C-Terminal bombesin sequence requirements for binding and effects on protein synthesis in Swiss 3T3 cells

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1. Synthetic peptides corresponding to the five, seven, nine and eleven C-terminal amino acids of the tetradecapeptide bombesin as well as bombesin itself and gastrin-releasing peptide have been evaluated in Swiss 3T3 cells in order to define the minimal peptide length needed for biological responsiveness. 2. Gastrin-releasing peptide, bombesin, the undecapeptide and nonapeptide had nearly equipotent abilities to compete for binding of labelled gastrin-releasing peptide to the cell receptors and showed half-maximal competition at 5–10 nM. The heptapeptide and pentapeptide were ineffective. 3. Cross-linking experiments demonstrated specific binding of gastrin-releasing peptide to a 100 kDa receptor subunit. 4. Total cell protein synthesis was stimulated equally by the nonapeptide and longer peptides with a half-maximal effect at 0.5 nM, while a more than 1000-fold higher concentration of the heptapeptide was required to produce a similar response. Comparable results were found when insulin was also present. 5. Neither an inhibition of protein breakdown nor a stimulation of DNA labelling could be demonstrated by bombesin or gastrin-releasing peptide. 6. We conclude that a C-terminal peptide ligand comprising more than seven but no more than nine amino acids is required to achieve high-affinity binding and receptor-mediated responses via the bombesin receptor.

INTRODUCTION

Bombesin, a tetradecapeptide isolated from frog skin (Anastasi *et al.*, 1972), has been shown to exhibit a wide range of biological activities in mammals that include facilitation of peptide hormone release from endocrine glands (Deschodt-Lanckman *et al.*, 1976; Jensen *et al.*, 1978), stimulation of gastrointestinal smooth muscle contraction (Erspamer & Melchiorri, 1975), inhibition of gastric acid secretion (Taché *et al.*, 1980) as well as stimulation of mitogenesis (Rozengurt & Sinnett-Smith, 1983). Mammalian counterparts of bombesin that include the 27-amino-acid gastrin-releasing peptide (GRP), fragments of that peptide and also the decapeptide neuromedin C have been isolated from brain, lung and gut (McDonald *et al.*, 1978; Minamino *et al.*, 1984; Reeve *et al.*, 1983). All these peptides have homologous C-terminal regions and share an identical C-terminal heptapeptide. Receptor binding experiments with either ¹²⁵I-labelled GRP (Zachary & Rozengurt, 1985) or ¹²⁵I-labelled [Tyr⁴]bombesin (Jensen *et al.*, 1978) indicate that the peptides noted above interact with the same receptor, as do the related peptides litorin and neuromedin B that contain one or two substitutions in the C-terminal heptapeptide.

In the present report we have examined the bombesin sequence requirements for both binding to Swiss 3T3 fibroblasts and effects on protein metabolism in this cell line. For this purpose, synthetic peptides that consist of the C-terminal five, seven, nine and eleven amino acids of bombesin (see Table 1) have been evaluated together with the complete bombesin molecule and GRP.

MATERIALS AND METHODS

Peptides

Solid phase peptide synthesis (Merrifield, 1963) using the Applied Biosystems Model 430A synthesizer with *t*-butoxycarbonyl amino acids and a *p*-4-methylbenzhydryl amine resin, and cleavage of the peptide with trifluoromethanesulphonic acid, were accomplished according to the manufacturer's directions (Clark-Lewis *et al.*, 1986; Bergot *et al.*, 1986). The peptides formed (Table 1) were purified by adsorption to a Waters C₁₈ μ Bondapak column and elution with an acetonitrile gradient in 0.1% trifluoroacetic acid. Two absorbance peaks were obtained upon chromatography of each peptide. Since amino acid analyses of material in both peaks were consistent with the predicted sequence, and since the first-eluting peak could be converted with dithiothreitol (0.6 M) to give a peptide with an elution position identical with that of the second peak, it seemed likely that the amidated methionyl residue in the first peak was present as a sulfoxide derivative. In the second peak this residue was in the reduced form. Peptides eluting at positions of the second peak were used for all experiments.

GRP (porcine, synthetic) was obtained from Sigma and ¹²⁵I-labelled GRP (2000 Ci/mmol) from Amersham International. The insulin used was Actrapid (Novo). Insulin-like growth factor-1 was a homogenous preparation isolated from bovine colostrum (Francis *et al.*, 1986); bovine brain basic fibroblast growth factor was generously provided by Dr. P. Böhlen (Universität Zurich) and recombinant human epidermal growth

Abbreviations used: GRP, gastrin-releasing peptide; B11, B9, B7 and B5, the C-terminal eleven, nine, seven and five amino acids of bombesin; W:DME, a 1:1 mixture of Waymouth's MB 721/1 medium and Dulbecco's modified Eagle's Minimal Essential Medium.

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Table 1. Amino acid sequences of synthetic peptides

Only the C-terminal 14 amino acids of GRP are given.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bombesin	<Glu	Gln	Arg	Leu	Gly	Asn	Gln	Trp	Ala	Val	Gly	His	Leu	Met-NH ₂
B11				Leu	Gly	Asn	Gln	Trp	Ala	Val	Gly	His	Leu	Met-NH ₂
B9						Asn	Gln	Trp	Ala	Val	Gly	His	Leu	Met-NH ₂
B7								Trp	Ala	Val	Gly	His	Leu	Met-NH ₂
B5										Val	Gly	His	Leu	Met-NH ₂
GRP	-Met	Tyr	Pro	Arg	Gly	Asn	His	Trp	Ala	Val	Gly	His	Leu	Met-NH ₂

factor by Dr. C. George-Nascimento (Chiron Corp., Emeryville, CA, U.S.A.).

Cell culture

Swiss 3T3 cells obtained from Dr. H. Herschman (UCLA School of Medicine, Los Angeles, CA, U.S.A.) were grown at 37 °C and 5% CO₂ in 12- or 24-place multiwell dishes containing Alpha modified Eagle's Minimal Essential Medium supplemented with 10% fetal bovine serum (Gibco) plus 60 mg of penicillin G and 100 mg of streptomycin sulphate (Glaxo) per litre.

Binding experiments

Confluent cell monolayers maintained in 12-place multiwell dishes without a medium change for 5–8 days were washed twice over a 60 min period with 0.1 M-Hepes, pH 7.5, containing 0.12 M-NaCl, 1.2 mM-MgSO₄, 8 mM-glucose and 0.5% bovine serum albumin (RIA grade; Sigma). Binding of ¹²⁵I-labelled GRP was measured using 0.5 ml of the same medium containing 1 × 10⁴ c.p.m. of GRP together with the indicated concentration of competing ligand. After incubation for 3 h at 15 °C, the monolayers were washed twice at 0 °C with Hanks' salts containing 0.5% bovine serum albumin and twice with Hanks' salts. The cells were dissolved in 0.5 M-NaOH containing 0.1% Triton X-100 for radioactivity and protein (Dulley & Grieve, 1975) measurements.

The procedure for receptor–ligand crosslinking (Kasuga *et al.*, 1981) was identical with that described above except that 5 × 10⁵ c.p.m. was added to each well. After the incubation and washes had been completed, 0.5 ml of 0.5 mM-disuccinimidyl suberate in albumin-free binding buffer was added and the cells were left for 15 min at 15 °C. The reaction in each well was quenched with 1.5 ml of 0.1 M-Tris containing 1 mM-EDTA at pH 7.4. After 5 min at 15 °C, this solution was replaced by 100 µl of a dissociating mixture composed of 2% (w/v) SDS, 10% (w/v) glycerol, 100 mM-dithiothreitol, 0.01% Bromophenol Blue and 62.5 mM-Tris/HCl at pH 6.8. After a further 30 min the cell lysate was transferred to a Microfuge tube and heated at 95 °C for 10 min. Portions (25 µl) of the dissociated samples were subjected to SDS/polyacrylamide gel electrophoresis on 10% (w/v) acrylamide gels (Laemmli, 1970). Staining, gel drying and autoradiography were carried out as described previously (Ballard *et al.*, 1986).

Measurement of protein synthesis

All procedures were identical with those described by Ballard *et al.* (1986) except that a 1:1 mixture of Waymouth's MB 721/1 medium and Dulbecco's modi-

fied Eagle's Minimal Essential Medium (W:DME) replaced Eagle's Minimal Essential Medium. The leucine concentration was adjusted to 2 mM for labelling. Rates, calculated as the percentage of monolayer protein synthesized over the 4 h measurement period, were expressed relative to the values obtained either in the absence of any additions or to that observed in the presence of 10 nM-insulin.

Measurement of protein breakdown

The procedure was identical with that described by Ballard *et al.* (1986) except for the substitution of W:DME medium for Eagle's Minimal Essential Medium and the inclusion of 5% fetal bovine serum during the initial 18 h labelling period. Rates were expressed as the percentage of labelled protein degraded over a 4 h period.

Thymidine incorporation into DNA

Confluent, quiescent monolayers in 24-place multiwell dishes that had been left for 7–9 days from subculturing without a medium change were washed twice over 2 h with W:DME medium and incubated for 18 h in the same medium plus the appropriate growth factor. Subsequently the cells were labelled for 4 h by the addition of 1 µCi (5 nmol) of [³H]thymidine (New England Nuclear) per well and harvested as described for the protein synthesis measurements (Ballard *et al.*, 1986). DNA labelling was expressed as the thymidine incorporation per well.

RESULTS

Binding experiments

Confluent monolayers of Swiss 3T3 cells containing 300–350 µg of protein bound approx. 15% of the labelled GRP added. This binding was reduced to 3% of added label in the presence of 1 µM non-radioactive GRP (Fig. 1). Concentration curves for the competition of GRP, bombesin and the truncated bombesin peptides for ¹²⁵I-labelled GRP binding (Fig. 1) indicated that bombesin, B11 and B9 were essentially equivalent to GRP on a molar basis except that slightly higher concentrations of B11 or B9 were required to inhibit tracer binding by 50%. Neither B7 nor B5 had any effect on the binding of labelled GRP at a concentration as high as 1 µM.

Crosslinking of labelled GRP to Swiss 3T3 cells followed by electrophoresis of the solubilized protein under reducing conditions and autoradiography of the dried gels gave one band with a molecular mass of approx. 100 kDa (Fig. 2, lane 1) when compared with

Fig. 1. Ligand competition for labelled GRP binding to Swiss 3T3 monolayers

Peptides at the indicated concentrations were added to Swiss 3T3 cells in the presence of 10^4 c.p.m. of ^{125}I -labelled GRP and binding was measured as described in the text. Values are means $\pm$ S.E.M. for eight determinations and are expressed as percentages of the binding in the absence of unlabelled ligand. The arrows indicate concentrations of ligand giving 50% inhibition of binding. Left scales apply to the GRP, B11 and B7 curves; right scales are for the bombesin, B9 and B5 curves.

molecular mass standards. Evidence that this band represents the receptor or receptor subunit is provided (Fig. 2) by the data showing competition for binding with 100 nM-GRP (lane 2), 100 nM-bombesin (lane 3) or 100 nM-B9 (lane 5) as well as the lack of competition observed when 100 nM-B7 (lane 6) was added. The lower concentration of bombesin tested (lane 4) gave only marginal competition for binding of labelled GRP to the putative receptor protein, a result also found with 1 nM-GRP and 1 nM-B9 (results not shown). Since none of the bands with molecular masses between those of the putative receptor and free GRP showed any loss of intensity with high concentrations of GRP, bombesin or B9, they probably represent non-specific binding of the radioligand.

Effects on protein metabolism

The addition of growth factors to cell monolayers incubated in serum-free media leads to a stimulation of protein synthesis that can be readily detected over a 4-h period. Thus with Swiss 3T3 cells the rate was increased 2.24 ± 0.06 (S.E.M. for 12 determinations) -fold by the addition of 10% fetal bovine serum from a fractional rate of 3.9% of cell protein synthesized during the measurement period. Similarly, 10 nM-insulin, a sub-optimal concentration for this cell line, increased protein synthesis by 1.47 ± 0.06 -fold (see below).

The effects of GRP, bombesin and the C-terminal peptides on protein synthesis were evaluated both in the

Fig. 2. Autoradiograph of a dried SDS/polyacrylamide electrophoresis gel after cross-linking ^{125}I -labelled GRP to Swiss 3T3 cells

The concentrations of non-radioactive ligands were: lane 1, no addition; lane 2, 100 nM-GRP; lane 3, 100 nM-bombesin; lane 4, 1 nM-bombesin; lane 5, 100 nM-B9; lane 6, 100 nM-B7. The positions of molecular mass markers in adjacent lanes are indicated. The band corresponding to the putative receptor is indicated by an arrow.

absence and presence of 10 nM-insulin. When added in the absence of other growth factors, GRP, bombesin, B11 or B9 increased the rate of protein synthesis by a maximum of 0.5–0.7-fold (Fig. 3). In each case half-maximal effects occurred at approx. 5×10^{-10} M. No response was found with B5 and only a modest yet statistically significant ($P < 0.01$) stimulation at the highest concentration of B7 (Fig. 3). Very similar results were obtained in the presence of insulin (results not shown). Thus the addition of either GRP, bombesin, B11 or B9 produced a 1.3-fold increase in the rate of protein synthesis with half-maximal responses noted at approx. 1 nM. The overall rate achieved in the presence of 10 nM-insulin plus optimal concentrations of the bombesin-related peptides was twice that found with control medium, an indication of additivity between the two groups of growth factors. No response could be detected with B7 or B5 above that found with insulin alone.

Co-ordinate effects of growth factors to inhibit protein breakdown as well as to stimulate protein synthesis are frequently observed in cultured cells (Ballard & Gunn, 1985; Ballard & Read, 1985; Ballard *et al.*, 1986). Indeed, this dual response also occurs when either fetal bovine serum or insulin is added to Swiss 3T3 cells (Table 2; see also the previous section). However, neither GRP nor any of the bombesin peptides had any effect on protein breakdown in this cell line either in the absence or the presence of 10 nM-insulin (Table 2).

Fig. 3. Effects of peptides on protein synthesis in Swiss 3T3 cells

The protein synthesis rate was calculated as the percentage stimulation at the indicated concentration of each peptide above that occurring in W:DME medium and is expressed as means $\pm$ S.E.M. for eight determinations. Arrows indicate concentrations giving half-maximal stimulation. Left scales apply to the GRP, B11 and B7 curves; right scales are for the bombesin, B9 and B5 curves.

Table 2. Effects of growth factors on protein breakdown in Swiss 3T3 cells

Values are means $\pm$ S.E.M. with the numbers of determinations given in parentheses.

Additions	Protein degraded in 4 h (%)	
	– Insulin	+ Insulin (10 nM)
None	12.6 $\pm$ 0.3 (7)	12.0 $\pm$ 0.1 (14)
Fetal bovine serum, 10 %	8.8 $\pm$ 0.1 (7)	–
Insulin, 1 μ M	8.9 $\pm$ 0.3 (5)	–
GRP, 1 μ M	12.6 $\pm$ 0.4 (3)	11.7 $\pm$ 0.7 (3)
Bombesin, 1 μ M	12.1 $\pm$ 0.3 (3)	11.6 $\pm$ 0.2 (3)
B11, 1 μ M	12.6 $\pm$ 0.4 (3)	12.2 $\pm$ 0.5 (3)
B9, 1 μ M	12.7 $\pm$ 0.3 (3)	12.1 $\pm$ 0.3 (3)
B7, 1 μ M	12.4 $\pm$ 0.1 (3)	12.2 $\pm$ 0.3 (3)
B5, 1 μ M	12.5 $\pm$ 0.4 (3)	12.3 $\pm$ 0.1 (3)

Thymidine incorporation into DNA

Rozengurt & Sinnett-Smith (1983) have reported that bombesin, but not insulin, produced a substantial stimulation of DNA synthesis in quiescent cultures of Swiss 3T3 cells. Furthermore, when the two growth factors were added together both thymidine incorporation and the proportion of labelled nuclei were increased to about the same extent as occurred with 10 % fetal bovine serum. Our results are clearly different. Thus bombesin at concentrations as high as 100 nM had no

Table 3. Effects of bombesin on thymidine incorporation in Swiss 3T3 cells

Values are means $\pm$ S.E.M. for nine determinations at each concentration.

Additions	$10^{-3} \times$ Incorporation/well (c.p.m.)	
	– Insulin	+ Insulin (1 μ M)
None	4.1 $\pm$ 0.3	9.9 $\pm$ 0.5
Fetal bovine serum, 10 %	84.8 $\pm$ 4.9	–
Bombesin		
10^{-11} M	4.4 $\pm$ 0.4	11.8 $\pm$ 1.0
10^{-10} M	3.9 $\pm$ 0.4	12.8 $\pm$ 0.6
10^{-9} M	4.2 $\pm$ 0.4	14.5 $\pm$ 0.7
10^{-8} M	4.6 $\pm$ 0.4	16.7 $\pm$ 1.1
10^{-7} M	4.2 $\pm$ 0.4	16.2 $\pm$ 1.3

Table 4. Growth factor effects on thymidine incorporation in Swiss 3T3 cells

Values are means $\pm$ S.E.M. for four determinations.

Additions	$10^{-3} \times$ Incorporation (c.p.m.)	Stimulation (-fold)
None	2.9 $\pm$ 0.1	1.0
Fetal-bovine serum, 10 %	57.7 $\pm$ 1.5	20.2
Insulin, 1 μ M	16.1 $\pm$ 0.9	5.6
Insulin-like growth factor-1, 10 nM	19.7 $\pm$ 0.8	6.9
Basic fibroblast growth factor, 1 nM	46.8 $\pm$ 3.7	16.3
Epidermal growth factor, 1 nM	24.8 $\pm$ 1.6	8.7
GRP, 1 μ M	4.6 $\pm$ 0.3	1.6
Bombesin, 1 μ M	4.5 $\pm$ 0.3	1.6

effect on thymidine incorporation (Table 3), while the addition of 10 % fetal bovine serum produced a 20-fold stimulation. Insulin (1 μ M) increased DNA labelling 2.4-fold, so that an increase to 4-fold above the quiescent rate was noted when a 10 nM or higher bombesin concentration was also present (Table 3). In a second series of experiments (Table 4) in which 10 % fetal bovine serum produced a similar 20-fold stimulation to that shown in Table 3, the addition of 1 μ M-bombesin or 1 μ M-GRP gave a slight (1.6-fold) but significant effect. However, the increased rate of DNA labelling was much less than found with 1 μ M-insulin (5.6-fold), 10 nM-bovine insulin-like growth factor-1 (6.9-fold), 1 nM-bovine basic fibroblast growth factor (16.3-fold) or 1 nM-human epidermal growth factor (8.7-fold).

DISCUSSION

Swiss 3T3 cells were used for an analysis of the C-terminal sequence requirements for bombesin binding and responsiveness because large effects of bombesin on DNA synthesis have been demonstrated with this cell line (Rozengurt & Sinnett-Smith, 1983; Zachary &

Rozengurt, 1985). As is evident from the data presented in Tables 3 and 4, DNA synthesis measured here with Swiss 3T3 cells was only minimally stimulated by the addition of either bombesin or GRP and no synergism with insulin could be detected, again in contrast with the data reported by Rozengurt & Sinnott-Smith (1983). Similar negative results were observed (not shown) when thymidine incorporation was measured using the 28 h labelling protocol described by Rozengurt & Sinnott-Smith (1983) or when labelled nuclei were counted.

It seems likely that our different results are a consequence of heterogeneity in the cell line, both because we found slightly greater effects of bombesin on DNA synthesis and no insulin responsiveness in parallel experiments with Swiss 3T3 cells obtained from another source (batch 363 from Commonwealth Serum Laboratories, Parkville, Victoria, Australia) and also because several other research groups have recently reported substantial mitogenic effects of bombesin in Swiss 3T3 cells (Metcalfe *et al.*, 1985; Palumbo *et al.*, 1986; Isacke *et al.*, 1986; Letterio *et al.*, 1986; Corps *et al.*, 1987).

Although DNA labelling in the Swiss 3T3 cells used in the present study was not substantially increased by bombesin either in the presence or absence of insulin, the same cells bound ^{125}I -labelled GRP with the specificity expected for a true receptor. Thus unlabelled GRP or bombesin at nanomolar concentrations competed for tracer binding, while both binding and competition indicated a receptor subunit of approx. 100 kDa. Further evidence for a functional GRP/bombesin receptor was provided by the substantial stimulation of protein synthesis at low concentrations of GRP or bombesin (Fig. 3). The observation that protein breakdown was not inhibited by these peptides, even though the pathway could be inhibited by insulin or by serum, suggests that these latter growth-promoting agents initiate a more complete cascade of events on the route to cell division. Whereas the concomitant stimulation of protein synthesis and inhibition of protein breakdown following growth factor addition to cells is a more usual response (Ballard & Gunn, 1985; Ballard *et al.*, 1986), a selective response on one pathway has been noted once previously in a study of cellular senescence (Ballard & Read, 1985). In that study, protein synthesis was resistant to growth factor action while the protein breakdown pathway was inhibited as effectively as in young cells. It seems pertinent that both in the ageing study and in the bombesin experiments reported here the addition of growth factor did not lead to a stimulation of DNA synthesis, suggesting perhaps that a marked increase in cellular protein content produced by dual effects on protein synthesis and degradation is needed for cell division to occur.

The receptor experiments showed that GRP, bombesin, B11 and B9 all competed for ^{125}I -labelled GRP binding to the Swiss 3T3 cells. Approx. 2-fold higher concentrations of B9 or B11 were required to inhibit tracer binding by 50 %, while neither B7 nor B5 competed even at concentrations 1000-fold higher than were effective with GRP, bombesin, B11 or B9. This sharp break in receptor binding between the heptapeptide and nonapeptide is also found with effects on protein synthesis. In those measurements all the peptides containing the C-terminal nonapeptide of bombesin led to a stimulation of protein synthesis. A significant level of stimulation occurred when $1\text{ }\mu\text{M}$ -B7 was added to the

cells, at least in the absence of insulin (Fig. 3). The difference between this response by the heptapeptide and the lack of heptapeptide competition for GRP binding can be explained by the 10-fold higher concentration needed for ligand competition, a result also reported in bombesin investigations with other cell types (Jensen *et al.*, 1978). The inference that a heptapeptide concentration greater than $1\text{ }\mu\text{M}$ would have displaced the tracer is also supported by the measurements of Zachary & Rozengurt (1985), where very high concentrations of the C-terminal heptapeptide did reduce the binding of labelled GRP.

Although systematic studies on the minimal C-terminal requirement for binding to and responsiveness of the GRP/bombesin receptor have not been carried out previously, several investigators have evaluated effects produced by partially homologous naturally-occurring peptides. The peptides tested were neuromedin B, a molecule differing by substitutions of leucine, threonine and phenylalanine at positions 7, 10 and 13 (of bombesin, Table 1) from the C-terminal decapeptide of bombesin; litorin, a nonapeptide equivalent to the C-terminal nine amino acids of bombesin but with substitutions of pyroglutamyl and phenylalanyl residues at positions 6 and 13 respectively (of bombesin, Table 1), and GRP10, the C-terminal decapeptide of GRP isolated from canine intestine (Reeve *et al.*, 1983) that has histidine replacing glutamine at position 7 (of bombesin). Of these three peptides, litorin showed near equal potency with bombesin to stimulate amylase output and calcium efflux from pancreatic fragments (Deschodt-Lanckman *et al.*, 1976), insulin secretion by pancreatic islet cells (Swope & Schonbrunn, 1984) as well as rubidium uptake and calcium release by Swiss 3T3 cells (Mendoza *et al.*, 1986). On the other hand, concentrations of neuromedin B at least 10-fold higher than bombesin were needed to stimulate DNA synthesis (Zachary & Rozengurt, 1985), rubidium uptake or calcium efflux (Mendoza *et al.*, 1986) in Swiss 3T3 cells or to increase the levels of gastrin and gastrin inhibitory peptide in rats (Greeley *et al.*, 1986). GRP-10 was also much less potent than bombesin as a mediator of gastrin release *in vivo* (Greeley *et al.*, 1986; Orloff *et al.*, 1984). In another investigation where the synthetic bombesin C-terminal nonapeptide was tested on pancreatic fragments, it was shown to be equivalent with bombesin in its ability to stimulate amylase output and calcium release (Deschodt-Lanckman *et al.*, 1976). Finally, pharmacological actions by the N-terminal octapeptide of bombesin and marginal effects by the heptapeptide on smooth muscle preparations have been demonstrated by Broccardo *et al.* (1975). We conclude from these results and those described in the present study that: (a) more than seven but no more than nine C-terminal amino acids in the bombesin sequence are required for high-affinity receptor binding and biological responsiveness, (b) substitution of glutamine, the eighth amino acid from the C-terminus, with either leucine or histidine leads to reduced potencies of decapeptides, but not of the much longer GRP molecule, (c) substitution of phenylalanine for leucine at position 13 of the bombesin sequence does not reduce activity, and (d) receptor-binding and sequence requirements for function are very similar between a number of cell types.

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