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BOMBESIN BINDING AND BIOLOGICAL EFFECTS ON PANCREATIC ACINAR AR42J CELLS

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The effects of bombesin on amylase release and the receptor binding of ^{125}I-[Tyr^4]bombesin in the rat pancreatic acinar carcinoma cell line AR42J were examined. Bombesin-like peptides stimulated amylase release from AR42J cells in a dose-dependent manner; a maximal 2-fold stimulation occurred at a bombesin concentration of 300 pM. Binding of ^{125}I-[Tyr^4]-bombesin to AR42J cells was specific, saturable and temperature dependent. The relative potencies with which various structurally related peptides stimulated amylase release correlated well with their relative abilities to compete for the bombesin receptor.

Much of current understanding of the receptors for bombesin-like peptides and their mechanisms of action comes from studies conducted with pancreatic acinar cells. Bombesin-like peptides stimulate pancreatic amylase release (1,2,3), and may have trophic actions on the pancreas (4). Specific receptors have been demonstrated for bombesin on pancreatic acinar cell membranes (5,6). Activation of pancreatic bombesin receptors leads to increases in $^{45}Ca^{2+}$ efflux and cyclic GMP accumulation (1). To better understand the biological actions and the regulation of bombesin receptors it would be useful to study a pancreatic acinar cell line. We, therefore, have investigated the AR42J cell line as an in vitro model for studying bombesin receptors on pancreatic acinar cells.

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MATERIALS AND METHODS

Materials

The following were purchased: bovine plasma albumin (fraction V) from Reheis, Chicago, IL; soybean trypsin inhibitor (type 1-S), HEPES, and dexamethasone from Sigma Chemical Co., St. Louis, MO; penicillin, streptomycin, amphotericin B, and fetal calf serum from the Cell Culture Facility, University of California, San Francisco, San Francisco, CA; biorad protein reagent from Bio-Rad Laboratories, Richmond, CA; ^{125}Iodine from New England Nuclear, Boston, MA; bombesin, [Tyr^4]bombesin, litorin, gastrin-releasing peptide (GRP) and bombesin[8-14] from Bachem, Torrance CA. Synthetic cholecystokinin octapeptide (CCK8) was a gift from Squibb, Princeton, NJ. All other chemicals and reagents were of analytical grade.

Amylase secretion

Measurement of peptide-induced amylase release was by a modification of the method reported previously (7). Cultures were washed twice then incubated at 37°C in a medium consisting of DME-H21 with 10 mM HEPES (pH 7.40) enriched with 0.1 mg/ml soybean trypsin inhibitor, 5 mg/ml bovine plasma albumin, and gassed with 100% O_2. Various concentrations of bombesin or its related peptides were then added. After 40 min the medium was removed, analyzed for amylase content, and amylase release was calculated as the amount of amylase present in the medium as a percentage of initial cell content. To determine cell amylase content the cells were washed twice with 2 ml of 154 mM NaCl at 4° C and scraped into 1 ml of buffer containing 0.05 M $NaPO_4$ and 0.05 M NaCl, pH 6.9. After sonication the resultant cell homogenate was assayed for amylase by the method of Jung (8), protein content by the method of Bradford (9) using Biorad reagent, and DNA content was determined using 33258 Hoechst dye (10). Statistical analyses were carried out utilizing the Students t-test.

^{125}I-[Tyr^4]Bombesin Receptor Binding

Bombesin was radioiodinated by a method to be described in detail elsewhere (S. Vigna, to be published). Briefly, Tyr^4-bombesin was labeled with ^{125}Iodine using Iodogen beads. Monoiodinated bombesin was separated from other forms by HPLC then reduced by treatment with dithiothreitol. This reduced form was repurified over HPLC to yield the final labeled ligand which consisted of pure monoiodinated reduced ^{125}I-bombesin which was biologically active.

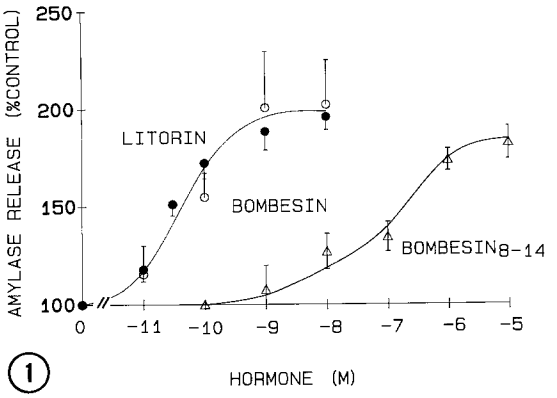
AR42J cells (0.5-1.0 x 10^6 cells per dish) were incubated in 1 ml of HEPES-buffered Ringer (HR) containing 5 mg/ml bovine plasma albumin, 0.1 mg/ml soybean trypsin inhibitor, and 1 mg/ml bacitracin. ^{125}I-[Tyr^4]bombesin (10 pM) and various concentrations of unlabeled ligand were added as indicated. At specified times, the incubation medium was removed, and the cell layer was washed twice at 4°C with 154 mM sodium chloride. The radioactivity associated with the cells was measured in a gamma-scintillation counter; an aliquot of the incubation medium was also counted to determine the total radioactivity. Binding was normalized per mg DNA or converted to per 10^6 cells using the known DNA/cell (7). Nonspecific binding was determined in the presence of an excess of unlabeled hormone (100 nM bombesin).

Degradation of ^{125}I-[Tyr^4]bombesin in the medium was monitored by the precipitation of binding supernatants with trichloroacetic acid added at a final concentration of 10%.

RESULTS

Bombesin stimulated an increase in amylase secretion, from 14 ± 2 to 28 ± 4% (n=11) of initial content per 40 min. One-half maximal stimulation occurred at a bombesin concentration of 30 pM and maximal stimulation occurred at 300 pM (Fig. 1). Litorin and bombesin[8-14] also stimulated amylase release in AR42J cells to the same maximal extent as bombesin. Litorin was equipotent with bombesin in stimulating amylase release, whereas, bombesin[8-14] was 10,000-fold weaker. In other experiments GRP was equipotent and equally as effective as bombesin (data not shown).

To characterize bombesin receptors on AR42J cells we utilized [Tyr^4]bombesin which was monoiodinated and purified using HPLC. When AR42J cells were incubated with 10 pM ^{125}I-[Tyr^4]bombesin in HEPES-Ringer at 37°C binding was one-half maximal after 20 min and maximal within 45-60 min (Fig. 2). The average specific binding was 15 ± 2% of the



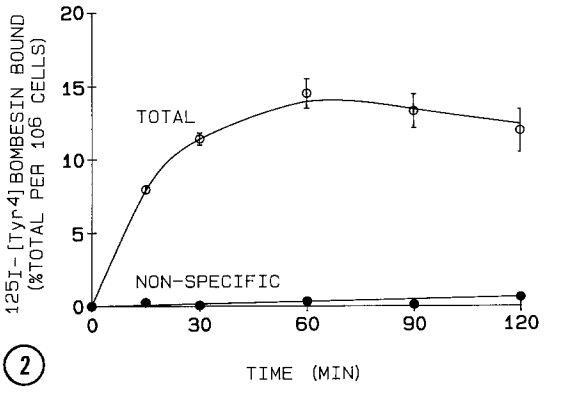


Figure 1. Concentration dependence of amylase release stimulated by bombesin and related peptides for AR42J cells. Amylase release over 40 min is plotted as a function of the concentration of bombesin (●), litorin (0), or bombesin[8-14] (△) in the medium. Results shown are the mean ± S.E. for 5-11 experiments.

Figure 2. Time-course of ^{125}I-[Tyr^4]bombesin binding to AR42J cells. Binding to AR42J cells is expressed as the percent of total radioactivity that is bound per 10^6 cells; nonspecific binding was determined in the presence of 100 nM unlabeled bombesin. Values are the mean ± S.E. for 3 experiments.

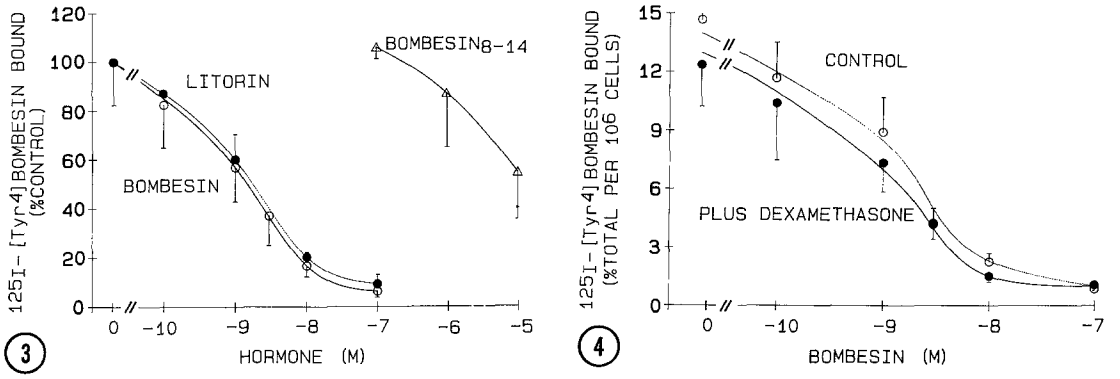


Figure 3. Specificity of inhibition of ^{125}I[Tyr^4]bombesin binding to AR42J cells by bombesin and related peptides. Binding of 10 pM ^{125}I[Tyr^4]bombesin was carried out at 37°C for 60 min in the presence of various concentrations of bombesin, litorin, or bombesin[8-14]. Values are expressed as per cent of control ^{125}I[Tyr^4]bombesin bound per mg DNA and represent the mean ± S.E. of 3-9 experiments.

Figure 4. Competitive inhibition of ^{125}I-[Tyr^4]bombesin binding to AR42J cells raised in the presence (●) or absence (○) of 100 nM dexamethasone. Values are expressed as per cent of total ^{125}I-[Tyr^4]bombesin bound per 10^6cells and represent the mean ± S.E. of 5-7 experiments.

total radioactivity per 10^6 cells (n=8). Nonspecific binding (labeled hormone plus an excess of unlabeled hormone) was 0.8 ± 0.2% of the total radioactivity per 10^6 cells. Degradation of ^{125}I-[Tyr^4]bombesin by AR42J cells, as measured by the appearance of radioactivity soluble in trichloroacetic acid, did not exceed 10% of the total. When binding was conducted at 22°C or 4°C the time-course was slower and the binding at all times was lower than that observed at 37°C (data not shown). Maximal binding was not reached within 4 h at 4°C.

^{125}I-[Tyr^4]bombesin binding to AR42J cells was competitively inhibited by increasing concentrations of unlabeled bombesin (Fig. 3). The results of eight experiments indicated that one-half maximal inhibition occurred with 1 nM bombesin. In similar experiments litorin and bombesin[8-14] also competitively inhibited ^{125}I-[Tyr^4]bombesin binding; litorin was equipotent with bombesin, while bombesin[8-14],

which lacks the first seven C-terminal amino acids of bombesin, was 10,000-fold less potent (Fig. 3).

Previously we reported that cells which had been pretreated with dexamethasone showed increased binding of ^{125}I-CCK (11). Therefore, we examined the binding of ^{125}I-bombesin to AR42J cells which had been raised in the presence or absence of dexamethasone for 48 h. Pretreatment with dexamethasone had no effect on the maximal amount of ^{125}I-bombesin bound per cell nor did it affect the concentration of unlabeled bombesin required for one-half maximal inhibition of ^{125}I-bombesin binding (Fig. 4). Dexamethasone, however, had its previously reported effect to increase amylase content (data not shown) (7).

DISCUSSION

The AR42J cell line was derived from rat acinar pancreas, synthesizes and secretes amylase (7) and has been shown to have functional receptors for CCK (11) substance P (12) and insulin (13). In the present study we investigated the binding of radioiodinated bombesin to the AR42J cells and the effects of bombesin-like peptides on amylase release from these cells. We found that bombesin, litorin or bombesin[8-14] stimulated amylase release from AR42J cells in a dose-dependent manner and that the relative potencies with which bombesin, litorin, and bombesin[8-14] stimulated amylase release agreed well with the relative potencies with which they inhibited the binding of ^{125}I-[Tyr^4]bombesin. For a given peptide, however, a 100-fold higher concentration was required for one half-maximal inhibition of binding than was required for one half-maximal stimulation of amylase release. This phenomenon has been reported previously (5) and is likely explained by an excess of

receptors such that occupancy of only a fraction of receptors is required for full biological action.

Pretreatment of the AR42J cells with glucocorticoids had no effect on the binding of bombesin. These results are in contrast to our previously published results indicating an increase in CCK receptors after pretreatment of AR42J cells with glucocorticoids (12). Thus, effects of glucocorticoids on CCK receptors is specific and not a general phenomenon.

In conclusion, AR42J cells have functional bombesin receptors which have specificities for ligands similar to those found on normal rat pancreatic acinar cells. Bombesin receptors on AR42J cells are not regulated by glucocorticoids. The AR42J cell line should provide a useful in vitro system for further studies on the structure, regulation, and function of bombesin receptors.

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