8 was performed with the nonlinear model-fitting program (LIGAND) using the binding affinities of CCK-8 determined above. The result showed that the dose-inhibition curve was significantly better fit (P < 0.01) with a three-site model than with a two-site model (Fig. 9). One site, R2 (Table 3), had an affinity of 0.20 ± 0.10 nM and a binding capacity of 116 ± 15 fmol/mg protein, which are quite similar to those seen with gastrin-17-I inhibition of binding of 125I-gastrin-I (0.21 ± 0.04 nM, 184 ± 29 fmol/mg protein). Since 125I-gastrin-I binds only to gastrin (CCK-B) receptors (28), this demonstrates that the R2 site represents binding to gastrin (CCK-B) receptors. R1 and R3 had 424 and 25,000 times lower affinities for gastrin, respectively, than did the R2 site. These values are consistent with the low affinity and very low affinity for gastrin exhibited by high affinity CCK-A receptors and low affinity CCK-A receptors, respectively. These conclusions are further supported by comparing the high affinity binding capacity demonstrated with the ability of both CCK-8 and
Data were best fit by a model having two binding sites, and 7 were analyzed by using a nonlinear model-fitting program (LIGAND). The unlabeled gastrin-17-I. Saturable binding is expressed as a percentage of radioactivity bound in the absence of unlabeled gastrin-17-I. Results are means from five separate experiments. In each experiment, each value was determined in duplicate. Vertical bars, SE.

Table 2. Affinities and binding capacities from dose-inhibition curve for the ability of CCK-8 to inhibit 125I-BH-CCK-8 binding in DSL-6 pancreatic carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High affinity</th>
<th>Low affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$ (nM)</td>
<td>0.51 ± 0.03</td>
<td>2.753 ± 1.153</td>
</tr>
<tr>
<td>$B_{max}$ (fmol/mg protein)</td>
<td>324 ± 63</td>
<td>3,899 ± 1,497</td>
</tr>
</tbody>
</table>

Fig. 8. Ability of gastrin-17-I to inhibit binding of 125I-BH-CCK-8 to DSL-6 pancreatic carcinoma tissue sections. Sections were incubated for 4 h at 22°C, pH 6.0, with 25 pM 125I-BH-CCK-8 alone or with the indicated concentrations of unlabeled gastrin-17-I. Saturable binding is expressed as a percentage of radioactivity bound in the absence of unlabeled gastrin-17-I. Results are means from five separate experiments. In each experiment, each value was determined in duplicate. Vertical bars, SE.

Table 3. Affinities and binding capacities from dose-inhibition curve for the ability of gastrin-17-I to inhibit 125I-BH-CCK-8 binding in DSL-6 pancreatic carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$K_d$ (nM)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$</td>
<td>214 ± 47</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td>$B_{max}$</td>
<td>224 ± 16</td>
<td>5,695 ± 2,335</td>
</tr>
</tbody>
</table>

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gastrointestinal proliferative agents on cancer cells (9-12). In addition, studies using the nonpeptide gastrin receptor antagonist proglumide to prevent the growth-promoting effects of gastrin demonstrate that mediation of the effects is via a gastrin receptor (29-31).

Several investigators have previously demonstrated the presence of CCK receptors on pancreatic carcinoma cells. Logsdon (32) demonstrated high affinity CCK receptors in the AR42J cell line, which like DSL-6 is derived from a azaserine-induced acinar cell carcinoma. He also noted that CCK receptors on AR42J cells seemed to be different from those in normal rat pancreas. Scemama et al. (18) reported that AR42J cells possess both CCK-A and CCK-B receptor subtypes and that CCK-B receptor occupation is related to stimulation of ornithine decarboxylase activity. Likewise, Seva et al. (19) demonstrated that occupation of the CCK-B receptor subtype in AR42J cells led to an increase in cell proliferation as measured by [3H]thymidine incorporation.

In our study, the 125I-gastrin-I binding data indicated that gastrin (CCK-B) receptors are extensively expressed in DSL-6 pancreatic carcinoma. No saturable binding could be detected in normal pancreas, although the binding assays utilized do not rule out the possibility of the expression of a minute quantity of gastrin (CCK-B) receptors. It is our belief that these receptors are first expressed in significant amounts in DSL-6 during the process of carcinogenesis at the point of malignant transformation. The relative abilities of L365,260 and L364,718 to inhibit binding of 125I-gastrin-I further demonstrated that these receptors are of the CCK-B subtype.
Previous studies characterizing CCK receptors on dispersed pancreatic acini or pancreatic tissue sections using $^{125}$I-BH-CCK-8 or various $^{125}$I-labeled carboxyl-terminal fragments or analogues of CCK have demonstrated two classes of CCK binding sites in rats, mice, guinea pigs, dogs, and the AR42J cell line (17, 33–37), one with a high affinity for CCK and the other with a low affinity. Recently, it was demonstrated that $^{125}$I-BH-CCK-8 binds to three classes of receptors on dispersed pancreatic acini of guinea pigs, not two as reported previously (16). Two of these three classes of receptors bound CCK-8 with equal high affinity. One of these two high affinity CCK binding sites also had a high affinity for gastrin-17-I and the other had a 355-fold lower affinity for gastrin-17-I. These two sites are referred to as CCK-B and high affinity CCK-A receptor subtypes, respectively. The third class had low affinity for both CCK-8 and gastrin-17-I. In the present study, we also demonstrated two classes of receptors for CCK by computer analysis of the dose-inhibition curve of the ability of CCK-8 to inhibit the binding of $^{125}$I-BH-CCK-8, which was significantly better fit by a three-site model than by a two-site model. Two of these three sites were determined to correspond to the high affinity CCK binding site. One of them, $R_2$ (Table 3), had the highest affinity for gastrin-17-I ($K_a = 0.20 \pm 0.10$ nM). It appears to be the CCK-B receptor subtype. The other, $R_3$ (Table 3), had a 1000-fold lower affinity for gastrin-17-I ($K_a = 214 \pm 47$ nM). It appears to be the high affinity CCK-A receptor subtype. The CCK-B receptors constitute 34% of the binding capacity of high affinity CCK receptors. In addition, a site, $R_3$ (Table 3), with the lowest affinity for gastrin-17-I ($K_a = 5695 \pm 2335$ nM) was found to correspond to the low affinity CCK-A binding site. It appears to be the low affinity CCK-A receptor subtype.

Carcinogenesis is a multistage process that is generally believed to include an initiation phase, in which initial cellular DNA damage and gene mutation occur, followed by promotion and progression phases. The development of carcinoma depends on further alteration in the initiated cell during the promotion phase. We surmise that the novel expression of gastrin (CCK-B) receptors in DSL-6 pancreatic carcinoma may be a result of a gene mutation or amplification during initiation and may provide a mechanism for promotion and/or progression of tumor cell growth. In conclusion, DSL-6 pancreatic carcinoma expresses three subtypes of CCK receptors. Gastrin (CCK-B) receptors, which were not detected in normal rat pancreas, constitute about one third of the total high affinity CCK receptors in DSL-6 pancreatic carcinoma. The expression of gastrin (CCK-B) receptors may be generated by gene mutation or amplification during carcinogenesis and may play an important role in promoting tumor growth.

ACKNOWLEDGMENTS

We are grateful for the advice and support of Robert T. Jensen, M.D., Digestive Disease Branch, NIDDK, NIH. In addition, we appreciate the excellent technical assistance of Nancy Rosen.
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