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High-affinity receptors for peptides of the bombesin family in Swiss 3T3 cells

(growth factors/gastrin-releasing peptide/platelet-derived growth factor/bombesin antagonist)

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ABSTRACT **Gastrin-releasing peptide (GRP) labeled with ^{125}I at tyrosine-15 (^{125}I-GRP) binds to intact quiescent Swiss 3T3 cells in a specific and saturable manner. Scatchard analysis indicates the presence of a single class of high-affinity binding sites of $K_d = 0.5 \times 10^{-9}$ M and a value for the number of sites per cell of about 100,000. ^{125}I-GRP binding was not inhibited by other mitogens for these cells, and cell lines that are mitogenically unresponsive to GRP do not exhibit specific GRP binding. Structure–activity relationships show a close parallel between the ability of a range of GRP-related peptides to both inhibit GRP binding and to stimulate mitogenesis. Further, GRP binding is selectively blocked in a competitive fashion by a novel bombesin antagonist, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]substance P. In addition, this compound selectively inhibits GRP and bombesin-induced mitogenesis. These results demonstrate that the mitogenic response of Swiss 3T3 cells to peptides of the bombesin family is mediated by a class of receptors distinct from those of other mitogens for these cells.**

A rapidly increasing number of new regulatory peptides have been identified in the brain, gastrointestinal tract, and other tissues (1–3). These peptides appear to behave as local hormones or neurotransmitters, acting in a paracrine or autocrine fashion on adjacent cells. Recently, we reported that the amphibian tetradecapeptide bombesin (4), which has potent pharmacological and physiological effects in animals (5–8), is a mitogen for Swiss 3T3 cells at nanomolar concentrations (9).

Peptides related to bombesin, including gastrin-releasing peptide (GRP) and the neuromedins, are widely distributed in normal mammalian tissues (10–16) and also are found in high levels in human pulmonary (17–20) and thyroid (21) tumors. The finding that bombesin is mitogenic (9) and the wide distribution of endogenous mammalian analogues of bombesin strongly suggest that this family of peptides may play a role in the control of both normal and abnormal animal cell growth.

An important step in the elucidation of the mechanism of bombesin-stimulated mitogenesis is to determine the presence of specific receptors for this peptide in Swiss 3T3 cells. We have used biologically active, radiolabeled GRP, the mammalian counterpart of bombesin, to establish the presence of receptors for this molecule.

In the present paper, we show that the mitogenic stimulation of Swiss 3T3 cells by peptides of the bombesin family is mediated by a single class of high-affinity receptors. This receptor is distinct from those of other mitogens for these cells. Further, a substance P (SP) analogue recently described as a bombesin antagonist (22) selectively blocks both GRP binding and GRP-induced mitogenesis. Since there is evidence that bombesin-like peptides may contribute to the self-stimulatory (autocrine) growth of small-cell carcinoma of the lung (18–20, 23, 24, 47), our findings may suggest novel clinical approaches to the treatment of this important cancer.

MATERIALS AND METHODS

Cell culture procedures (9, 25, 26) and assays of DNA synthesis by [^{3}H]thymidine incorporation (26) were carried out as described.

Binding Assay. For binding at 37°C, confluent and quiescent cultures of Swiss 3T3 cells in 33-mm dishes were washed twice with Dulbecco's modified Eagle's medium (DME medium) and incubated with 1 ml of binding medium, which consisted of 1:1 (vol/vol) DME and Waymouth media supplemented with 1 mg of bovine serum albumin per ml, 50 mM 2-[bis(2-hydroxyethyl)-2-amino]ethanesulfonic acid (Bes) (pH 7.0), and GRP labeled with ^{125}I at tyrosine-15 (^{125}I-GRP) at the concentrations indicated. After 30 min of incubation (unless otherwise stated), cultures were washed rapidly four times with cold (4°C) wash solution (0.15 M NaCl/5 mM KCl/0.02 M Na_2HPO_4/1.8 mM KH_2PO_4, pH 7.2, supplemented with bovine serum albumin at 1 mg/ml). Washed cultures were extracted in 0.5 ml of 0.1 M NaOH containing 2% Na_2CO_3 and 1% $NaDodSO_4$, and total cell-associated radioactivity was determined in a γ counter. Nonspecific binding, defined as the cell-associated radioactivity not displaced in the presence of 360 nM GRP, was proportional to the concentration of ^{125}I-GRP and varied from 23% of total binding at the highest concentrations (3 nM) of ^{125}I-GRP to 5% at low concentrations (0.05 nM). For binding at 4°C, cells were washed once with DME medium at 4°C and incubated at 4°C for 5 min prior to the binding assay. The binding assay at 4°C was performed in 0.14 M NaCl/5 mM KCl/0.01 M Na_2HPO_4/1.8 mM KH_2PO_4/1.8 mM $CaCl_2$/1 mM $MgCl_2$/25 mM Hepes, pH 7.0, supplemented with bovine serum albumin at 1 mg/ml. All values shown are the average of duplicate determinations that agree within 13%.

Materials. ^{125}I-GRP (≈2000 Ci/mmol; 1 Ci = 37 GBq) and [^{3}H]thymidine were obtained from the Radiochemical Centre. Bombesin and insulin were obtained from Sigma. The 14- to 27-amino acid fragment of GRP [GRP-(14–27)], neuromedin B, and the 1- to 16-amino acid fragment of GRP [GRP-(1–16)] were obtained from Peninsula Laboratories (San Carlos, CA). The complete GRP molecule of 1–27 amino acids [GRP-(1–27)], bombesin-(8–14), and [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP were obtained from Bachem Fine Chemicals. Fibroblast-derived growth factor (FDGF) was isolated and purified from the medium conditioned by simian virus 40-infected baby hamster kidney cells as described (27, 28). The serum used was fetal bovine (GIBCO). Platelet-derived growth factor (PDGF) was of porcine origin and was partially

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Abbreviations: GRP, gastin-releasing peptide; SP, substance P; FDGF, fibroblast-derived growth factor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; PGE, prostaglandin E.

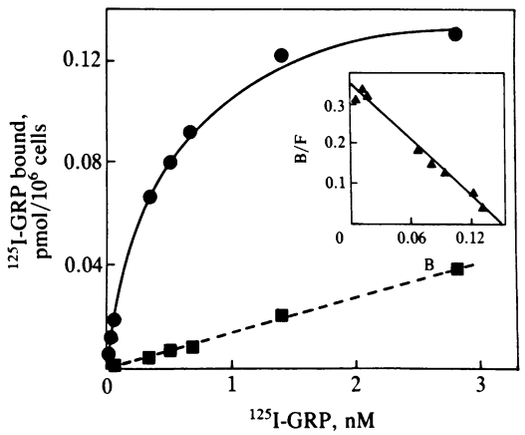

FIG. 1. Concentration-dependence of ^{125}I-GRP binding to intact quiescent Swiss 3T3 cells at 37°C. ●, Specific cell-associated radioactivity obtained at the indicated concentrations of radiolabeled ligand; ■, nonspecific binding. (*Inset*) Scatchard plot of the same data: bound (B) radiolabeled ligand is expressed in pmol per 10^6 cells; the concentration of ^{125}I-GRP in the medium (F) is in pmol/ml (nM). All other experimental details were as described.

purified as described (29). All other materials used were of reagent grade.

RESULTS

^{125}I-GRP Binds to Specific High-Affinity Sites in Swiss 3T3 Cells. When intact, quiescent Swiss 3T3 cells were incubated with ^{125}I-GRP at 37°C, cell-associated radioactivity increased rapidly, reaching a maximum value at 30 min and decreasing afterwards. At 4°C, binding occurred slowly, reaching equilibrium after 8 hr. Fig. 1 shows the specific ^{125}I-GRP binding to Swiss 3T3 cells as a function of the concentration of the radiolabeled ligand. Specific GRP binding was saturable, while nonspecific GRP binding increased linearly with increasing ligand concentration. Scatchard analysis (Fig. 1 *Inset*) of these data indicated the presence of a single class of high-affinity sites of $K_d = 0.5 \times 10^{-9}$ M and a value for the number of binding sites per cell of ≈100,000. Similar experiments performed at 4°C also showed saturable binding, the presence of a single class of high-affinity sites of a slightly higher K_d (0.9×10^{-9} M), and a value for the number of sites per cell of 88,000. The affinity of these binding sites agrees well with values obtained for bombesin binding to pancreatic acinar (30) and pituitary cells (31).

^{125}I-GRP binding was not inhibited by other mitogens for cultured fibroblasts. Swiss 3T3 cells were incubated with a very low concentration of ^{125}I-GRP (0.028 nM) in the presence of saturating concentrations of PDGF (29, 32), FDGF (27), epidermal growth factor (EGF) (33), vasopressin (34), phorbol 12,13-dibutyrate (33), insulin (33, 34), and two neuropeptides, vasoactive intestinal peptide and SP, that exhibit slight COOH-terminal homology with bombesin. ^{125}I-GRP binding was reduced to 5% of total cell-associated radioactivity by unlabeled GRP (1 μg/ml); in contrast, none of the other factors tested caused a significant decrease in ^{125}I-GRP binding, even at this low concentration of labeled ligand (Table 1). This result strongly suggests that peptides of the bombesin family interact with receptors that are different from those that recognize other mitogens for 3T3 cells.

Table 1. Specificity of GRP binding to Swiss 3T3 cells

Addition	^{125}I-GRP binding, pmol × 10^3 per 10^6 cells
—	7.7
GRP	0.4
PDGF	7.2
FDGF	7.5
EGF	7.9
Vasopressin	7.6
Phorbol 12,13-dibutyrate	6.5
Insulin	7.0
VIP	7.1
SP	7.0
PGE_1	7.4

^{125}I-GRP (0.028 nM; 2.46×10^6 cpm/pmol) binding to confluent cultures of Swiss 3T3 cells was measured at 37°C either in the absence or presence of the following factors at the concentrations indicated: GRP (360 nM), PDGF (1 μg/ml), FDGF (1 μg/ml), EGF (10 ng/ml), vasopressin (50 ng/ml), phorbol 12,13-dibutyrate (200 nM), insulin (1 μg/ml), vasoactive intestinal peptide (VIP) (1 μg/ml), SP (1 μg/ml), or PGE_1 (500 ng/ml). Identical results were obtained when the cultures were incubated for 5 min instead of 30 min.

Mitogenesis Is Mediated by High-Affinity Sites for Peptides of the Bombesin Family. The availability of a range of peptides structurally related to GRP (see Table 2) enabled us to examine the structure–activity relationships for their ability both to inhibit ^{125}I-GRP binding and to stimulate DNA synthesis. The addition of unlabeled GRP, GRP-(14–27), neuromedin B, or the 8- to 14-amino acid fragment of bombesin [bombesin-(8–14)] to 3T3 cells inhibited specific ^{125}I-GRP binding in a concentration-dependent manner (Fig. 2 *Lower*). The K_i values for GRP, GRP-(14–27), neuromedin B, and bombesin-(8–14) were, respectively, 0.7, 0.33, 7.6, and 888×10^{-9} M. In contrast, the NH_2-terminal fragment of GRP [GRP-(1–16)] did not inhibit binding at concentrations up to 5 μg/ml. It is noteworthy that ^{125}I-GRP stimulated DNA synthesis in Swiss 3T3 cells with the same potency as did unlabeled GRP.

Fig. 2 *Upper* shows the stimulation of DNA synthesis by the same peptides. There was a close parallelism between the relative mitogenic potencies of these agonists and their ability to competitively displace specifically bound ^{125}I-GRP. Bombesin, litorin, and neuromedin C also inhibited GRP binding and stimulated mitogenesis with very similar dose–response curves to that for GRP. These results strongly suggest that the mitogenic stimulation of 3T3 cells by peptides of the bombesin family is mediated by a specific class of high-affinity sites. In addition, these findings also indicate that the stereospecific requirements for binding to this receptor are satisfied by a peptide either identical with or very similar to the highly conserved COOH-terminal heptapeptide of this family of peptides (see Table 2).

To further strengthen our conclusion, we investigated the binding of ^{125}I-GRP to other cell lines, including BALB/c 3T3, Swiss 3T6, and whole-mouse embryo fibroblasts, that

Table 2. Amino acid sequence of peptides of the bombesin family

GRP-(1–27)	Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-*Trp-Ala-Val-Gly-His-Leu-Met*
Bombesin	Glu-Gln-Arg-Leu-Gly-Asn-Gln-*Trp-Ala-Val-Gly-His-Leu-Met*
GRP-(14–27)	Met-Tyr-Pro-Arg-Gly-Asn-His-*Trp-Ala-Val-Gly-His-Leu-Met*
Litorin	Glu-Gln-*Trp-Ala-Val-Gly-His-Phe-Met*
Neuromedin C	Gln-Asn-His-*Trp-Ala-Val-Gly-His-Leu-Met*
Neuromedin B	Gly-Asn-Leu-*Trp-Ala*-Thr-*Gly-His-Phe-Met*
Bombesin-(8–14)	*Trp-Ala-Val-Gly-His-Leu-Met*
GRP-(1–16)	Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro

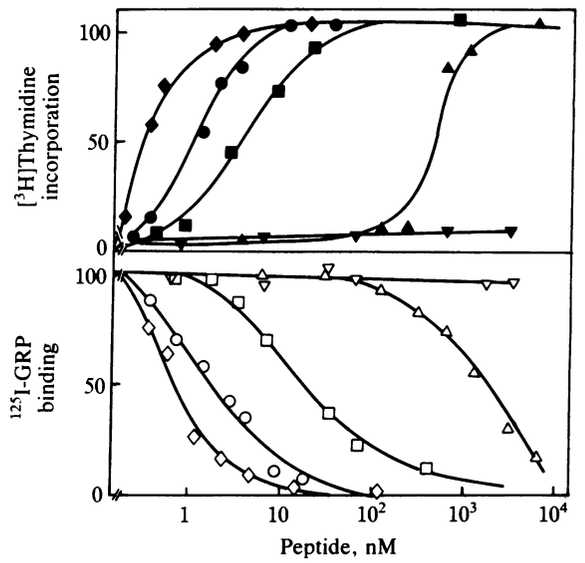

FIG. 2. Stimulation of DNA synthesis (*Upper*) and inhibition of specific ^{125}I-GRP binding (*Lower*) in intact, quiescent Swiss 3T3 cells by GRP (○, ●), GRP-(14–27) (◇, ◆), neuromedin B (□, ■), bombesin-(8–14) (△, ▲), and GRP-(1–16) (▽, ▼). The assay for DNA synthesis was performed in the presence of insulin at 1 μg/ml. Each point represents the mean (n = 6; SEM < 8%) of values for [^{3}H]thymidine incorporation obtained from duplicate cultures in different experiments, expressed as a percentage of the effect obtained in the presence of a saturating concentration (6.2 nM) of bombesin and insulin at 1 μg/ml. Binding of ^{125}I-GRP is expressed as a percentage of the specific binding (0.017 pmol per 10^6 cells; n = 21; SEM < 6%) in the absence of any unlabeled competitor.

are mitogenically stimulated by growth factors such as PDGF but are unresponsive to bombesin or GRP. We found that the inability of peptides of the bombesin family to produce a mitogenic response correlates with the absence of specific GRP binding.

A Selective Antagonist Blocks GRP Binding and Mitogenesis. SP exhibits some homology with the mitogenically active COOH-terminal region of bombesin. However, SP did not inhibit ^{125}I-GRP binding (Table 1) and also failed to stimulate DNA synthesis in Swiss 3T3 cells at concentrations up to 10 μg/ml (ref. 9 and unpublished data). The SP analogue [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (35) has been reported recently to be an inhibitor of bombesin binding and bombesin-stimulated amylase secretion in pancreatic acinar cells (22). To further elucidate the role of GRP binding sites in mitogenesis, we examined the effect of this putative antagonist on GRP binding and bombesin-induced mitogenesis. Fig. 3 *Left* shows that [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP caused a concentration-dependent decrease in the specific binding of a fully mitogenic concentration of ^{125}I-GRP (1.5 nM). Half-maximal inhibition of binding was achieved at 7 μM, and, at the highest concentration of antagonist used (100 μM), binding was reduced to 5% of its maximum value. The K_i for the antagonist is 2×10^{-6} M.

The binding of different concentrations of ^{125}I-GRP was measured in the presence and absence of 25 and 50 μM [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. A double-reciprocal plot of these data (Fig. 3 *Right*) shows that, although the antagonist does not significantly alter the number of binding sites for ^{125}I-GRP, it markedly reduces the affinity of the receptor for its ligand in a competitive fashion. Furthermore, the addition of antagonist to Swiss 3T3 cells preincubated with ^{125}I-GRP caused a rapid (1.5 min) release of cell-associated radioactivity into the medium.

The mitogenic effect of bombesin and GRP is strikingly inhibited by the addition of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (Fig. 4). The antagonist at a concentration of 100 μM markedly increased (by 5- to 10-fold) the concentrations of bombesin and GRP required to produce half-maximal stimulation of DNA synthesis. The fact that inhibition is completely reversed at higher concentrations of bombesin and GRP demonstrates that the antagonist is not toxic to 3T3 cells.

The selectivity of the inhibition of mitogenesis induced by peptides of the bombesin family by [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP was shown by testing its effect on the stimulation of DNA synthesis by other mitogens. The mitogenic stimulation of 3T3 cells by PDGF, FDGF, EGF, phorbol 12,13-dibutyrate, and the cyclic AMP-increasing agents cholera toxin, prostaglandin E_1 (PGE_1), and 8-Br-cAMP (36–38) was not significantly impaired by 75 μM [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP (Table 3). In contrast, the same concentration of the antagonist decreased the stimulation of DNA synthesis by GRP (1.4 nM) to only 8%.

Bombesin and PDGF Interact with Distinct Receptors. The possibility that PDGF and peptides of the bombesin family interact with a common receptor is suggested by the striking similarity exhibited by these growth factors in their ability to stimulate mitogenesis in the absence of other growth-promoting agents. We have already shown (Table 1) that satu-

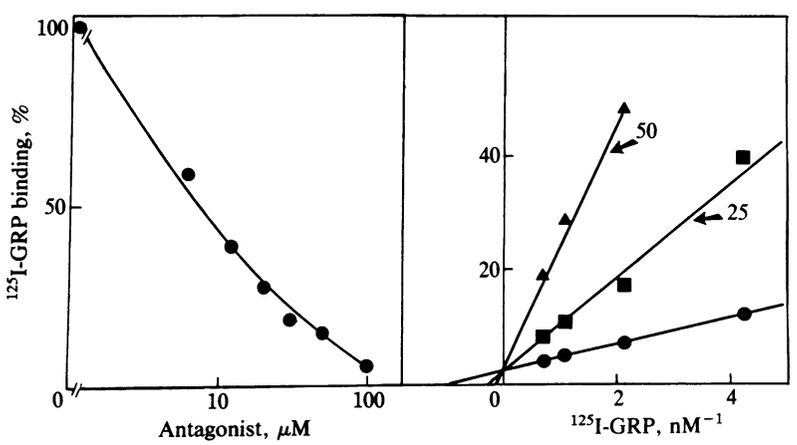

FIG. 3. (*Left*) Inhibition of specific ^{125}I-GRP binding to intact, quiescent Swiss 3T3 cells by the antagonist, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. Binding of ^{125}I-GRP (0.1 μCi/ml; 1.5 nM) measured at 37°C is expressed as a percentage of the specific binding in the absence of the antagonist. All other experimental details were as described. (*Right*) Effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP on the affinity of binding sites in 3T3 cells for ^{125}I-GRP. Quiescent 3T3 cells were incubated with different concentrations of ^{125}I-GRP in the absence (●) or presence of either 25 (■) or 50 μM (▲) [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP. The figure shows a double-reciprocal plot of the data obtained from this experiment.

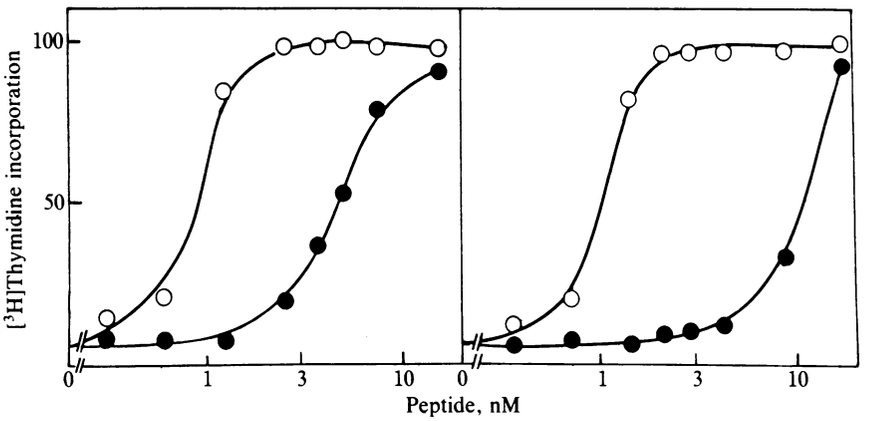

FIG. 4. Inhibition of bombesin (*Left*)- and GRP (*Right*)-induced mitogenesis by [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. Quiescent 3T3 cells were incubated in the presence of insulin at 1 μg/ml and various concentrations of either bombesin or GRP, either in the absence (○) or presence (●) of 100 μM [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. All other experimental details were as described.

rating concentrations of PDGF do not inhibit the binding of trace levels of ^{125}I-GRP. However, the availability of a bombesin antagonist (Figs. 3 and 4) provided us with an independent means of distinguishing between the receptors for PDGF and peptides of the bombesin family. Fig. 5 shows the effect of different concentrations of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP on the mitogenic effect of GRP (1.4 nM), PDGF (250 ng/ml), and EGF (2.5 ng/ml). In contrast to GRP, the effect of PDGF was only slightly reduced by the antagonist, whereas that of EGF was unaffected.

DISCUSSION

The results presented here demonstrate that the interaction of peptides of the bombesin family with specific, high-affinity sites is a primary event in their mitogenic stimulation of quiescent fibroblasts. The binding of ^{125}I-GRP to Swiss 3T3 cells is specific, saturable, and reversible. Scatchard analysis of GRP binding at 37°C indicates the presence of a single class of binding sites of $K_d = 0.5 \times 10^{-9}$ M. A similar K_d value was obtained when binding was performed at 4°C.

An important aspect in the study of hormone receptors is the comparison between the ability of hormonal analogues to bind to high-affinity sites and their potency in eliciting a biological response (38–41). Our results demonstrate that the relative ability of peptides of the bombesin family to inhibit ^{125}I-GRP binding closely corresponds to their mitogenic potency. These structure–activity relationships strongly suggest that the high-affinity sites described here are responsible for mediating the mitogenic response elicited by these peptides. Our conclusion is further supported by the finding that cell lines that do not respond mitogenically to bombesin also do not bind ^{125}I-GRP.

The use of specific antagonists has been of considerable importance in defining the role of specific receptors in hormonal action. Recently, a SP analogue was reported as the first known bombesin antagonist (22). Despite the fact that it was originally described as a SP antagonist (35), the inability of Swiss 3T3 cells to respond to SP allowed us to use this putative bombesin inhibitor to strengthen our view that peptides of the bombesin family interact with receptors that are different from those of other mitogens for these cells, and in particular, PDGF. Bombesin, PDGF, and the PDGF-like growth factor FDGF (27, 36), in contrast to other growth-promoting agents, are able to stimulate mitogenesis in the absence of any exogenously added factors (9). In addition, we have observed a striking similarity between these mitogens in their ability to elicit a number of diverse early responses,

Table 3. Selectivity of the effect of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP on GRP-induced mitogenesis in Swiss 3T3 cells

Factor	[3H]Thymidine incorporation, cpm × 10^{-6}	
	No additions	With SP analogue
Insulin	0.05	0.05
GRP	1.69	0.13
PDGF	1.67	1.33
FDGF	1.55	1.58
EGF	1.63	1.59
Phorbol 12,13-dibutyrate	1.51	1.33
Cholera toxin	0.54	0.55
PGE_2	0.45	0.38
8-Br-cAMP	1.19	1.20

[3H]Thymidine incorporation was measured as described in the presence of insulin at 5 μg/ml and the following factors at the concentrations indicated: GRP (1.43 nM), PDGF (250 ng/ml), FDGF (250 ng/ml), EGF (2.5 ng/ml), phorbol 12,13-dibutyrate (35 ng/ml), cholera toxin (100 ng/ml), PGE_2 (500 ng/ml), or 8-Br-cAMP (2.5 mM) either in the presence or absence of 75 μM [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP.

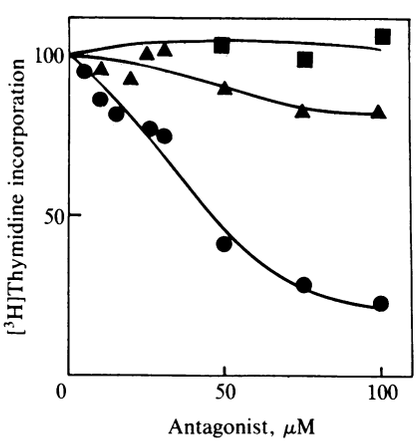

FIG. 5. Concentration-dependence of the effect of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP on the mitogenic response of 3T3 cells to GRP, PDGF, and EGF. 3T3 cells were incubated with insulin at 5 μg/ml and 1.4 nM GRP (●), 250 ng of PDGF per ml (▲), or 2.5 ng of EGF per ml (■) in the presence of different concentrations of the antagonist [D-Arg[1], D-Pro[2], Trp[7,9], Leu[11]]SP. Values for the incorporation of [3H]thymidine into acid-precipitable material are expressed as the percentage of the effect obtained in the absence of [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]]SP. Each point represents the mean of four determinations for GRP and PDGF and two for EGF.

including the stimulation of monovalent and divalent ion fluxes, the inhibition of EGF binding, an increase in the level of intracellular cAMP, and an increase in the phosphorylation of a protein of M_r 80,000 (refs. 42–44; unpublished data). Thus, it was possible that bombesin was interacting with the PDGF receptor rather than a distinct class of binding site. We have shown that (*i*) PDGF at a saturating concentration does not inhibit the binding of low concentrations of ^{125}I-GRP; (*ii*) cell lines that are mitogenically stimulated by PDGF but unresponsive to GRP do not exhibit ^{125}I-GRP binding; (*iii*) the bombesin antagonist [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP blocks both bombesin and GRP-induced mitogenesis but has only a slight effect on the stimulation of DNA synthesis by PDGF. These results support the conclusion that the mitogens, PDGF, and bombesin interact with distinct receptors.

There is now intense interest in the production of growth factors by tumor cells that play a role in sustaining abnormal cell proliferation by self-stimulatory (autocrine) growth circuits (9, 23, 38, 41). In view of the potent mitogenic effects of bombesin, it has been suggested that the ectopic production of this peptide by small-cell carcinoma of the lung may play an important role as part of an autocrine circuit that contributes to the rapid growth of this important tumor (9). This suggestion has been supported by the recent findings that bombesin is a growth factor for normal bronchial epithelial cells (45) and small-cell lung carcinoma cells (46) and that anti-bombesin/GRP monoclonal antibodies inhibit the growth of these cells both *in vitro* and in nude mice (23, 24, 47). The results presented here show that a bombesin antagonist selectively blocks both bombesin and GRP-induced mitogenesis in Swiss 3T3 cells. This raises the possibility that antagonists for peptides of the bombesin family could be of considerable importance in future approaches to the clinical treatment of small-cell carcinoma of the lung.

1. Grossman, M. I. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2341–2343.
2. Costa, M. & Furness, J. B. (1982) *Br. Med. Bull.* **38**, 247–252.
3. Polak, J. M. & Bloom, S. R. (1982) *Br. Med. Bull.* **38**, 303–307.
4. Anastasi, A., Erpsamer, V. & Bucci, M. (1971) *Experientia* **27**, 166–167.
5. Rivier, J. E. & Brown, M. R. (1978) *Biochemistry* **17**, 1766–1771.
6. Tache, Y., Vale, W., Rivier, J. & Brown, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5511–5519.
7. Gibbs, J., Fauser, D. J., Rowe, E. A., Rolls, B. J., Rolls, E. T. & Maddison, S. P. (1979) *Nature (London)* **282**, 208–210.
8. Swope, S. L. & Schonbrunn, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1822–1826.
9. Rozengurt, E. & Sinnett-Smith, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2936–2940.
10. McDonald, T. J., Jornvall, H., Nilsson, G., Vagne, M., Ghatei, M., Bloom, S. R. & Mutt, V. (1979) *Biochem. Biophys. Res. Commun.* **90**, 227–233.
11. Wharton, J., Polak, J. M., Bloom, S. R., Ghatei, M. S., Solicia, E., Brown, M. R. & Pearse, A. E. G. (1978) *Nature (London)* **273**, 769–770.
12. Walsh, J. A., Wong, H. C. & Dockray, G. J. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2315–2319.
13. Moody, T. W. & Pert, C. B. (1979) *Biochem. Biophys. Res. Commun.* **90**, 7–14.
14. Ghatei, M. A., Sheppard, M. N., O'Shaughnessy, D. J., Adrian, T. E., McGregor, G. P., Polak, J. M. & Bloom, S. R. (1982) *Endocrinology* **111**, 1248–1254.
15. Minamino, N., Kangawa, K. & Matsuo, H. (1983) *Biochem. Biophys. Res. Commun.* **114**, 541–548.
16. Minamino, N., Kangawa, K. & Matsuo, H. (1984) *Biochem. Biophys. Res. Commun.* **124**, 925–932.
17. Moody, T. W., Pert, C. B., Gazdar, A. F., Carney, D. N. & Minna, J. D. (1981) *Science* **214**, 1246–1248.
18. Wood, S. M., Wood, J. R., Ghatei, M. A., Lee, Y. C., O'Shaughnessy, D. & Bloom, S. R. (1981) *J. Clin. Endocrin. Metab.* **53**, 1310–1312.
19. Erisman, M. D., Linnoila, R. I., Hernandez, O., DiAugustine, R. P. & Lazarus, L. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2379–2383.
20. Roth, K. A., Evans, C. J., Weber, E., Barchas, J. D., Bostwick, D. G. & Bersch, K. G. (1983) *Cancer Res.* **43**, 5411–5415.
21. Matsubayashi, S., Yanaihara, C., Ohrubo, M., Fukata, S., Hayashi, Y., Tamai, H., Nakagawa, T., Miyauchi, A., Kuma, K., Abe, K., Suzuki, T. & Yanaihara, N. (1984) *Cancer* **53**, 2472–2477.
22. Jensen, R. T., Jones, S. W., Falkers, K. & Gardner, J. D. (1984) *Nature (London)* **309**, 61–63.
23. Minna, J. D. (1984) *Proc. Am. Assoc. Cancer Res.* **25**, 393–394.
24. Sporn, M. B. & Roberts, A. B. (1985) *Nature (London)* **313**, 745–747.
25. Todaro, G. J. & Green, H. (1963) *J. Cell Biol.* **17**, 299–313.
26. Dicker, P. & Rozengurt, E. (1980) *Nature (London)* **287**, 607–612.
27. Rozengurt, E., Collins, M., Brown, K. D. & Pettican, P. (1982) *J. Biol. Chem.* **257**, 3680–3686.
28. Bourne, H. R. & Rozengurt, E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4555–4559.
29. Rozengurt, E., Stroobant, P., Waterfield, M. D., Deuel, T. F. & Keehan, M. (1983) *Cell* **34**, 265–272.
30. Jensen, R. T., Moody, T., Pert, C., Rivier, J. E. & Gardner, J. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6139–6143.
31. Westendorf, J. M. & Schonbrunn, A. C. (1983) *J. Biol. Chem.* **258**, 7527–7535.
32. Ross, R. & Vogel, A. (1978) *Cell* **14**, 203–210.
33. Dicker, P. & Rozengurt, E. (1978) *Nature (London)* **276**, 723–726.
34. Rozengurt, E., Legg, A. & Pettican, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1284–1287.
35. Lundberg, J. M., Saria, A., Brodin, E., Rosell, S. & Folkers, K. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1120–1124.
36. Dicker, P., Pohjanpelto, P., Pettican, P. & Rozengurt, E. (1981) *Exp. Cell Res.* **135**, 221–227.
37. Rozengurt, E. (1980) *Curr. Top. Cell. Regul.* **17**, 59–88.
38. Rozengurt, E. (1983) *Mol. Biol. Med.* **1**, 169–181.
39. Catt, K. J., Harwood, J. P., Aguitera, G. & Dufani, M. L. (1979) *Nature (London)* **280**, 109–116.
40. Kahn, C. R. (1976) *J. Cell Biol.* **70**, 261–286.
41. Rozengurt, E. & Collins, M. (1983) *J. Pathol.* **141**, 309–331.
42. Rozengurt, E., Rodriguez-Pena, A. & Smith, K. A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7244–7248.
43. Rodriguez-Pena, A. & Rozengurt, E. (1985) *EMBO J.* **4**, 71–76.
44. Rozengurt, E. (1985) in *Molecular Mechanisms of Transmembrane Signalling*, eds. Cohen, P. & Houslay, M. (Elsevier Science, Amsterdam), pp. 429–452.
45. Willey, J. C., Lechner, J. F. & Harris, C. C. (1984) *Exp. Cell Res.* **153**, 245–248.
46. Weber, S., Zuckarman, J. E., Bostwick, D. G., Bersch, K. G., Sikic, B. I. & Raffin, T. A. (1985) *J. Clin. Invest.* **75**, 306–309.
47. Cuttitta, F., Carney, D. N., Mulshine, J., Moody, T. W., Fedorko, J., Fischler, A. & Minna, J. O. (1985) *Nature (London)* **316**, 823–826.