Proc. Natl. Acad. Sci. USA
Vol. 77, No. 4, pp. 2079–2083, April 1980
Cell Biology

Interaction of cholecystokinin with specific membrane receptors on pancreatic acinar cells

(pancreatic secretagogues/amylase secretion/calcium transport/cyclic GMP)

R. T. JENSEN, G. F. LEMP, AND J. D. GARDNER

Digestive Diseases Branch, National Institute Arthritis, Metabolism and Digestive Disease, National Institutes of Health, Bethesda, Maryland 20205

Communicated by G. Gilbert Ashwell, January 3, 1980

ABSTRACT **We have prepared ^{125}I-labeled cholecystokinin and have examined the kinetics, stoichiometry, and chemical specificity with which the labeled peptide binds to dispersed acini from guinea pig pancreas. Binding of ^{125}I-labeled cholecystokinin was reversible, temperature-dependent, saturable, specific, and localized to the plasma membrane. Each acinar cell possessed approximately 9000 binding sites, and binding of the labeled peptide to these sites could be inhibited by cholecystokinin and structurally related peptides (e.g., gastrin and caerulein) as well as by nonpeptide competitive antagonists of the action of cholecystokinin. Binding was not inhibited by other pancreatic secretagogues such as secretin, vasoactive intestinal peptide, glucagon, physalaemin, eledoisin, kassinin, substance P, carbamoylcholine, litorin, or ranatensin or by bovine pancreatic polypeptide, atropine, neurotensin, leucine-enkephalin, methionine-enkephalin, or cyclic somatostatin. With agonists as well as antagonists there was a good correlation between occupation of cholecystokinin binding sites and changes in acinar cell function. With each of six different peptide agonists maximal stimulation of enzyme secretion occurred with 40% receptor occupation and occupation of the remaining 60% caused a progressive decrease in stimulated amylase release. Agonists, but not antagonists, accelerated the dissociation of bound ^{125}I-labeled cholecystokinin, and these findings suggest that, in pancreatic acini, radiolabeled cholecystokinin binds to at least one class of interacting binding sites whose affinities are influenced by the extent to which these sites are occupied by agonists but not the extent to which they are occupied by antagonists.**

Cholecystokinin (CCK) increases pancreatic enzyme secretion by virtue of its ability to mobilize cellular calcium (for review see refs. 1 and 2), and this calcium mobilization is accompanied by increased cellular cyclic GMP (cGMP) (3–6) and changes in the electrical properties of the acinar cell plasma membrane (7, 8). Previous attempts to examine the interaction of CCK with its cell-surface receptors have been hampered by the low specific activity of the tritium-labeled ligand (9–11). As a result, correlations between changes in binding of the labeled peptide and changes in cell function have been poor (10, 11). In the present study we have prepared an ^{125}I-labeled derivative of CCK with a specific activity sufficiently high (900–1300 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) to be used to examine the interaction of CCK and structurally related peptides with their receptors on dispersed pancreatic acini.

MATERIALS AND METHODS

Unless stated otherwise the standard incubation solution contained 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM KH_2PO_4, 1.2 mM $MgCl_2$, 11.5 mM glucose, 5 mM sodium fumarate, 5 mM sodium glutamate, 5 mM sodium pyruvate, 0.5 mM $CaCl_2$, 2 mM glutamine, 1% (wt/vol) albumin, 0.01% trypsin inhibitor, 1% (vol/vol) amino acid mixture, 1% (vol/vol) essential vitamin mixture, and 5 mM theophylline.

^{125}I-Labeled natural porcine CCK (^{125}I-CCK-33) was prepared by using the modification of the procedure of Bolton and Hunter (12) described by Rehfeld (13). CCK-33 (1.3 nmol) was dissolved in 4 μl of 2.5 mM acetic acid and then mixed with 20 μl of 50 mM sodium borate, pH 10.0. [^{125}I]Iodinated *N*-succinimidyl-3-(4-hydroxyphenyl)propionate (1.0 mCi; 1500 Ci/mmol; New England Nuclear) was dried under a gentle stream of N_2 and combined with the CCK-33-containing solution. The mixture was incubated at 0°C for 30 min, after which 250 μl of 0.2 M glycine in 50 mM sodium borate, pH 10.0, was added and the incubation was continued at 0°C for 5 min. At the end of the incubation, 500 μl of 6 M guanidine hydrochloride and then 250 μl of 0.1% gelatin in 0.1 M acetic acid, pH 4.5, were added to the reaction tube. The radiolabeled peptide was isolated by applying the reaction mixture to a column of Sephadex G-50 superfine (1.5 × 50 cm) that had been equilibrated with 0.1% gelatin in 0.1 M acetic acid (pH 4.5). The column was eluted with the gelatin/acetic acid solution at a flow rate of 7.5 ml/hr, and the elution profile of ^{125}I was essentially the same as that illustrated in figure 1 of ref. 13. The fractions containing ^{125}I-CCK-33 (those corresponding to fractions 69–74 in figure 1 in ref. 13) were combined. The specific activities of the various preparations of ^{125}I-CCK-33 used for the present studies were 900–1300 Ci/mmol and their biological activities, determined from their abilities to increase amylase release from pancreatic acini (14), were at least 75% of the ability of native CCK-33.

Dispersed acini from guinea pig pancreas were prepared by using the procedure described previously (14) and were suspended in standard incubation solution. Binding of ^{125}I-CCK-33 (15), amylase release (14), outflux of ^{45}Ca (16), and cellular cGMP (17) were measured with the procedures described in detail previously.

RESULTS

Binding of ^{125}I-CCK-33 to dispersed pancreatic acini at 37°C was maximal by 45 min and did not change significantly from 45 to 90 min (Fig. 1 *Upper*). Reducing the incubation temperature from 37°C to 4°C decreased binding of ^{125}I-CCK-33 to approximately 50%. At 37°C, adding 0.1 μM CCK-8 decreased binding to 8% and higher concentrations of CCK-8

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CCK, cholecystokinin; CCK-33, natural porcine CCK (sulfated at Tyr-27); CCK-8, synthetic COOH-terminal octapeptide of CCK-33; CCK-7, synthetic COOH-terminal heptapeptide of CCK-33; des(SO_3H)-CCK-7, synthetic unsulfated COOH-terminal heptapeptide of CCK-33; Tyr-Hnl(SO_3H)-CCK-6, *N*-*t*-butyloxycarbonyl-tyrosyl-hydroxynorleucine sulfate-L-methionyl-glycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide; cGMP, guanosine 3′,-5′-monophosphate; Bt_2cGMP, N^2,$O^{2'}$-dibutyryl cGMP; $O^{2'}$BtcGMP, $O^{2'}$-monobutyryl cGMP; N^2BtcGMP, N^2-monobutyryl cGMP; $EC_{1/2}$, concentration for half-maximal effect.

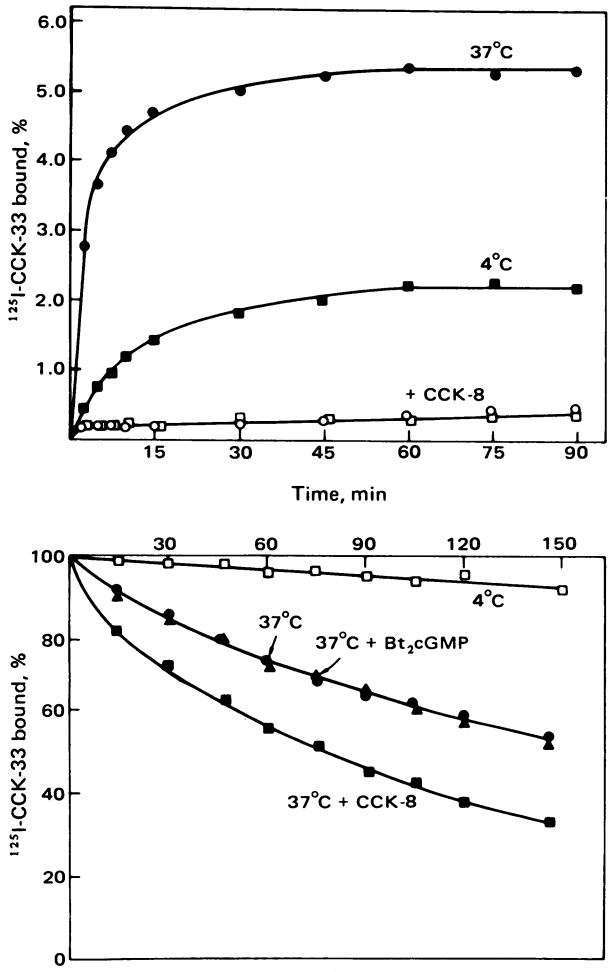

FIG. 1. Time course for binding of ^{125}I-CCK-33 to pancreatic acini. (*Upper*) Acini were incubated with 50 pM ^{125}I-CCK-33 at 37°C (●, ○) or 4°C (■, □) with or without 0.1 μM CCK-8. Binding is expressed as the percentage of the added radioactivity. In each experiment each value was determined in triplicate and this experiment is representative of four others. (*Lower*) Acini were incubated with 50 pM ^{125}I-CCK-33 for 30 min at 37°C, washed to remove free radioactivity, resuspended in fresh incubation solution, and reincubated under the conditions specified. When present, CCK-8 was 0.1 μM and N^2,$O^{2'}$-dibutyryl cGMP (Bt_2cGMP) was 1 mM. Values are expressed as a percent of the saturable binding at the beginning of the second incubation. In each experiment each value was determined in triplicate and results given are means from five separate experiments.

caused no further decrease. In terms of its ability to inhibit binding of ^{125}I-CCK-33 (Fig. 2) and to produce functional changes (Fig. 3; ref. 18), CCK-8 is equal in efficacy to CCK-33 but is 10 times more potent. Because CCK-8 is more potent than CCK-33 and because our supplies of CCK-33 were limited, CCK-8 (0.1 μM) was used to measure nonsaturable binding of ^{125}I-CCK-33. Unless specified otherwise, all subsequent values for binding of ^{125}I-CCK-33 are for "saturable binding," i.e., binding measured with radiolabeled CCK-33 alone *minus* binding measured with 0.1 μM CCK-8.

To examine the possibility that the association of radioactivity with pancreatic acini reflects uptake of the tracer into the cytoplasm, acini were incubated with ^{125}I-CCK-33 for 30 min at 37°C, washed to remove free radioactivity, lysed with at least 100 vol of iced distilled water, and centrifuged at 100 × *g* for 5 min. Under these conditions, which have been shown to sediment plasma membranes from pancreatic acinar cells (19), at least 94% of the total radioactivity was associated with the sediment.

To examine the reversibility of the binding reaction, acini were incubated with ^{125}I-CCK-33 for 30 min at 37°C, washed to remove free radioactivity, and resuspended in fresh incubation solution. At 37°C, 47% of the bound radioactivity dissociated by 150 min (Fig. 1 *Lower*). Reducing the incubation temperature from 37°C to 4°C reduced the rate of dissociation to 6% in 150 min. Adding 0.1 μM CCK-8 increased the rate of dissociation of bound ^{125}I-CCK-33 to 67% in 150 min, whereas adding Bt_2cGMP, a competitive antagonist of the action of CCK (20), did not alter the dissociation rate.

Approximately 10 min were required to process the acini for determination of bound ^{125}I-CCK-33 by centrifugation and resuspension. To examine the possibility that a significant amount of bound radioactivity dissociated during the time required to process the samples, binding was measured by centrifuging the acini through a layer of silicone oil (processing time, 40 sec) or by collecting and washing the acini on a filter by suction filtration (processing time, 30 sec). Values for saturable binding of ^{125}I-CCK-33 obtained by using silicone oil or filtration were not significantly different from values obtained by repeated centrifugation and resuspension; therefore, any dissociation of bound ^{125}I-CCK-33 during the time required to process the samples must have been complete within 30 sec.

Various agents were tested for their abilities to inhibit binding of ^{125}I-CCK-33 to pancreatic acini. Secretin, vasoactive intestinal peptide, glucagon, physalaemin, eledoisin, kassinin, substance P, bovine pancreatic polypeptide, methionine-enkephalin, leucine-enkephalin, neurotensin, cyclic somatostatin, carbamoylcholine, atropine, bombesin, litorin, or ranatensin did not inhibit binding of ^{125}I-CCK-33 (not shown).

To examine the relationship between the ability of a peptide to bind to pancreatic acini and its ability to alter acinar cell function, we tested CCK-33 and six structurally related, COOH-terminal fragments or analogs of CCK-33 for their abilities to inhibit binding of ^{125}I-CCK-33 (Fig. 2) and their abilities to stimulate amylase secretion (Fig. 3) under identical incubation conditions. The curves describing the inhibition of binding of ^{125}I-CCK-33 caused by CCK-33 or one of the analogs were parallel and were relatively broad in that for each peptide, the maximally effective concentration was at least 1000 times greater than the threshhold concentration (Fig. 2). In terms of

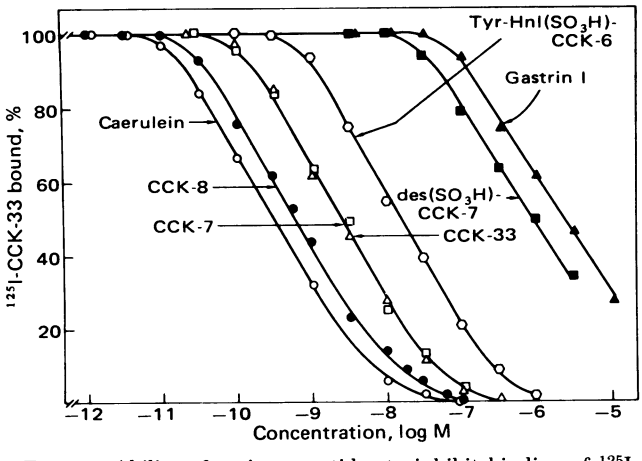

FIG. 2. Ability of various peptides to inhibit binding of ^{125}I-CCK-33 to pancreatic acini. Acini were incubated for 30 min at 37°C with 50 pM ^{125}I-CCK-33 plus the peptides indicated. Binding is expressed as the percentage of radioactivity that was saturably bound in the absence of added nonradioactive peptides. In each experiment each value was determined in triplicate and results given are means from eight separate experiments.

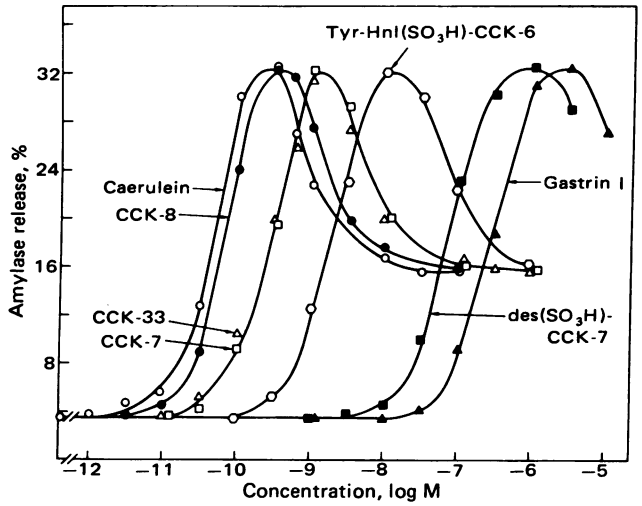

FIG. 3. Ability of various peptides to stimulate amylase release from pancreatic acini. Acini were incubated for 30 min at 37°C with the peptides indicated. Values are expressed as the percentage of the amylase activity in the cells at the beginning of the incubation that was released into the extracellular medium during the incubation. In each experiment each value was determined in duplicate and results given are means from nine separate experiments.

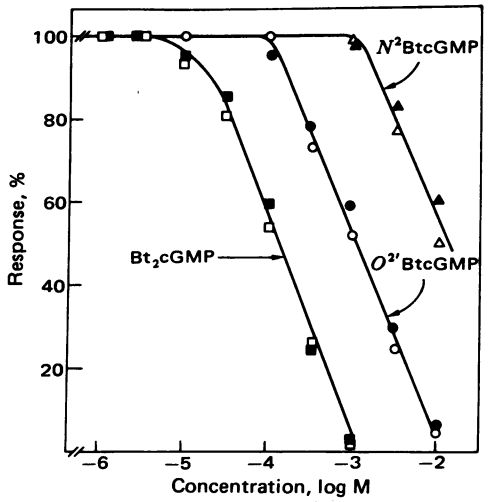

FIG. 4. Effect of butyryl derivatives of cGMP on the increase in amylase release caused by CCK-33 and on binding of ^{125}I-CCK-33. To measure amylase release, acini were incubated for 30 min at 37°C with or without 1 nM CCK-33 plus the indicated cyclic nucleotide derivative. Values for amylase release (closed symbols) were calculated as a percentage of the increase in amylase release caused by CCK-33 without cyclic nucleotide. To measure binding of ^{125}I-CCK-33, acini were incubated with 50 pM ^{125}I-CCK-33, 1 nM CCK-33, and the indicated cyclic nucleotide derivative. Values for binding (open symbols) were calculated as a percentage of radioactivity that was saturably bound in the absence of added cyclic nucleotides. In each experiment each value was determined in duplicate and results given are means from six separate experiments.

their abilities to inhibit binding of ^{125}I-CCK-33, the relative potencies of the peptides tested were caerulein (concentration for half-maximal effect, $EC_{1/2}$, 0.3 nM) > CCK_8 ($EC_{1/2}$, 0.6 nM) > CCK-7 = CCK-33 ($EC_{1/2}$, 2 nM) > Tyr-Hnl(SO_3H)-CCK-6 ($EC_{1/2}$, 17 nM) > des(SO_3H)CCK-7 ($EC_{1/2}$, 1 μM) > gastrin I ($EC_{1/2}$, 2 μM) (Fig. 2). The curves describing the stimulation of amylase release caused by CCK-33 or one of the analogs were parallel and were similar in configuration in that, as the peptide concentration was increased, amylase release increased, became maximal (8-fold increase), and then decreased to a plateau (3-fold increase) (Fig. 3). As occurred with binding of ^{125}I-CCK-33, each peptide altered amylase release over a relatively broad range of concentrations in that the peptide concentration above which there was no further change in amylase release was at least 1000 times greater than the threshhold peptide concentration. In terms of the maximal stimulation of amylase release, all peptides tested were equal in efficacy, and their relative potencies for stimulating amylase release were the same as their relative potencies for inhibiting binding of ^{125}I-CCK-33 (compare Fig. 3 with Fig. 2). For CCK-33 as well as for each of the analogs, maximal stimulation of amylase release occurred when inhibition of binding of ^{125}I-CCK-33 was approximately half-maximal (compare Figs. 2 and 3).

Previously (20) we have shown that butyryl derivatives of cyclic GMP are specific competitive antagonists of the action of CCK on pancreatic acinar cells. In the present study, with each nucleotide tested, there was a close correlation between its ability to inhibit binding of 1 nM ^{125}I-CCK-33 and its ability to inhibit the increase in amylase release caused by 1 nM CCK-33 (Fig. 4). The relative potencies of the cyclic nucleotides were Bt_2cGMP ($EC_{1/2}$, 0.1 mM) > $O^{2'}$BtcGMP ($EC_{1/2}$, 1 mM) > N^2BtcGMP ($EC_{1/2}$, 10 mM). The dose–response curves for the actions of butyryl cyclic nucleotides on binding of ^{125}I-CCK-33 and on CCK-33-stimulated amylase release spanned a narrower range of concentrations than did the dose–response curves for the actions of peptide agonists on binding and on amylase release (compare Fig. 4 with Figs. 2 and 3). That is, with each cyclic nucleotide tested, the maximally effective concentration was approximately 100 times greater than the threshhold concentration.

In addition to being able to inhibit binding of ^{125}I-CCK-33 (Fig. 2), CCK-33 and structurally related peptides were able to accelerate the dissociation of bound ^{125}I-CCK-33 [i.e., to "displace" bound radioactivity (Fig. 1 *Lower* and Fig. 5)]. The dose–response curves for the actions of CCK-33, CCK-8, and Tyr-Hnl(SO_3H)-CCK-6 on the dissociation of bound ^{125}I-CCK-33 were parallel and relatively broad in that for a given peptide the maximally effective concentration was at least 1000 times greater than the threshhold concentration (Fig. 5). For the three peptides tested, their relative potencies for displacing bound ^{125}I-CCK-33 were the same as their relative potencies

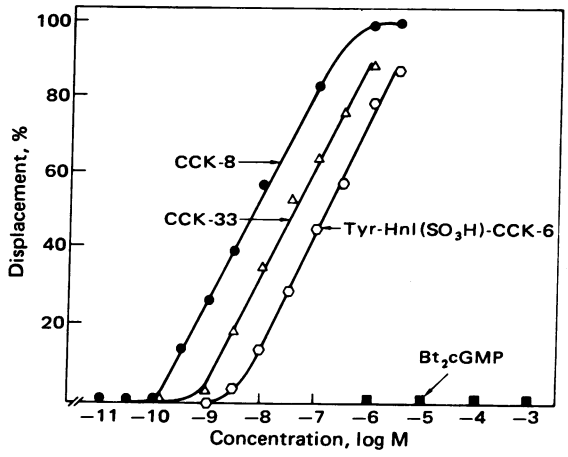

FIG. 5. Ability of various peptides to increase the dissociation of ^{125}I-CCK-33 bound to pancreatic acini. Acini were incubated for 30 min at 37°C with 50 pM ^{125}I-CCK-33, washed to remove free radioactivity, resuspended in fresh incubation solution, and reincubated for 90 min at 37°C with the peptides indicated. Displacement was calculated as a percentage of the increase in the dissociation of saturably bound ^{125}I-CCK-33 caused by 1 μM CCK-8. In each experiment each value was determined in triplicate and results given are means from four separate experiments.

for inhibiting binding of the tracer (compare Fig. 5 with Fig. 2). For a given peptide, however, the concentration required to produce half-maximal displacement of bound ^{125}I-CCK-33 was approximately 10 times greater than the concentration required to produce half-maximal inhibition of binding of ^{125}I-CCK-33. Bt_2cGMP, at concentrations as high as 1 mM did not displace bound ^{125}I-CCK-33 (Fig. 1 *Lower* and Fig. 5), and this finding indicates that the peptide-induced displacement illustrated in Figures 1 and 5 is not an artifact attributable to the ability of the peptide to inhibit rebinding of the dissociated radioactivity.

As one would anticipate from the broad dose–response curves for the action of CCK-33 and related peptides on binding of ^{125}I-CCK-33 (Fig. 2) and from the abilities of these peptides to cause displacement of bound ^{125}I-CCK-33 (Fig. 5), a Scatchard plot (21) of the ability of CCK-33 to inhibit binding of ^{125}I-CCK-33 was curvilinear with an upward concavity (Fig. 6). This curvilinearity can be accounted for, at least in part, by the ability of CCK-33 to displace bound ^{125}I-CCK-33 (22); however, in addition, pancreatic acini may possess two (or more) classes of binding sites whose numbers and affinities are such that they cannot be distinguished clearly on the basis of stoichiometric studies (22). By extrapolating the curve in Figure 6 to the abscissa (i.e., the concentrations of bound CCK-33 when bound/free equals zero) and assuming that the pancreas from one guinea pig has 1.5×10^8 acinar cells (4, 15), we calculated that each cell has at least 9000 binding sites that interact with CCK-33 and structurally related peptides.

Because, in addition to stimulating amylase release from pancreatic acini, CCK also increases cellular cGMP and outflux of ^{45}Ca (2), we compared the ability of CCK-8 to alter these three functions with its ability to inhibit binding of ^{125}I-CCK-33 (Fig. 7). CCK-8 caused a 5-fold increase in outflux of ^{45}Ca and a 17-fold increase in cGMP, and the dose–response curves for the action of CCK-8 on these two functions were identical (Fig. 6). As has been reported previously (4), supramaximal concentrations of CCK-8 did not cause submaximal stimulation of cGMP or calcium outflux (Fig. 7). Maximal stimulation of amylase release occurred with a concentration of CCK-8 (0.3 nM) that inhibited binding of ^{125}I-CCK-33 by 40%, whereas maximal stimulation of calcium outflux and cGMP occurred with a concentration of CCK-8 (3 nM) that inhibited binding of ^{125}I-CCK-33 by 77%.

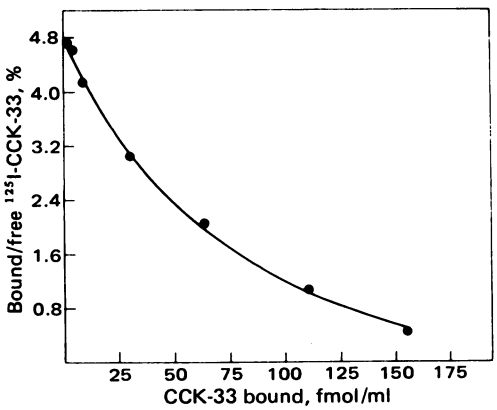

FIG. 6. Scatchard plot of binding of ^{125}I-CCK-33 to pancreatic acini. Acini were incubated for 30 min at 37°C with 50 pM ^{125}I-CCK-33 plus different concentrations of CCK-33. The units on the ordinate are for saturable binding of ^{125}I-CCK-33 expressed as a percentage of free ^{125}I-CCK-33. The units on the abscissa are for the concentration of saturably bound ^{125}I-CCK-33. In each experiment each value was determined in duplicate and results given are means of eight separate experiments.

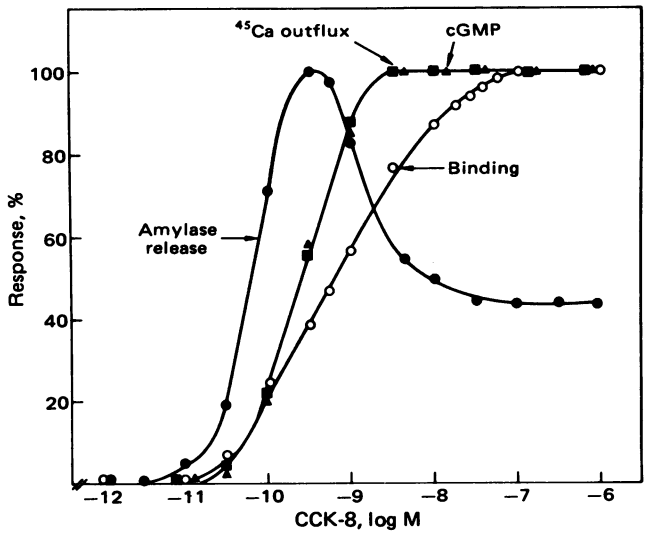

FIG. 7. Effect of CCK-8 on binding of ^{125}I-CCK-33, outflux of ^{45}Ca, accumulation of cGMP, and release of amylase. Acini were incubated for 30 min at 37°C to measure amylase release, cGMP, and binding of ^{125}I-CCK-33 and for 5 min at 37°C to measure outflux of ^{45}Ca. Results are expressed as the percentage of the response obtained with a maximally effective concentration of CCK-8 (i.e., 0.3 nM for amylase release and 0.1 μM for cyclic GMP) ^{45}Ca outflux, and inhibition of binding of ^{125}I-CCK-33. In each experiment each value was determined in duplicate and results given are means of at least five separate experiments.

DISCUSSION

The present results demonstrate that, in dispersed acini from guinea pig pancreas, binding of ^{125}I-CCK-33 is reversible, temperature-dependent, saturable, specific, and localized to the plasma membrane. The sites to which ^{125}I-CCK-33 binds also interact with pancreatic secretagogues that are structurally related to CCK (e.g., caerulein, gastrin, CCK-8, and CCK-7) and with competitive antagonists of the action of CCK (i.e., butyryl derivatives of cyclic GMP) but do not interact with pancreatic secretagogues that are structurally unrelated to CCK (e.g., carbachol, vasoactive intestinal peptide, secretin, bombesin, and substance P).

Several findings indicate that the sites on pancreatic acini that bind ^{125}I-CCK-33 are the receptors with which CCK and structurally related peptides interact to produce changes in acinar cell function. (*i*) There is a close correlation between the relative potencies with which seven different CCK-related peptides inhibit binding of ^{125}I-CCK-33 and the relative potencies with which these peptides increase amylase secretion. (*ii*) With three different competitive antagonists of the action of CCK, there is a close correlation between the ability of each antagonist to inhibit binding of ^{125}I-CCK-33 and its ability to inhibit CCK-stimulated amylase release. (*iii*) Although the dose–response curve for the action of CCK on amylase release is broad and has a biphasic contour, with each of seven different CCK-related peptides the range of concentrations over which a particular peptide inhibits binding of ^{125}I-CCK-33 is the same as that over which the peptide produces changes in amylase release. The present studies also illustrate the complex relationship that can exist between receptor occupation and changes in target cell function. For example, occupation of up to 40% of the receptors for CCK and related peptides causes progressive stimulation of amylase release and occupation of the remaining 60% causes a progressive reduction in stimulated amylase release.

Previous attempts to measure binding of CCK and related peptides to pancreatic acinar cells employed [^{3}H]caerulein with

a specific activity of 13 Ci/mmol and were unable to detect changes in binding at concentrations of peptides that clearly produced relevant changes in the functions of the target tissue (10, 11). For example, the *lowest* concentration of caerulein that produced a detectable change in binding, 2 nM, was at least 100 times *greater* than the concentration of caerulein that produced detectable stimulation of amylase release and was 20 times *greater* than that which produced detectable stimulation of calcium outflux (10, 11). In contrast, the present studies have been performed with a radiolabeled peptide with a specific activity that is approximately 100 times greater than that of [^{3}H]caerulein and show a good correlation between the concentrations at which agonists as well as antagonists inhibit binding and the concentrations at which they produce changes in cell function.

The present studies indicate that ^{125}I-CCK-33 binds to sites on pancreatic acini that mediate the biologic effects of CCK and structurally related peptides. These agonists are able to displace bound ^{125}I-CCK-33, and their dose–response curves for inhibition of binding of ^{125}I-CCK-33 are broad and give rise to curvilinear Scatchard plots. Cyclic nucleotide antagonists do not cause displacement of bound ^{125}I-CCK-33 and their dose–response curves for inhibition of binding of ^{125}I-CCK-33 are narrow. These results are compatible with the hypothesis that, in pancreatic acini, ^{125}I-CCK-33 binds to at least one class of interacting binding sites whose affinities are influenced by the extent to which these sites are occupied by agonists but not by the extent to which they are occupied by antagonists.

We thank Mary Ernst for typing the manuscript and Drs. Viktor Mutt, Miklos Bodanszky, Roberto de Castiglione, Miguel Ondetti, and T. M. Lin for generously donating some of the peptides used in these studies.

1. Jorpes, J. E. & Mutt, V. (1973) in *Secretin, Cholecystokinin, Pancreozymin and Gastrin*, eds. Jorpes, J. E. & Mutt, V. (Springer, New York), pp. 1–177.
2. Gardner, J. D. (1979) *Annu. Rev. Physiol.* **41**, 55–66.
3. Albano, J., Bhoola, K. D. & Harvey, R. F. (1976) *Nature (London)* **262**, 404–406.
4. Christophe, J. P., Frandsen, E. K., Conlon, T. P., Krishna, G. & Gardner, J. D. (1976) *J. Biol. Chem.* **251**, 4640–4645.
5. Haymovits, A. & Scheele, G. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 156–160.
6. May, R. J., Conlon, T. P., Erspamer, V. & Gardner, J. D. (1978) *Am. J. Physiol.* **235**, E112–E118.
7. Petersen, O. H. & Ueda, N. (1975) *J. Physiol.* **247**, 461–471.
8. Petersen, O. H. (1976) *Physiol. Rev.* **56**, 535–577.
9. Deschodt-Lanckman, M., Robberecht, P., Camus, J. & Christophe, J. (1978) *Eur. J. Biochem.* **91**, 21–29.
10. Christophe, J., DeNeef, P., Deschodt-Lanckman, M. & Robberecht, P. (1978) *Eur. J. Biochem.* **91**, 31–38.
11. Robberecht, P., Deschodt-Lanckman, M., Morgat, J. L. & Christophe, J. (1978) *Eur. J. Biochem.* **91**, 39–48.
12. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539.
13. Rehfeld, J. H. (1978) *J. Biol. Chem.* **253**, 4016–4021.
14. Peikin, S. R., Rottman, A. J., Batzri, S. & Gardner, J. D. (1978) *Am. J. Physiol.* **235**, E743–E749.
15. Jensen, R. T., Moody, T., Pert, C., Rivier, J. E. & Gardner, J. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6139–6143.
16. Gardner, J. D. & Hahne, W. F. (1977) *Biochim. Biophys. Acta* **471**, 466–476.
17. Lopatin, R. N. & Gardner, J. D. (1978) *Biochim. Biophys. Acta* **543**, 465–475.
18. Sjodin, L. & Gardner, J. D. (1977) *Gastroenterology* **73**, 1015–1018.
19. Christophe, J. P., Conlon, T. P. & Gardner, J. D. (1976) *J. Biol. Chem.* **251**, 4629–4634.
20. Peikin, S. R., Costenbader, C. L. & Gardner, J. D. (1979) *J. Biol. Chem.* **254**, 5321–5327.
21. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
22. Boeynaems, J. M. & Dumont, J. E. (1975) *J. Cyclic Nucl. Res.* **1**, 123–142.