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Interaction of bombesin and litorin with specific membrane receptors on pancreatic acinar cells

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

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ABSTRACT We have prepared ^{125}I-labeled [Tyr^4]bombesin 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 [Tyr^4]-bombesin was saturable, temperature-dependent, and reversible and reflected interaction of the labeled peptide with a single class of binding sites on the plasma membrane of pancreatic acinar cells. Each acinar cell possessed approximately 5000 binding sites, and binding of the tracer to these sites could be inhibited by [Tyr^4]bombesin [concentration for half-maximal effect (Kd), 2 nM], bombesin (Kd, 4 nM), or litorin (Kd, 40 nM) but not by eledoisin, physalemin, somatostatin, carbachol, atropine, secretin, vasocative intestinal peptide, neurotensin, or bovine pancreatic polypeptide. At high concentrations (>0.1 μM), cholecystokinin and caerulein each caused a small (15–20%) reduction in binding of lableled [Tyr^4]bombesin. With bombesin, litorin, and [Tyr^4]bombesin, there was a close correlation between the relative potency for inhibition of binding of labeled [Tyr^4]bombesin and that for stimulation of amylase secretion. For a given peptide, however, a 10-fold higher concentration was required for half-maximal inhibition of binding than for half-maximal stimulation of amylase secretion, calcium outflux, or cyclic GMP accumulation. These results indicate that dispersed acini from guinea pig pancreas possess a single class of receptors that interact with [Tyr^4]bombesin, bombesin, and litorin and that occupation of 25% of these receptors will cause a maximal biological response.

It has been shown (1–3) that several peptides isolated from amphibian skin (caerulein, bombesin, litorin, and physalemin) as well as a peptide isolated from the salivary gland of a Mediterranean octopod (eledoisin) can alter the function of acinar cells from rat or guinea pig pancreas. Like cholecystokinin and muscarinic cholinergic agents (1–8), each of these nonmammalian peptides increased amylase secretion, cyclic GMP (cGMP) accumulation, and outflux of exchangeable cellular calcium but did not alter cellular cyclic AMP (cAMP) (1). These actions of caerulein were not unanticipated because seven of its eight COOH-terminal amino acids are identical to those of the COOH-terminal octapeptide of cholecystokinin (9). The amino acid sequence of bombesin is similar to that of litorin and the amino acid sequence of physalemin is similar to that of eledoisin; however, the structures of each of these peptides differ substantially from those of cholecystokinin and caerulein (10, 11).

To explore further the actions of bombesin and structurally similar peptides on pancreatic acinar cells, we have examined the ability of a synthetic radiolabeled analogue of bombesin [^{125}I-labeled [Tyr^4]bombesin (^{125}I-[Tyr^4]bombesin)] to bind to dispersed acini prepared from guinea pig pancreas, the abilities of various peptides to inhibit this binding, and the relationship between a peptide's ability to inhibit binding of ^{125}I-[Tyr^4]bombesin and its ability to alter acinar cell function. Our results indicate that pancreatic acinar cells possess a single class of receptors that interact reversibly with bombesin and litorin but not with other pancreatic secretagogues and that occupation of approximately 25% of these receptors will cause a maximal biologic response.

MATERIALS AND METHODS

Male guinea pigs (175–225 g) were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health. Hepes, synthetic human gastrin, and cyclic somatostatin were from Calbiochem; carbachol, atropine sulfate, neurotensin, and collagenase (type VI *Clostridium histolyticum*, 830 units/mg) were from Sigma; ^{45}Ca (12.5 mCi/mg), ^{125}I-labeled succinyl cGMP, tyrosine methyl ester, and prereacted cGMP antiserum complex were from New England Nuclear; and carrier-free Na^{125} I was from Amersham/Searle. Bombesin, litorin, caerulein, eledoisin, and physalemin (purity >90%) were gifts from Roberto de Castiglione (Farmitalia, Milan, Italy). Porcine vasoactive intestinal peptide (VIP) and secretin were gifts from Vicktor Mutt (Gastrointestinal Hormone Research Unit, Karolinska Institutet). The COOH-terminal octapeptide of porcine cholecystokinin (CCK-OP) was a gift from Miguel Ondetti (Squibb Institute for Medical Research). Bovine pancreatic polypeptide (BPP) was a gift from T. M. Lin (Lilly Research Laboratories). [Tyr^4]bombesin was synthesized by the method described (12). In terms of peptide content, this material was >75% pure. 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 Na fumarate, Na glutamate, and Na pyruvate, 2.0 mM $CaCl_2$, 2 mM glutamine, 0.2% albumin, 0.01% soybean trypsin inhibitor, 1% amino acid mixture, and 1% essential vitamin mixture.

^{125}I-[Tyr^4]Bombesin was prepared by using a modification of the procedure described by Hunter and Greenwood (13). Equimolar amounts (0.5 nmol) of [Tyr^4]bombesin, ^{125}I, and chloramine-T were incubated in a total volume of 35 μl at ambient temperature for 60 sec and then 1.5 nmol of sodium metabisulfite was added. Radiolabeled peptide was separated from free ^{125}I by using Sephadex LH-20 (0.7 × 20 cm) equilibrated and eluted with methanol/acetic acid/water, 10:2:1 (vol/vol), as described (14). Assuming complete recovery of the peptide, the specific activity of ^{125}I-[Try^4]bombesin was 150 Ci/mmol.

Dispersed acini from guinea pig pancreas were prepared by using the previously described procedure (5) and, unless spec-

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Abbreviations: cAMP, cyclic AMP; cGMP, cyclic GMP; CCK-OP, COOH-terminal octapeptide of porcine cholecystokinin; VIP, porcine vasoactive intestinal peptide; BPP, bovine pancreatic polypeptide; Kd, concentration for half-maximal effect.

ified otherwise, were suspended in standard incubation solution containing 0.5 mM calcium, 5 mM theophylline, and 1% albumin.

To measure binding of ^{125}I-[Try^4]bombesin, dispersed acini from the pancreas of one animal were suspended in 25 ml of standard incubation solution containing 0.1% bacitracin. Samples of the cell suspension were gassed with 100% O_2 and incubated with 0.2 nM ^{125}I-[Tyr^4]bombesin (approximately 40,000 cpm/ml of cell suspension). At appropriate times, 0.5-ml samples were taken and the acini were washed three times by alternate centrifugation (10,000 × *g* for 15 sec in a Beckman model 153 Microfuge) and resuspension in standard incubation solution containing no radioactivity. Radioactivity associated with the acini was determined by using a Packard Auto-Gamma crystal scintillation detector. At some time during the incubation, an aliquot of the incubation mixture (i.e., acini plus incubation medium) was taken for determination of radioactivity and this value was used to calculate binding of ^{125}I-[Tyr^4]-bombesin as the percentage of the radioactivity present in the incubation medium. In each experiment, each value was determined in triplicate and the coefficient of variation (SD/mean) was always less than 6%.

Release of amylase from pancreatic acini was determined as described (4). Acini from the pancreas of one animal were suspended in 200 ml of incubation solution; 1.0 ml of this cell suspension was gassed with 100% O_2 and incubated with the appropriate agents for 60 min at 37°C. Amylase release was measured as the percentage of total amylase released into the incubation medium during the 60-min incubation. In each experiment, each value was determined in triplicate and the coefficient of variation for triplicate samples was always <10%.

Outflux of ^{45}Ca from dispersed pancreatic acini was measured as described (15). Acini from the pancreas of one animal were suspended in 40 ml of incubation solution, gassed with 100% O_2, and loaded with ^{45}Ca (1 μCi/ml) during a 90-min preincubation at 37°C. Outflux of ^{45}Ca was calculated from the loss of cellular ^{45}Ca during a 5-min incubation with 1.7 mM EDTA (6). In each experiment, each value was determined in triplicate and the coefficient of variation for triplicate samples was always <12%.

To determine cGMP, acini from the pancreas of one animal were suspended in 50 ml of incubation solution, gassed with 100% O_2, and incubated with the appropriate agents for 30 min at 37°C. cGMP was measured by using the modification of the procedure of Harper and Brooker (16) as described (17). In each experiment, each value was determined in quadruplicate and the coefficient of variation for quadruplicate samples was always <7%.

RESULTS

Binding of ^{125}I-[Tyr^4]bombesin to dispersed pancreatic acini at 37°C became maximal after 60 min, did not change significantly between 60 and 105 min, and decreased progressively thereafter (Fig. 1). Reducing the incubation temperature from 37 to 4°C caused a significant slowing of the binding reaction. Addition of 0.1 μM bombesin decreased binding by 85%, and higher concentrations of bombesin caused no further decrease. Results similar to those illustrated in Fig. 1 were also obtained with a suspension of dispersed single acinar cells prepared as described (6). Unless specified otherwise, all subsequent values for binding of ^{125}I-[Tyr^4]bombesin are for "specific binding"—i.e., binding measured without bombesin *minus* binding measured in the presence of 0.1 μM bombesin. To examine the possibility that the association of the tracer with pancreatic acini reflects uptake of the tracer into the cytoplasm, acini were incubated with ^{125}I-[Tyr^4]bombesin for 60 min at 37°C, washed to remove free radioactivity, lysed with 100 vol of iced distilled water, and centrifuged at 1000 × *g* for 5 min. Under these conditions, which have been shown (7, 18) to sediment plasma membranes from pancreatic acinar cells, >93% of the total radioactivity was associated with the sediment.

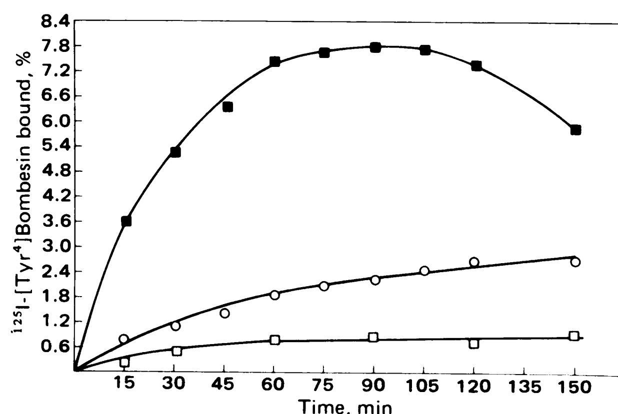

FIG. 1. Time course of binding of ^{125}I-[Tyr^4]bombesin to pancreatic acini. Pancreatic acini were incubated with 0.2 nM ^{125}I-[Tyr^4]bombesin at 37°C (■) or at 4°C (○) or with 0.1 μM bombesin at 37°C (□). Results are expressed as the percentage of added radioactivity bound. In each experiment, each value was determined in triplicate, and this experiment is representative of three others.

To examine the reversibility of the binding reaction, acini were incubated with ^{125}I-[Tyr^4]bombesin at 37°C for 65 min, washed to remove free radioactivity, and resuspended in fresh incubation solution. At 37°C, dissociation of bound ^{125}I-[Tyr^4]bombesin was a simple first-order process with a dissociation rate constant of 2.4%/min (Fig. 2). Addition of 0.1 μM bombesin did not alter the rate of dissociation of bound ^{125}I-[Tyr^4]bombesin. Reducing the incubation temperature from 37 to 4°C reduced the rate of dissociation to 0.3%/min. The

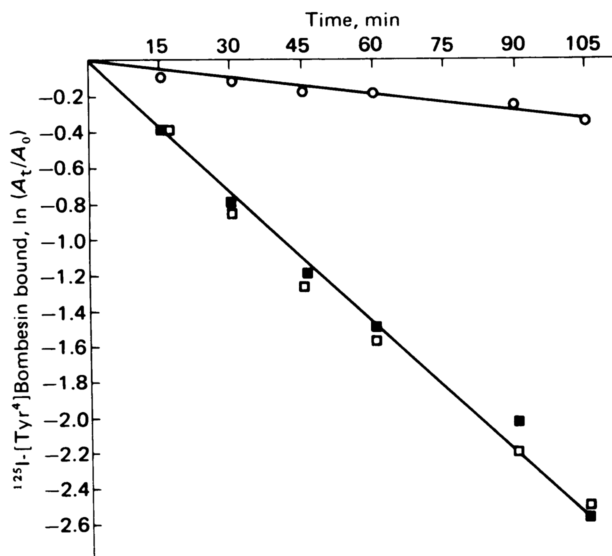

FIG. 2. Dissociation of specifically bound ^{125}I-[Tyr^4]bombesin from pancreatic acini. Pancreatic acini were preincubated with ^{125}I-[Tyr^4]bombesin (0.2 nM) for 65 min at 37°C. The cells were then washed to remove free radioactivity, resuspended in fresh incubation solution, and incubated at 37°C (■) or at 4°C (○) or with 0.1 μM bombesin at 37°C (□). Results are expressed as the natural logarithm of the fraction of ^{125}I-[Tyr^4]bombesin specifically bound at the beginning of the incubation (A_t/A_0). In each experiment, each value was determined in triplicate, and this experiment is representative of two others.

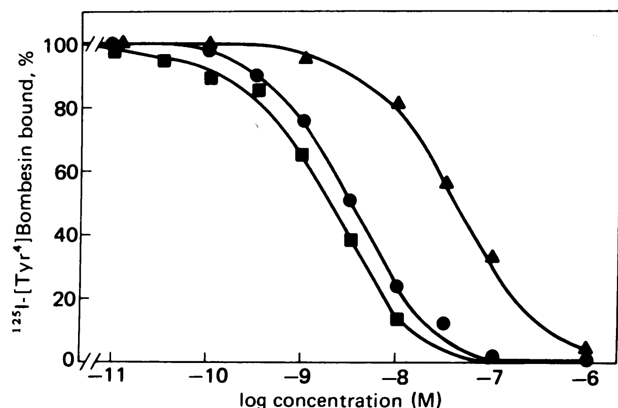

FIG. 3. Ability of various peptides to inhibit binding of ^{125}I-[Tyr^4]bombesin to pancreatic acini. Acini were incubated for 60 min at 37°C with ^{125}I-[Tyr^4]bombesin (0.2 nM) plus different concentrations of [Tyr^4]bombesin (■), bombesin (●), or litorin (▲). Binding of ^{125}I-[Tyr^4]bombesin is expressed as the percentage of radioactivity specifically bound in the absence of added nonradioactive peptides. In each experiment, each value was determined in triplicate and results given are means from four separate experiments.

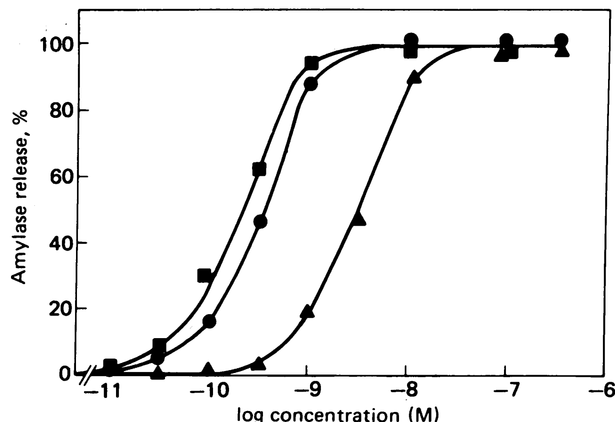

FIG. 4. Ability of various peptides to stimulate amylase release from pancreatic acini. Acini were incubated for 60 min at 37°C with different concentrations of the peptides. Values are expressed as the percentage of the amylase release caused by 10 nM bombesin. Basal amylase release was 4.2%/60 min; that with a maximally effective concentration of [Tyr^4]bombesin (■), bombesin (●), or litorin (▲), was 29.3%/60 min. In each experiment each value was determined in triplicate, and results are means from four separate experiments.

results at 4°C indicate that loss of bound radioactivity during the 4-min washing and resuspension procedure used to measure binding was negligible.

To examine the specificity of binding of ^{125}I-[Tyr^4]bombesin to dispersed pancreatic acini, various agents were tested for their ability to inhibit binding of the tracer. [Tyr^4]bombesin, bombesin, and litorin each inhibited binding of ^{125}I-[Tyr^4]-bombesin (Fig. 3); [Tyr^4]bombesin [concentration for half-maximal effect (Kd), 2 nM] was more potent than bombesin (Kd, 4 nM) which was more potent than litorin (Kd, 40 nM). Similar results were also obtained with a suspension of dispersed single acinar cells (6). Physalemin, eledoisin, VIP, BPP, secretin, somatostatin, neurotensin, gastrin, carbachol, and atropine did not inhibit binding of ^{125}I-[Tyr^4]bombesin (Table 1). At relatively high concentrations (0.1 μM), CCK-OP and caerulein each caused small but statistically significant inhibition of binding of ^{125}I-[Tyr^4]bombesin. Plotting the results in Fig. 3 in the form described by Scatchard (19) (bound/free vs. bound) gave a single straight line (not shown). If it is assumed that the pancreas from one guinea pig has 1.5×10^8 cells (6, 7, 18), each acinar cell has 5400 ± 1100 binding sites (mean ± SD; four experiments) and half of these sites are occupied at 2 nM [Tyr^4]bombesin.

[Tyr^4]Bombesin, bombesin, and litorin each caused a 7-fold increase in amylase secretion from dispersed pancreatic acini (Fig. 4), and the *relative* potencies with which these peptides increased amylase secretion were similar to those with which they inhibited binding of ^{125}I-[Tyr^4]bombesin (Fig. 3). That is, in stimulating amylase release, [Tyr^4]bombesin (Kd, 0.2 nM) was more potent than bombesin (Kd, 0.4 nM) which was more potent than litorin (Kd, 4 nM). On the other hand, for a given peptide, half-maximal stimulation of amylase secretion occurred at concentrations that were approximately 10% of those

Table 1. Effect of various agents on binding of ^{125}I-[Tyr^4]-bombesin to dispersed pancreatic acini

Agent added	^{125}I-[Tyr^4]Bombesin bound, percent of control
CCK-OP (0.1 μM)	73 ± 4*
Caerulein (0.1 μM)	72 ± 9*
Gastrin (1 μM)	100 ± 1
Physalemin (1 μM)	95 ± 5
Eledoisin (1 μM)	96 ± 6
BPP (1 μM)	101 ± 9
Neurotensin (1 μM)	97 ± 2
Somatostatin (1 μM)	110 ± 7
Secretin (0.1 μM)	105 ± 5
VIP (0.1 μM)	104 ± 8
Carbachol (1 mM)	104 ± 11
Atropine (1 mM)	113 ± 20

Dispersed pancreatic acini were incubated for 60 min at 37°C in standard incubation solution containing 0.5 mM calcium, 5 mM theophylline, 1% (wt/vol) albumin, 0.1% bacitracin, 0.2 nM ^{125}I-[Tyr^4]bombesin, and the indicated agents. Results are expressed as the percentage of ^{125}I-[Tyr^4]bombesin specifically bound with no additions (i.e., percentage of control). All values are means ± SD of three separate experiments; in each experiment, each value was determined in triplicate.

* Significantly different from control ($P < 0.05$) by Student's paired *t* test.

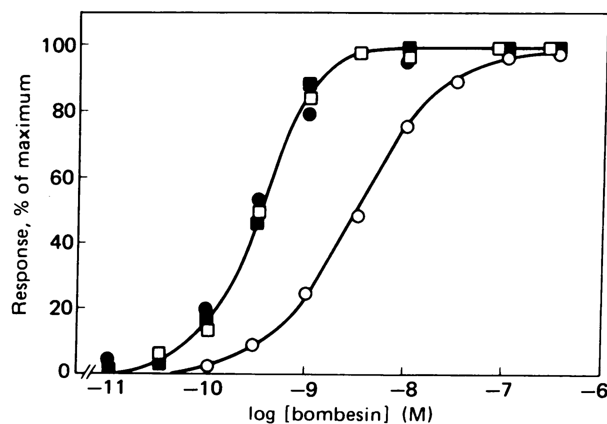

FIG. 5. Effect of bombesin on binding (○) of ^{125}I-[Tyr^4]bombesin, ^{45}Ca outflux (●), amylase release (■), and cGMP accumulation (□). In dispersed pancreatic acini, ^{125}I-[Tyr^4]bombesin binding and amylase release were determined at 37°C after a 60-min incubation. ^{45}Ca outflux was determined at 37°C after a 5-min incubation, and cellular cGMP was determined after a 30-min incubation at 37°C. Results are expressed as the percentage of the response obtained with a maximally effective concentration of bombesin (0.3 μM). Values for binding, ^{45}Ca outflux, and amylase release were determined in triplicate; values for cGMP accumulation were determined in quadruplicate. Results given are means of at least four separate experiments.

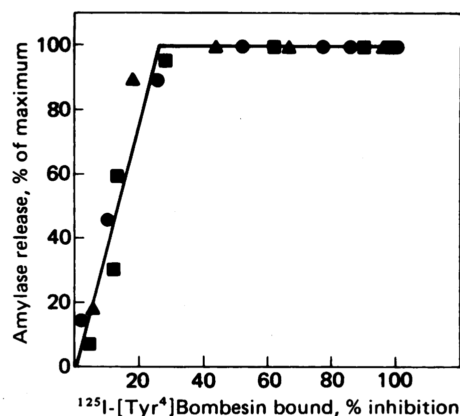

FIG. 6. Comparison of the ability of [Tyr^4]bombesin (■), bombesin (●), and litorin (▲) to stimulate amylase release and to inhibit binding of ^{125}I-[Tyr^4]bombesin in dispersed pancreatic acini. Amylase release and binding were determined at the end of a 60-min incubation at 37°C. Values for the stimulation of amylase release are from Fig. 4; those for inhibition of ^{125}I-[Tyr^4]bombesin binding are from Fig. 3.

required to cause half-maximal inhibition of binding of ^{125}I-[Tyr^4]bombesin (compare Fig. 4 with Fig. 3).

Because, in addition to increasing amylase secretion from pancreatic acinar cells, bombesin and litorin also increased outflux of ^{45}Ca as well as cellular accumulation of cGMP (1), we compared the abilities of bombesin to alter these functions with its ability to inhibit binding of ^{125}I-[Tyr^4]bombesin (Fig. 5). Bombesin caused a 4-fold increase in outflux of ^{45}Ca and a 23-fold increase in cGMP accumulation, and the dose-response curves for the action of bombesin on these two functions were identical to that for the action of bombesin on amylase secretion.

The dose-response curve for the action of bombesin on binding of ^{125}I-[Tyr^4]bombesin spanned a broader range of bombesin concentrations than did the dose-response curves for the action of bombesin on amylase secretion, cGMP accumulation, and ^{45}Ca outflux, and the Kd for bombesin inhibition of binding (4 nM) was 10 times greater than the Kd for the actions of bombesin on the other functions (0.4 nM) (Fig. 5). To compare the abilities of [Tyr^4]bombesin, bombesin, and litorin to inhibit binding of ^{125}I-[Tyr^4]bombesin with their abilities to stimulate amylase secretion, the percentage of maximal stimulation of amylase release was plotted as a function of the percentage inhibition of tracer binding (Fig. 6). As the inhibition of binding increased, the stimulation of amylase release increased linearly, and maximal stimulation of amylase release occurred with 25% inhibition of binding.

DISCUSSION

The present results demonstrate that dispersed acini from guinea pig pancreas bind a radiolabeled analogue of bombesin, ^{125}I-[Tyr^4]bombesin, with a high affinity. This binding can be localized to the plasma membrane and is temperature-dependent, saturable, and reversible. There are approximately 5000 binding sites per pancreatic acinar cell. The sites to which ^{125}I-[Tyr^4]bombesin binds have a high affinity for [Tyr^4]-bombesin, bombesin, and litorin.

The present results also indicate that the receptors on pancreatic acini with which bombesin and related peptides interact to increase outflux of ^{45}Ca, cGMP accumulation, and amylase release are functionally distinct from the receptors that mediate the actions of other pancreatic secretagogues. Secretin and VIP stimulate amylase secretion from pancreatic acini (4) as a result of their ability to interact with a common receptor to activate adenylate cyclase and increase cellular cAMP (4, 20). Neither bombesin nor litorin inhibits binding of ^{125}I-labeled VIP to pancreatic acinar cells and, as shown in the present study, neither secretin nor VIP inhibits binding of ^{125}I-[Tyr^4]-bombesin. Physalemin, eledoisin, carbachol, caerulein, and CCK-OP each increases ^{45}Ca outflux, cGMP accumulation, and amylase release in pancreatic acini (1–8). Physalemin and eledoisin, each of which has the same two COOH-terminal amino acids as do bombesin and litorin (leucine and methionine), as well as carbachol and atropine, did not alter binding of ^{125}I-[Tyr^4]bombesin. Caerulein and CCK-OP, when present at high concentrations (0.1 μM), were able to inhibit binding of ^{125}I-[Tyr^4]bombesin by approximately 25%. These concentrations, however, are approximately 300 times greater than those at which caerulein and CCK-OP cause maximal stimulation of amylase secretion from pancreatic acinar cells (4, 5).

There was good agreement between the *relative* potencies with which [Tyr^4]bombesin, bombesin, and litorin inhibited binding of ^{125}I-[Tyr^4]bombesin and the *relative* potencies with which these peptides increased amylase secretion. For a given peptide, however, 10-fold higher concentrations were required for half-maximal inhibition of binding than were required for half-maximal stimulation of amylase release. The simplest explanation for this difference is that pancreatic acinar cells possess a single class of receptors that interact with bombesin and litorin, and that occupation of 25% of these receptors will cause a maximal biologic response. We considered the possibility that the difference between the potency for occupation of binding sites and that for stimulation of amylase secretion occurred because maximal stimulation of amylase secretion could be produced by submaximal changes in some mediating substance or function. This type of phenomenon has been shown to occur with agents whose action is mediated by cAMP in that submaximal changes in cellular cAMP can cause maximal changes in one or more cellular functions (21, 22). Release of exchangeable cellular calcium and increased cellular cGMP are two postulated intermediate steps in the mechanism by which bombesin and related peptides increase amylase secretion from pancreatic acini (1–3). It seemed possible that submaximal changes in one of these functions might be able to cause maximal stimulation of amylase secretion. We found, however, that the dose-response curves for the action of bombesin on calcium outflux and on cGMP accumulation were identical with the curve for bombesin-stimulated amylase release. These findings indicate that, if enzyme secretion is maximal with submaximal changes in some bombesin-stimulated intermediate function, this intermediate function must precede bombesin-induced changes in calcium outflux and cGMP accumulation.

We also considered the possibility that radiolabeled [Tyr^4]-bombesin might be binding to duct cells or endocrine cells present in our preparation. This possibility seems unlikely because we obtained identical results for binding of ^{125}I-[Tyr^4]-bombesin when we used a suspension of dispersed single acinar cells that contained approximately 0.5% duct cells and 0.5% endocrine cells (6). Finally, we considered the possibility that the actions of bombesin and related peptides on amylase release, cGMP accumulation, and calcium outflux might result from these peptides interacting with a class of binding sites that we were unable to detect with our present techniques. Compared to the binding sites detected in the present study, these undetectable binding sites would have the same relative affinities but 10-fold greater absolute affinities for bombesin and related peptides. In addition, to go undetected with the technique used for the present study, these hypothetical binding sites would have to be present at a density of less than 100 sites per cell.

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