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Antiproliferative gastrin/cholecystokinin receptor antagonists target the 78-kDa gastrin-binding protein

(autocrine loop/colorectal carcinoma/gastrin receptor)

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ABSTRACT **Inhibition of colon carcinoma cell growth by the nonselective gastrin/cholecystokinin (CCK) receptor antagonists proglumide and benzotript provided evidence that gastrin functions as an autocrine growth factor. However, the molecular properties of the receptor mediating the antagonist effects have not been identified. A 78-kDa gastrin-binding protein (GBP), the sequence of which is related to the family of enzymes possessing enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, has been previously purified from porcine gastric mucosal membranes. I now report that covalent cross-linking of ^{125}I-labeled [Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP is inhibited by crotonyl-CoA and by acetoacetyl-CoA. Gastrin, CCK, and their analogues also inhibit cross-linking, and the spectrum of analogue affinities correlates better with the values previously reported for binding to the gastrin/CCK-C receptor than with the values reported for binding to either the CCK-A or the gastrin/CCK-B receptor. Cross-linking is also inhibited by proglumide and benzotript, but no inhibition is seen with either the CCK-A receptor-selective antagonist L364,718 or the gastrin/CCK-B receptor-selective antagonist L365,260. The affinities of antagonists for the GBP correlate well with their affinities for the gastrin/CCK-C receptor and with their potencies for inhibition of colon carcinoma cell growth. I conclude that the 78-kDa gastrin-binding protein is (*i*) a member of the hydratase/dehydrogenase family of fatty acid oxidation enzymes, (*ii*) the gastrin/CCK-C receptor, and (*iii*) the target for the antiproliferative action of two gastrin/CCK receptor antagonists.**

Evidence that a gastrin-like peptide acts as an autocrine growth factor in colorectal carcinoma has been accumulating steadily. First, gastrin mRNA has been demonstrated in normal and neoplastic colorectal mucosa by Northern blotting (1) and in colon carcinoma cell lines by the polymerase chain reaction (1–3). Second, an increase in progastrin production in colorectal tumor tissue compared with normal mucosa has been demonstrated in tumor sections by immunohistochemistry (4) and in tissue extracts by radioimmunoassay (1, 5, 6). Progastrin, but not mature amidated gastrin, was also produced by all (five/five) colonic carcinoma cell lines tested (1). Third, exogenous gastrin$_{17}$ or its derivatives enhanced the growth of xenografts of 60% of colon carcinomas tested (7, 8). However, many colon carcinoma cell lines did not increase either DNA (9) or protein synthesis (10) *in vitro* in response to exogenous gastrin, perhaps because they were already maximally stimulated by an autocrine gastrin-like peptide. In some cases previously unresponsive cells have been rendered responsive by synchronization (11–13).

The most convincing evidence for an autocrine role for a gastrin-like peptide has been provided by studies with gastrin/cholecystokinin (CCK) receptor antagonists (14). The antagonist proglumide, which does not discriminate clearly between the three known classes of gastrin/CCK receptors (Table 1), increased the survival time of mice bearing tumors derived from the mouse colon carcinoma line MC26 (27). Proglumide also reversed the increase in tumor volume observed in tumor-bearing mice after treatment with pentagastrin (7). Proliferation of six colon carcinoma cell lines *in vitro* was inhibited by the nonselective antagonists proglumide and benzotript (14), with IC_{50} values in the millimolar range (Table 1). Furthermore, in the case of the HCT 116 cell line, proliferation was also inhibited by an anti-gastrin antiserum (14). The high concentrations of gastrin required to reverse inhibition by both antagonists and antibodies (14) suggested that neither pancreatic CCK-A nor gastric gastrin/CCK-B receptors were involved (Table 1) but were consistent with the involvement of the low-affinity gastrin/CCK-C receptor first described on the surface of several gastric and colonic carcinoma cell lines (9). The failure of the selective antagonists L364,718 and L365,260 (Table 1) to inhibit proliferation of HCT 116 cells at concentrations as high as 1 μM confirmed that neither CCK-A nor gastrin/CCK-B receptors participated in the autocrine loop (22).

A 78-kDa GBP has been previously characterized in detergent extracts of porcine gastric mucosal membranes (24). The affinity of the GBP for gastrin$_{17}$, determined in a covalent cross-linking assay, was between 0.3 and 2 μM, depending on the detergent used for solubilization (24). Purification of the GBP permitted the determination of extensive tracts of amino acid sequence (28), which in turn allowed the cloning of the corresponding cDNA by a combination of the polymerase chain reaction with degenerate oligonucleotides and library screening (29). Surprisingly, the amino acid sequence translated from the GBP cDNA was related to the family of fatty acid oxidation enzymes possessing enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activity (30). However the structure of the gene encoding the GBP was clearly distinct from the gene encoding the rat peroxisomal bifunctional enzyme, implying that the relationship was distant (31). To investigate the possibility that the GBP might be involved in the autocrine effects of gastrin-like peptides on colorectal carcinoma cells, I have examined the effect of agonists and antagonists on the interaction between the GBP and gastrin. The good correlation observed between the IC_{50} values for antagonists in the GBP cross-linking assay and the IC_{50} values for inhibition of either gastrin binding to colon carcinoma cells or cell proliferation suggests that the GBP is the target for the inhibitory effects of gastrin/CCK receptor antagonists on cell growth.

MATERIALS AND METHODS

Gastrin$_{17}$, CCK$_8$, and CCK$_8$SO$_4$ were from Research Plus (Bayonne, NJ). Pentagastrin, gastrin$_4$, proglumide, ace-

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Abbreviations: CCK, cholecystokinin; GBP, gastrin-binding protein.

Table 1. Comparison of the 78-kDa gastrin-binding protein (GBP) with human gastrin and CCK receptors

Agonist or antagonist	IC_{50}, μM: For binding* CCK-A receptor (pancreas, gall bladder)	For binding* Gastrin/CCK-B receptor (stomach, brain)	For binding* Gastrin/CCK-C receptor (HCT 116 cells)	For cell proliferation† (HCT 116 cells)	For cell proliferation† (HT 29 cells)	For cross-linking 78-kDa GBP receptor (stomach)
Agonist						
$Gastrin_{17}$	0.08 (15) 1.8 (16)	6.4×10^{-3} (19) 9.4×10^{-4} (20) 2.6×10^{-3} (21)	1.6 (14)	—	—	0.23 ± 0.15
CCK_8	0.14§ (17)	1.9×10^{-3} (20) 4.7×10^{-3} (21)	13.2 (14)	—	—	4.5 ± 1.0
CCK_8SO_4	2×10^{-5} (15) 3×10^{-3} (16)	3.0×10^{-3} (19) 1.4×10^{-4} (20) 1.1×10^{-4} (21)	ND	—	—	5.8 ± 3.2
Pentagastrin	ND	ND	100 (14)	—	—	210 ± 90
$Gastrin_4$	ND	3.2×10^{-2} (20) 3.0×10^{-2} (21)	ND	—	—	370 ± 280
Antagonist						
Proglumide	600¶ (18)	900§ (18)	8600 (14)	5000 (14)	14,000 (23)	5100 ± 3600
Benzotript	102¶ (18)	59§ (18)	400 (14)	500 (14)	3,400 (23)	200 ± 120
L364,718	1×10^{-4} (15) 8×10^{-4} (16)	0.5 (19) 0.15 (20)	ND	4 (22)	>100 (23)	>200
L365,260	9 (15) 0.02 (16)	1.0×10^{-2} (19) 3.8×10^{-3} (20)	ND	15 (22)	22 (23)	>200

Values are for the human receptors unless indicated otherwise. ND, not determined. Reference numbers are given in parentheses.

*IC_{50} values for the binding of agonists and antagonists to the CCK-A, gastrin/CCK-B, and gastrin/CCK-C receptors were determined by competition for ^{125}I-labeled (^{125}I)CCK_{33} (18), ^{125}I-CCK_8 (15–17, 19–21), or ^{125}I-$gastrin_{17}$ (14) binding to pancreatic membranes (17, 18), to gastric glands (18), to HCT 116 colon carcinoma cells (14), or to intact COS or Chinese hamster ovary cells transfected with cDNAs encoding the human gallbladder CCK-A receptor (15, 16) or the human brain gastrin/CCK-B receptor (19–21).

†IC_{50} values for the inhibition of proliferation of HCT 116 (14, 22) and HT 29 (23) colon carcinoma cells by antagonists were determined from cell counts (14, 22) or by colorimetric assay (23).

‡IC_{50} values (mean ± SD, calculated from at least three sets of data) for inhibition of cross-linking of ^{125}I-[Nle^{15}]$gastrin_{2,17}$ to the 78-kDa GBP in detergent extracts of porcine gastric mucosal membranes (24) were determined from the data presented in Fig. 1 and from similar experiments by least squares fitting with the programs EBDA (25) and LIGAND (26).

§Guinea pig receptor.

¶Rat receptor.

toacetyl-CoA, crotonyl-CoA, pepstatin, benzamidine, hexamethylphosphoramide, and aprotinin were from Sigma. Benzotript was from Calbiochem. The antagonists L364,718 and L365,260 were generous gifts from V. J. Lotti (Merck Sharp & Dohme). Na^{125}I was from NEN.

The 78-kDa GBP was partially purified from detergent extracts of porcine gastric mucosal membranes (24, 28) and crosslinked to ^{125}I-[Nle^{15}]$gastrin_{2,17}$ with disuccinimidyl-suberate (24). The following protease inhibitors were included in all buffers to prevent proteolysis: pepstatin, 1 μM; benzamidine, 1 mM; hexamethylphosphoramide, 0.1% (wt/vol); aprotinin, 500 units/ml. The products of the cross-linking reaction were separated by polyacrylamide gel electrophoresis, and radioactivity associated with the 78-kDa GBP was detected and quantitated with a PhosphorImager (Molecular Dynamics). Initial estimates of IC_{50} values, and of the levels of ^{125}I-$gastrin_{2,17}$ in the absence of competitor, were obtained by fitting the data with the program EBDA (25) and were refined with the program LIGAND (26).

RESULTS

Inhibition by Fatty Acid Derivatives. A substrate for enoyl-CoA hydratase, crotonyl-CoA, and a product of 3-hydroxyacyl-CoA dehydrogenase, acetoacetyl-CoA, both inhibit cross-linking of ^{125}I-$gastrin_{2,17}$ to the GBP (Fig. 1). The mean IC_{50} values from three separate experiments are 45 ± 26 μM for crotonyl-CoA and 180 ± 150 μM for acetoacetyl-CoA. No inhibition is observed with NADH, the cofactor of the dehydrogenase reaction, at concentrations as high as 1.5 mM.

Definition of the Gastrin-Binding Site. $Gastrin_{17}$ and its derivatives pentagastrin and $gastrin_4$ all inhibit cross-linking of ^{125}I-$gastrin_{2,17}$ to the 78-kDa GBP (Fig. 2*A*). The IC_{50}

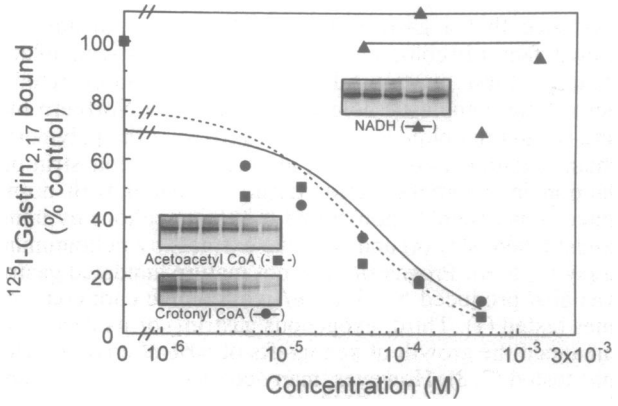

FIG. 1. Binding of fatty acyl-CoA derivatives to the 78-kDa GBP. Cross-linking of ^{125}I-[Nle^{15}]$gastrin_{2,17}$ to the 78-kDa GBP was measured by PhosphorImager (*Insets*) in the presence of increasing concentrations of crotonyl-CoA, a substrate of enoyl-CoA hydratase, or of acetoacetyl-CoA, a product of 3-hydroxyacyl-CoA dehydrogenase, and expressed as a percentage of the value obtained in the absence of competitor. Lines of best fit for inhibition by acetoacetyl-CoA and crotonyl-CoA were obtained with the program LIGAND (26). Values for IC_{50} and for the predicted ordinate intercept were as follows: crotonyl-CoA, 43 μM, 76%; acetoacetyl-CoA, 74 μM, 70%. No inhibition was observed with NADH, a cofactor for 3-hydroxyacyl-CoA dehydrogenase.

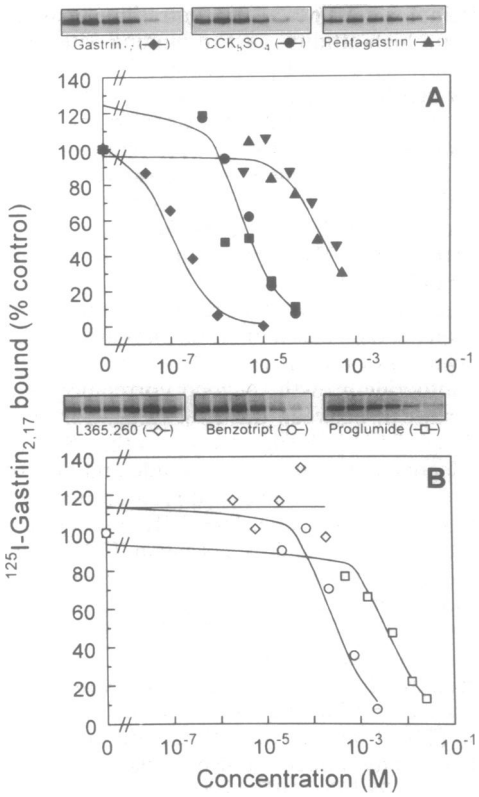

FIG. 2. Gastrin analogues and antagonists inhibit covalent cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP. Cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP in the presence of increasing concentrations of gastrin analogues (*A*, closed symbols) and gastrin/CCK receptor antagonists (*B*, open symbols) was measured by PhosphorImager (*Insets*) and expressed as a percentage of the value obtained in the absence of competitor. The following values for IC_{50} and for the predicted ordinate intercept were obtained with the program LIGAND (26): gastrin$_{17}$, 0.11 μM, 102%; CCK_8 (■), 5.6 μM, 98%; CCK_8SO_4, 3.5 μM, 125%; pentagastrin, 200 μM, 96%; gastrin$_4$ (▼), 320 μM, 98%; proglumide, 4.1 mM, 94%; benzotript, 250 μM, 114%. Lines of best fit are not shown for CCK_8 or gastrin$_4$ for clarity. No inhibition was observed with the CCK-A receptor-selective antagonist L364,718 (data not shown) or with the gastrin/CCK-B receptor-selective antagonist L365,260.

values, which are dependent on chain length, are in good agreement with the IC_{50} values reported previously for inhibition of binding of ^{125}I-gastrin$_{17}$ to the gastrin/CCK-C receptor on the HCT 116 colon carcinoma cell line (Table 1). In contrast, the IC_{50} values for the 78-kDa GBP differ markedly from the IC_{50} values for the CCK-A and gastrin/CCK-B receptors (Table 1). The similarity between the IC_{50} values for the 78-kDa GBP and the IC_{50} values for the gastrin/CCK-C receptor, and the dissimilarity between the IC_{50} values for the 78-kDa GBP and the IC_{50} values for the gastrin/CCK-B receptor, are particularly apparent when the data are presented graphically (Fig. 3). No significant difference is observed in the IC_{50} values for CCK_8 and CCK_8SO_4 (Table 1).

Inhibition by Gastrin/CCK Receptor Antagonists. The non-selective antagonists proglumide and benzotript inhibit cross-linking of ^{125}I-gastrin$_{2,17}$ to the GBP (Fig. 2*B*), with IC_{50} values of 5.1 mM and 200 μM, respectively (Table 1). As with gastrin and its derivatives, a better correlation is observed between the IC_{50} values for the 78-kDa GBP and the IC_{50} values for inhibition of ^{125}I-gastrin$_{17}$ binding to the gastrin/CCK-C receptor than between the IC_{50} values for the 78-kDa GBP and the IC_{50} values for the gastrin/CCK-B receptor (Fig. 3). Furthermore, neither the CCK-A receptor antagonist L364,718 nor the gastrin/CCK-B receptor antagonist

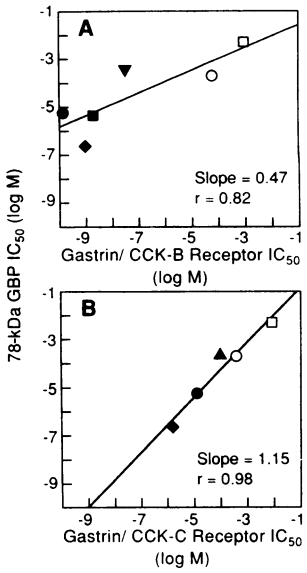

FIG. 3. Antiproliferative gastrin/CCK receptor antagonists target the 78-kDa GBP. The mean IC_{50} values (Table 1) for the inhibition of cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP by gastrin analogues (closed symbols as in Fig. 2) and antagonists (open symbols as in Fig. 2) were determined as described in the legend to Fig. 2 and compared with the IC_{50} values for inhibition of binding of iodinated (^{125}I) Bolton–Hunter-labeled CCK_8 to the cloned human gastrin/CCK-B receptor expressed in COS cells (analogues) (20) and of ^{125}I-CCK_{33} to the gastrin/CCK-B receptor on guinea pig gastric glands (antagonists) (18) (*A*) or inhibition of binding of ^{125}I-gastrin$_{17}$ to the gastrin/CCK-C receptor on HCT 116 colon carcinoma cells (14) (*B*). Lines of best fit were obtained by linear regression.

L365,260 has any effect on cross-linking at concentrations as high as 200 μM (Fig. 2*B*). A good correlation is also observed between the IC_{50} values for the 78-kDa GBP and the IC_{50} values for inhibition of HCT 116 and HT 29 colon carcinoma cell proliferation by proglumide and benzotript (Table 1).

DISCUSSION

The 78-kDa GBP Is a Member of the Enoyl-CoA Hydratase/3-Hydroxyacyl-CoA Dehydrogenase Family. The sequence translated from overlapping cDNA clones (29) isolated with oligonucleotides based on the amino acid sequences from the 78-kDa GBP (28) revealed a distant relationship with the family of fatty acid oxidation enzymes possessing enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities (30). I now report that crotonyl-CoA, a substrate of the enoyl-CoA hydratase reaction, and acetoacetyl-CoA, a product of the 3-hydroxyacyl-CoA dehydrogenase reaction, both inhibit cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP (Fig. 1), with IC_{50} values similar to the K_d values reported for the peroxisomal bifunctional enzyme (32). Since the putative substrate and product of the enzyme encoded by the cloned cDNA both inhibit binding of gastrin to the GBP, I conclude that the cloned cDNA does indeed encode the 78-kDa GBP.

So far, it has not been possible to demonstrate enoyl-CoA hydratase or 3-ketoacyl-CoA hydrogenase activities with crotonyl-CoA and acetoacetyl-CoA, respectively, as substrates in preparations of the 78-kDa GBP from porcine gastric mucosal membranes (data not shown). Perhaps the GBP, like the mitochondrial trifunctional enzyme (33), is only active with longer-chain acyl-CoAs. The failure to demonstrate either activity in GBP preparations does not appear to be caused by prevention of substrate binding by partial denaturation due to the detergent required for solubilization, since both crotonyl-CoA and acetoacetyl-CoA inhibit gastrin

cross-linking in the presence of 0.5% Triton X-100 (Fig. 1). Because acetoacetyl-CoA is a competitive inhibitor of the enoyl-CoA hydratase activity of the peroxisomal bifunctional enzyme (32) as well as a product of the 3-hydroxyacyl-CoA dehydrogenase reaction, further work will be required to establish whether gastrin binds to one or both of the enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase active sites.

Affinity of the 78-kDa GBP for Gastrin and Gastrin Analogues. The IC_{50} values determined for inhibition of cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP by gastrin$_{17}$, CCK_8, and their analogues are dependent on chain length (Fig. 2*A*, Table 1). As with the gastrin/CCK-B receptor, binding is still observed with the tetrapeptide Trp-Met-Asp-Phe amide. The similarity of the IC_{50} values for inhibition by CCK_8 and CCK_8SO_4 (Table 1) confirms that the tyrosine residue that is sulfated in CCK_8SO_4 and that is seven residues from the C terminus of CCK does not form part of the binding determinant.

The evidence available from radioimmunoassay of tissue and colon carcinoma cell line extracts suggests that colorectal tumors overproduce progastrin but are unable to process it to amidated gastrin$_{17}$ (1, 5, 6). Any receptor for autocrine gastrin should therefore not require an amidated gastrin C terminus for binding. The 78-kDa GBP meets this requirement since the IC_{50} value (0.19 μM) for inhibition of cross-linking by glycine-extended gastrin$_{17}$ (the last processing intermediate before amidation) is not significantly different from the IC_{50} value for amidated gastrin$_{17}$ (0.23 μM) (unpublished data). Additional experiments will be required to determine whether further extension of the C or N terminus of gastrin$_{17}$ results in tighter binding to the 78-kDa GBP.

Identity of the 78-kDa GBP and the Gastrin/CCK-C Receptor. The IC_{50} values for gastrin analogues in the cross-linking assay agree well with the values previously reported for the gastrin/CCK-C receptor on gastrointestinal cell lines (9, 14). Thus the slope of the line in the logarithm–logarithm plot (Fig. 3*B*) is 1.15, a value that compares favorably with the value of 1 expected for identical receptors. In contrast, the IC_{50} values for gastrin analogues in the cross-linking assay differ markedly from the values (Table 1) reported for binding to the CCK-A (data not shown) and gastrin/CCK-B receptors [slope = 0.47 on a logarithm–logarithm plot (Fig. 3*A*)]. Similarly, for the antagonists proglumide and benzotript a better correlation is observed for the gastrin/CCK-C receptor (Fig. 3*B*) than for either the CCK-A receptor (Table 1) or the gastrin/CCK-B receptor (Fig. 3*A*). I therefore conclude that the 78-kDa GBP is the gastrin/CCK-C receptor.

Nevertheless, the correlation coefficient (0.82) for the comparison of the IC_{50} values for the 78-kDa GBP and the gastrin/CCK-B receptor is reasonable (Fig. 3*A*). In fact, the slope of 0.47 is close to the value predicted (0.5) for a dimeric gastrin/CCK-B receptor containing two molecules of the 78-kDa GBP and one or two molecules of the recently cloned cDNA belonging to the serpentine receptor family (19–21). In this context it is worthwhile noting that binding to the gastrin/CCK-B receptor has generally been measured in COS or Chinese hamster ovary (CHO) cells expressing the cloned serpentine receptor cDNAs (19–21) and that CHO and COS cells express an intrinsic 78-kDa GBP (T. Mantamadiotis and G.S.B., unpublished data). Other evidence supporting the presence of multiple subunits in CCK-A and gastrin/CCK-B receptors has been reviewed previously (34).

Role of Gastrin/CCK Receptors in Autocrine Growth. The available evidence suggests that CCK-A and gastrin/CCK-B receptors are not involved in the autocrine growth of all colon carcinoma cell lines, even though both receptors can transmit a mitogenic signal in other cell types (21, 35). Thus the CCK-A receptor- and gastrin/CCK-B receptor-selective antagonists L364,718 and L365,260 do not inhibit the growth of colon carcinoma cell lines at concentrations up to 1 μM (22). In addition, high-affinity gastrin-binding sites have been described in only a small proportion of gastric and colonic carcinoma cell lines [2/2 (36); 1/10 (37)]. The presence of high-affinity gastrin-binding sites on primary human colon cancer tumors is still controversial, with an early report claiming 57% of 67 specimens were positive (38), and a later report finding no positives in 9 specimens (39). Gastrin/CCK-B receptor mRNA has been detected in only 14% of 57 colorectal tumors by RNase protection assays (A. Imdahl and G.S.B., unpublished data).

The effects of gastrin/CCK receptor antagonists on the proliferation of colon carcinoma cell lines are consistent with the involvement of the gastrin/CCK-C receptor (the 78-kDa GBP) in autocrine growth. A good correlation is observed (data not shown) between the IC_{50} values for inhibition of cross-linking of ^{125}I-[Nle^{15}]gastrin$_{2,17}$ to the 78-kDa GBP by the nonselective antagonists proglumide and benzotript and the IC_{50} values for inhibition of cell proliferation by the same antagonists (14, 23) (Table 1). The CCK-A receptor-selective antagonist L364,718 and the gastrin/CCK-B receptor-selective antagonist L365,260 previously have been reported to have no effect on colon carcinoma cell proliferation at concentrations as high as 1 μM (22). Furthermore, the inhibition observed at higher concentrations of L364,718 was not reversed by addition of gastrin$_{17}$ or CCK_8SO_4 and, hence, was not caused by binding to cell surface gastrin or CCK receptors (22). I now report that neither L364,718 nor L365,260 has any effect on cross-linking of ^{125}I-[Nle^{15}]-gastrin$_{2,17}$ to the 78-kDa GBP at concentrations up to 200 μM. The failure of the CCK-A receptor- and gastrin/CCK-B receptor-selective antagonists L364,718 and L365,260 to inhibit GBP cross-linking or cell growth at concentrations up to 1 μM, and the good correlation between the IC_{50} values for inhibition of GBP cross-linking and of cell growth by the nonselective antagonists proglumide and benzotript, are consistent with the conclusion that inhibition of cell growth by gastrin/CCK receptor antagonists is mediated by the gastrin/CCK-C receptor.

As well as implicating the gastrin/CCK-C receptor in the autocrine growth of colon carcinoma cell lines, the current observations suggest a possible mechanism for the inhibitory effects of gastrin/CCK receptor antagonists on cell proliferation. Competition between antagonists and gastrin, and between acyl-CoA derivatives and gastrin, in the GBP cross-linking assay implies that antagonists will compete for substrate binding to the 78-kDa GBP. If the GBP possesses enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities with longer-chain acyl-CoA derivatives as substrates, as suggested by the binding of short-chain acyl-CoA derivatives (Fig. 1) and by sequence comparisons with other members of the hydratase/dehydrogenase family (30), then presumably antagonist inhibition of fatty acid metabolism will block cell growth, because of a reduction either in energy supply or in the availability of essential lipids. Although the competition observed between gastrin and acyl-CoA derivatives suggests that gastrin might also inhibit one or both activities, gastrin is able to reverse the inhibition of HCT 116 cell proliferation by proglumide (14), implying that gastrin itself does not block any essential function of the 78-kDa GBP. Direct measurements of the effect of gastrin on the enzyme activities of the purified 78-kDa GBP are required to resolve this inconsistency.

Is the 78-kDa GBP a component of an extracellular autocrine loop involving gastrin? The inhibition of HCT 116 cell proliferation by proglumide and benzotript, and the reversal of inhibition by concentrations of gastrin in the millimolar range (14), are consistent with this conclusion. However, no such reversal of proglumide inhibition by gastrin is observed with LIM 1215 or LIM 2412 cells (40), although the observed

competition between antagonists and gastrin for the 78-kDa GBP implies that reversal should be observed if the GBP is accessible to exogenous gastrin. One possible explanation for this discrepancy is that the 78-kDa GBP, the mRNA for which is expressed in all colon carcinoma cell lines tested so far (data not shown), may appear on the cell surface in only a subset of colon carcinoma cell lines. The observation that only HCT 116 and LoVo cells from five colon carcinoma cell lines tested secreted progastrin (1) is also consistent with the hypothesis that only a subset of cell lines utilizes gastrin in an extracellular autocrine loop. Generation of a panel of antibodies completely blocking the gastrin-binding site of the 78-kDa GBP should define the role of cell surface GBP in autocrine growth.

Anti-GBP antibodies would also define more precisely the subcellular localization of the 78-kDa GBP. Cross-linking of ^{125}I-gastrin$_{2,17}$ to detergent extracts of subcellular membrane fractions prepared from porcine gastric mucosa by differential centrifugation reveals the 78-kDa GBP in the microsomal fraction (data not shown). This observation is consistent with previous localization of the 78-kDa GBP to the exterior surface of the plasma membrane by binding of ^{125}I-gastrin$_{17}$ to intact gastrointestinal cell lines (9, 14) and by reversal of proglumide inhibition of HCT 116 cells by exogenous gastrin$_{17}$ (14). However the possibility still exists that some of the 78-kDa GBP is also located on internal membranes in some colon carcinoma cells and that this intracellular GBP is the target of intracellular gastrin in LIM 1215 and LIM 2412 cells (40). The hydrophobicity of the majority of gastrin/CCK receptor antagonists should permit ready access to intracellular targets.

The demonstration that the 78-kDa GBP is a target for the antiproliferative effects of gastrin/CCK receptor antagonists has revealed an unexpected mechanism for inhibition of cell growth. Although further work will be required to elucidate whether binding of gastrin to the 78-kDa GBP plays any role in the autocrine growth of colon carcinoma cells, covalent cross-linking of ^{125}I-gastrin$_{2,17}$ to the purified 78-kDa GBP will provide a convenient assay with which to isolate more potent and selective GBP antagonists capable of blocking cell growth.

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