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[D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P, a potent bombesin antagonist in murine Swiss 3T3 cells, inhibits the growth of human small cell lung cancer cells *in vitro*

(gastrin-releasing peptide/vasopressin/growth factors/neuropeptides)

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ABSTRACT **In the search for a more potent bombesin antagonist, we found [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P to be effective in mouse fibroblasts and to inhibit the growth of small cell lung cancer, a tumor that secretes bombesin-like peptides that may act as autocrine growth factors. In murine Swiss 3T3 cells, [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P proved to be a bombesin antagonist as judged by the following criteria: (*i*) inhibition of DNA synthesis induced by gastrin-releasing peptide and other bombesin-like peptides; (*ii*) inhibition of ^{125}I-labeled gastrin-releasing peptide binding to the bombesin/gastrin-releasing peptide receptor; (*iii*) reduction in cross-linking of the M_r 75,000–85,000 protein putatively a component of the bombesin/gastrin-releasing peptide receptor; (*iv*) blocking of early cellular events that precede mitogenesis—calcium mobilization and inhibition of epidermal growth factor binding. [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P was 5-fold more potent than the antagonist [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]substance P. [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P also inhibits mitogenesis induced by vasopressin but not that induced by a variety of other mitogens. Both antagonists reversibly inhibited the growth of small cell lung cancer *in vitro* in a concentration-dependent manner. Peptide antagonists could, therefore, have far-reaching therapeutic implications.**

Regulatory peptides that act as local hormones or neurotransmitters are increasingly implicated in the control of cell proliferation (1). The amphibian tetradecapeptide bombesin and structurally related mammalian peptides, including gastrin-releasing peptide (GRP), are potent mitogens for Swiss 3T3 cells (2, 3). The peptides bind to high-affinity cell-surface receptors in these cells (3) and elicit a complex array of early biological responses (4), including enhanced inositol phospholipid breakdown and mobilization of Ca^{2+} from intracellular stores (5–7), stimulation of Na^+/H^+ antiport activity (6), activation of protein kinase C (8–10), inhibition of ^{125}I-labeled epidermal growth factor (EGF) binding (8, 9, 11), and induction of the cellular oncogenes c-*fos* and c-*myc* (12–14). Zachary and Rozengurt (15) identified a surface protein in Swiss 3T3 cells with M_r 75,000–85,000 as a putative component of the bombesin/GRP receptor.

The widely distributed tachykinin substance P, which has slight amino acid sequence homology with bombesin (Fig. 1), neither inhibits the binding of GRP nor stimulates DNA synthesis in Swiss 3T3 cells (2, 3). However, [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]substance P (peptide A in Fig. 1), which was synthesized as a substance P antagonist (16), was found to block the secretory effects of bombesin in pancreatic acinar cells (17) and to antagonize the growth-promoting effects of bombesin in Swiss 3T3 cells (3, 6, 9, 17, 18). This antagonist also inhibits mitogenesis stimulated by the neurohypophyseal hormone vasopressin (19). It appears that peptide A interacts with several independent cellular receptors to block their biological effects.

Bombesin-like peptides are present in high concentrations in small cell lung cancer (SCLC) (20–22). They can stimulate the growth of SCLC cells *in vitro* (23, 24) and may act as autocrine growth factors (2, 25). The dilutional hyponatraemic syndrome, which commonly complicates SCLC, is associated with ectopic production of vasopressin (26, 27). Thus, the identification of more potent antagonists, which recognize the receptors for both peptides, could be of considerable interest in future clinical approaches to the treatment of SCLC.

Here we report that [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P (peptide D in Fig. 1) is 5-fold more potent than peptide A in preventing the cellular effects of GRP and vasopressin in mouse 3T3 cells and in inhibiting the growth of SCLC cells in serum-free medium.

MATERIALS AND METHODS

Swiss 3T3 cells were maintained in culture, and assays of DNA synthesis were performed by measuring [^{3}H]thymidine incorporation as described (2, 28). Receptor binding assays utilized ^{125}I-labeled GRP and EGF (3, 9). Cross-linking of ^{125}I-labeled GRP to a M_r 75,000–85,000 surface protein was carried out as described (15). Cytosolic [Ca^{2+}] was measured by fluorimetry by using the indicator fura-2 (6). SCLC lines were obtained from the American Type Culture Collection.

Radiochemicals were obtained from Amersham. Bombesin, GRP, insulin, and vasopressin were from Sigma. Peptides A, B, C, D, E, F, and K (Fig. 1) were obtained from Peninsula Laboratories (San Carlos, CA), and peptides A, G, H, and J were from Bachem Fine Chemicals (Torrance, CA).

RESULTS

Peptide D Is a Potent Inhibitor of GRP-Mediated Mitogenesis. To identify a more potent antagonist of bombesin-like peptides, we have tested 10 substance P antagonists at 50 μM (Fig. 1) for their ability to inhibit mitogenesis stimulated by GRP [the mammalian homologue of bombesin (29)] in Swiss 3T3 cells. Peptide D was clearly the most potent GRP antagonist. In contrast, peptides B, C, E, F, G, H, J, and K

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Abbreviations: EGF, epidermal growth factor; GRP, gastrin-releasing peptide; PDGF, platelet-derived growth factor; peptide A, [D-Arg^1,D-Pro^2,D-Trp^{7,9},Leu^{11}]substance P; peptide D, [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P; SCLC, small cell lung cancer.

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Bombesin	pGlu	Gln	Arg	Leu	Gly	Asn	Gln	Trp	Ala	Val	Gly	His	Leu	Met	NH_2
				1	2	3	4	5	6	7	8	9	10	11	
Substance P					Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met	NH_2
Antagonist A					**DArg**	**DPro**	**Lys**	**Pro**	**Gln**	**Gln**	**DTrp**	**Phe**	**DTrp**	**Leu**	**Leu**	**NH_2**
B					DArg	Pro	Lys	Pro	Gln	Gln	DTrp	Phe	DTrp	Leu	Leu	NH_2
C					Arg	DPro	Lys	Pro	Gln	Gln	DPhe	Phe	DTrp	Leu	Met	NH_2
D					**DArg**	**Pro**	**Lys**	**Pro**	**DPhe**	**Gln**	**DTrp**	**Phe**	**DTrp**	**Leu**	**Leu**	**NH_2**
E					Arg	DPro	Lys	Pro	Gln	Gln	DTrp	Phe	DTrp	Leu	Met	NH_2
F								DPro	Gln	Gln	DTrp	Phe	DTrp	DTrp	Met	NH_2
G										Arg	DTrp	MePhe	DTrp	Leu	Met	NH_2
H					DArg	DPro	Lys	Pro	Gln	Gln	DPhe	Phe	DHis	Leu	Met	NH_2
J								HArg	Gly	Gln	DTrp	Phe	Gly	Asp	$(OtBu)_2$	
K								DPro	Gln	Gln	DTrp	Phe	DTrp	Leu	Met	NH_2

FIG. 1. Amino acid sequences of the substance P antagonists studied. These peptides were tested as inhibitors of GRP-induced DNA synthesis in Swiss 3T3 cells in the presence of [^{3}H]thymidine at 1 μCi/ml (1 Ci = 37 GBq) (1 μM), insulin at 1 μg/ml, GRP at 2.7 nM, and the substance P antagonist to be tested at 50 μM (details as in Fig. 2). The peptides were also screened at 50 μM for inhibition of vasopressin-induced DNA synthesis and binding of ^{125}I-labeled GRP to Swiss 3T3 cells. Peptide D was the most potent in repeated assays. Similar results were obtained with peptide A from Bachem Fine Chemicals and Peninsula Laboratories. Peptide D synthesized in the Imperial Cancer Research Fund Laboratories by J. Rothbard gave similar results to the peptide obtained from Peninsula Laboratories. Substance P itself exhibited no antagonist activity.

were less potent than either peptide A or peptide D. Spantide (peptide B) had no antagonist activity even at 100 μM. None of the peptides stimulated DNA synthesis when tested at 20 μM with insulin at 1 μg/ml—i.e., none exhibited any agonist activity.

Following the identification of peptide D as the most promising GRP antagonist, we compared its potency with that of peptide A. Fig. 2 shows that peptide D at 20 μM markedly increased the concentration of GRP required to produce half-maximal stimulation of DNA synthesis whereas addition of peptide A also at 20 μM had only a slight effect. Inhibition of DNA synthesis by peptide D was completely reversed by high concentrations of GRP, indicating that its inhibitory effect was competitive and reversible. The dose–response curves for the two antagonists in the presence of 3.6 nM GRP are shown in Fig. 2 *Right*. Half-maximal inhibition of DNA synthesis was obtained with 22 μM peptide D and 118 μM peptide A.

Specificity of Peptide D Inhibition of Mitogenesis. Fig. 3 *Left* shows that peptide D added at 50 μM did not inhibit DNA synthesis stimulated by fully mitogenic doses of phorbol 12,13-dibutyrate, cholera toxin with isobutylmethylxanthine, EGF, or platelet-derived growth factor (PDGF) in the presence of insulin. In contrast, the same concentration of peptide D markedly decreased the stimulation of DNA synthesis by bombesin and GRP-(14–27)-peptide, which share a highly conserved COOH-terminal heptapeptide with GRP. Furthermore, addition of peptide D at 20 μM inhibited the increase in cell number caused by treatment of serum-depleted cells with GRP and insulin, whereas cell proliferation stimulated by EGF and insulin was only slightly affected (Fig. 3 *Right*). Thus peptide D, like peptide A (3), inhibited the reinitiation of DNA synthesis and cell division in a selective manner.

Peptide A has been shown to block specific binding of vasopressin to its receptor in Swiss 3T3 cells and vasopressin-induced mitogenesis (18, 19). Peptide D at 50 μM inhibited vasopressin-induced DNA synthesis in Swiss 3T3 cells (Fig. 3). In a detailed comparison, addition of peptides A and D at 20 μM increased the concentration of vasopressin required to achieve half-maximal DNA synthesis from 0.6 nM (vasopressin alone) to 17 nM with peptide A and 55 nM with peptide D.

Peptide D Binds Competitively to the GRP Receptor. The preceding results demonstrate that peptide D is a potent inhibitor of GRP-induced DNA synthesis that exhibits spec-

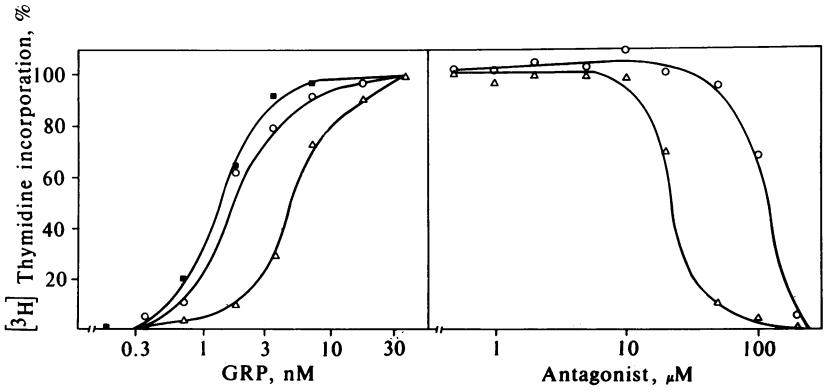

FIG. 2. Inhibition of GRP-induced DNA synthesis by peptides A and D. (*Left*) Confluent and quiescent cultures of Swiss 3T3 cells in 35-mm plastic dishes were washed twice with Dulbecco's modified Eagle's medium (DMEM) then incubated at 37°C in 2 ml of a 1:1 mixture of DMEM/Waymouth medium containing [^{3}H]thymidine at 1 μCi/ml (1 μM), insulin at 1 μg/ml, and various concentrations of GRP in the absence (■) or presence of 20 μM peptide A (○) or 20 μM peptide D (△). After 40 hr DNA synthesis was estimated by the incorporation of [^{3}H]thymidine into acid-precipitable material. Values are expressed as a percentage of [^{3}H]thymidine incorporation obtained with a saturating level of GRP (36 nM) in the absence of antagonist (100% = 8.4 × 10^5 cpm per dish). Each point represents the mean of duplicate determinations. (*Right*) Cultures of Swiss 3T3 cells were washed and incubated as above, except that the concentration of GRP was fixed at 3.6 nM with various concentrations of peptides A (○) and D (△). Values are expressed as a percentage of [^{3}H]thymidine incorporation obtained in the absence of antagonists (100% = 8.6 × 10^5 cpm per dish). Each point represents the mean of four determinations.

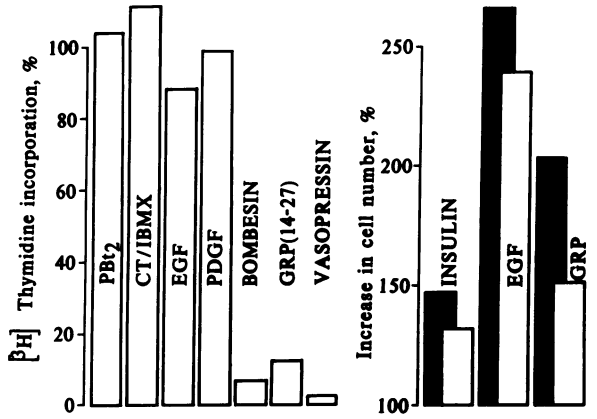

FIG. 3. Effect of peptide D on the stimulation of DNA synthesis and cell division by various mitogens. (*Left*) DNA synthesis assays (as in Fig. 2) were performed with [^{3}H]thymidine at 1 μCi/ml (1 μM) and insulin at 1 μg/ml with or without peptide D at 50 μM and the following factors at the concentrations indicated: phorbol 12,13-dibutyrate (PBt_2) at 50 ng/ml; cholera toxin (CT) at 100 ng/ml with isobutylmethylxanthine (IBMX) at 10 μM; EGF at 0.5 nM; PDGF at 0.2 nM; bombesin at 1.2 nM; GRP-(14–27)-peptide at 1.2 nM; vasopressin at 14 nM. Each addition (in the absence of antagonist) caused stimulation of DNA synthesis comparable to that achieved with 10% (vol/vol) fetal bovine serum. Results are expressed as the percentage of DNA synthesis obtained in the presence of peptide D. Each point represents the mean of two to six determinations. (*Right*) Effects of peptide D on the proliferation of Swiss 3T3 cells stimulated by EGF or GRP. The cells were cultured at low density (7.5×10^4 per 35-mm dish) in 2.5 ml of DMEM containing 3.5% (vol/vol) fetal bovine serum. After 3 days of incubation at 37°C the dishes contained 3.4×10^6 cells (mean). They were washed twice with DMEM and then transferred to 2 ml of a 1:1 mixture of DMEM/Waymouth medium containing bovine serum albumin at 1 mg/ml and insulin at 1 μg/ml, with or without 20 μM peptide D and either 0.5 nM EGF or 2.7 nM GRP. After 3 days of incubation, cells were removed from the dishes with a trypsin solution and counted by using a Coulter Counter. Results are expressed as the percentage increase in cell number in the absence (solid bars) or presence (open bars) of peptide D. Each point represents the mean of three determinations.

ificity against other mitogens. To elucidate its mechanism of action, we examined the effect of peptide D on the specific binding of ^{125}I-labeled GRP to Swiss 3T3 cells. Fig. 4 *Left* shows that both peptides A and D caused a concentration-dependent inhibition in the specific binding of ^{125}I-labeled GRP (1 nM). Half-maximal inhibition of binding was achieved with 2.3 μM peptide D and 14 μM peptide A, a 6.1-fold difference in potency.

The binding of various concentrations of ^{125}I-labeled GRP was measured in the absence and presence of 10 μM peptide D. A double-reciprocal plot of these data (Fig. 4 *Center*) shows that peptide D markedly reduces the affinity of the receptors for ^{125}I-labeled GRP, although the number of binding sites is unchanged. This is consistent with results obtained with peptide A (3) and strongly suggests that these peptides bind competitively to the GRP receptor.

To further substantiate these findings, we investigated the effects of the two antagonists on the affinity labeling of the M_r 75,000–85,000 protein that is a putative bombesin receptor (15) (Fig. 4 *Right*). They were both able to differentially inhibit the M_r 75,000–85,000 protein obtained by cross-linking ^{125}I-labeled GRP to Swiss 3T3 cells with ethylene glycol bis(succinimidylsuccinate). Half-maximal inhibition (obtained from the autoradiographs by scanning densitometry) was achieved with 5.5 μM peptide D and 20 μM peptide A.

Peptide D Inhibits the Early Events Elicited by GRP. One of the earliest events stimulated by addition of bombesin or GRP to quiescent Swiss 3T3 cells is an increase in cytosolic Ca^{2+} concentration (4). Fig. 5 *Left* shows that the increase in cytosolic Ca^{2+} caused by the addition of GRP (1 nM) to quiescent Swiss 3T3 cells was prevented by the addition of peptide A at 20 μM but not at 5 μM. In contrast peptide D was effective at 5 μM. These effects were specific and reversible since peptide D at 5 μM did not prevent a response to PDGF and because the effects of the antagonist in preventing Ca^{2+} mobilization in response to 5 nM GRP was reversed by addition of GRP at 50 nM.

Inhibition of ^{125}I-labeled EGF binding by GRP, which is mediated by the protein kinase C pathway (9), was reversed in a concentration-dependent fashion by peptides A and D (Fig. 5 *Right*). Half-maximal reversal of inhibition was obtained with 8.7 μM peptide D and 30 μM peptide A.

Peptide A and Peptide D Inhibit the Growth of SCLC Cell Lines. SCLC is known to secrete bombesin-like peptides (20–22) that might act as autocrine growth factors (2, 25).

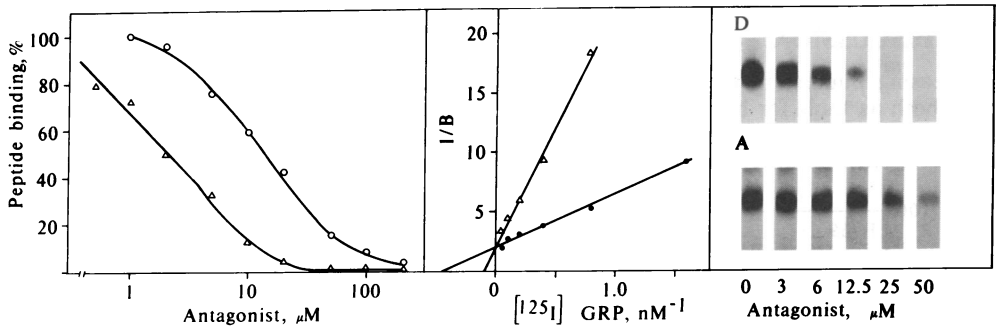

FIG. 4. Effects of peptides A and D on the binding of ^{125}I-labeled GRP ([^{125}I]GRP) to Swiss 3T3 cells. (*Left*) Inhibition of specific ^{125}I-labeled GRP binding by peptides A and D. Confluent and quiescent cells were washed twice with DMEM then incubated at 37°C in 1 ml of binding medium (3) containing ^{125}I-labeled GRP at 1 nM and various concentrations of peptide A (○) and D (△). Cell-associated ^{125}I-labeled GRP binding was measured after 30 min. Values are expressed as percentage of the specific binding obtained in the absence of antagonists. Nonspecific binding was determined by the addition of 300-fold excess of unlabeled GRP. Each point represents the mean of three determinations. (*Center*) Effect of peptide D on the affinity of binding sites in Swiss 3T3 cells for ^{125}I-labeled GRP. Cells were incubated in binding medium containing various concentrations of ^{125}I-labeled GRP in the absence (●) or presence (△) of 10 μM peptide D. Specific binding (B) is expressed in pmol per 10^6 cells and is shown in a double-reciprocal plot. Each point represents the mean of duplicate determinations. (*Right*) Effects of peptides A and D on the affinity labeling of the bombesin receptor-associated M_r 75,000–85,000 protein. Confluent cultures of Swiss 3T3 cells were washed twice with DMEM and incubated at 15°C in 1 ml of binding medium (pH 7.0) (15) containing 0.5 nM ^{125}I-labeled GRP and various concentrations of the antagonists. After 2 hr, the cultures were washed twice with binding medium then incubated in 1 ml of binding medium containing 6 mM ethylene glycol bis(succinimidylsuccinate) at pH 7.4 for 15 min at 15°C. The cultures were then washed twice with cold and solubilized in 0.1 ml of 2× sample buffer (15), then immediately heated at 100°C for 5 min, and electrophoresed on a 10% polyacrylamide gel.

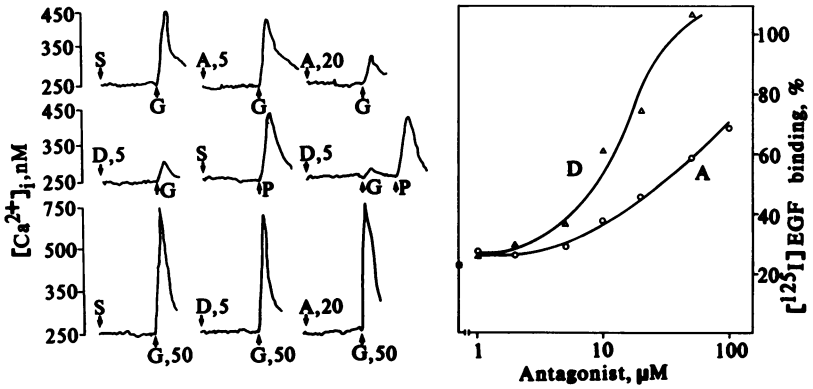

FIG. 5. Effects of peptides A and D on the early cellular responses stimulated by GRP. (*Left*) Effects on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Quiescent Swiss 3T3 cells grown on Cytodex 2 beads (Pharmacia) were washed twice with DMEM and incubated for 10 min with fura-2 tetraacetoxymethyl ester at 1 μM, then washed three times, and suspended in 2 ml of electrolyte solution (6) in the fluorimeter at 37°C and stirred. Fluorescence was recorded continuously in a Perkins–Elmer LS5 luminescence spectrometer with an excitation wavelength of 335 nm and emission wavelength of 510 nm. After a period of equilibration, the following additions were made: solvent (S); peptide A at 5 μM (A, 5) and 20 μM (A, 20); peptide D at 5 μM (D, 5). After 3 min, GRP was added at 1 nM (G) or at 50 nM (G, 50); PDGF at 1 nM (P). (*Right*) Peptides A and D reverse the inhibition of ^{125}I-labeled EGF binding induced by GRP. Swiss 3T3 cells were incubated for 1 hr at 37°C in 1 ml of binding medium (9) containing 0.2 nM ^{125}I-labeled EGF and 3.6 nM GRP alone (■) or in the presence of various concentrations of peptide A (○) or peptide D (△). Values are expressed as percentages of the specific binding obtained with 0.2 nM ^{125}I-labeled EGF alone. The nonspecific binding was obtained by the addition of 500-fold excess of unlabeled EGF. Each point represents the mean of six determinations.

Thus it was plausible but not proven that an antagonist to bombesin/GRP would inhibit SCLC growth, so we tested the effects of peptides A and D on SCLC cells *in vitro*.

Fig. 6 shows that the growth of the SCLC lines H69, H128, and H417 in serum-free medium was abolished by addition of peptide A at 150 μM, a concentration that reversibly inhibits GRP-induced mitogenesis in Swiss 3T3 cells. The inhibition of growth by the antagonist in SCLC cell lines was reversed by washing the cells and resuspending them in serum-free medium. In contrast, normal growth was not restored by addition of GRP at 72 nM with antagonist.

The effects of peptides A and D on H69 cells are compared in Fig. 7. The cells achieve 10-fold increase in number in ≈12 days in serum-free medium (*Inset*). Both antagonists inhibited growth in a dose-dependent manner; half-maximal effect was seen with 24 μM peptide D and with 82 μM peptide A. The difference in potency is of the same order of magnitude as that demonstrated in Swiss 3T3 cells.

DISCUSSION

There has been increasing interest in the use of peptide hormone antagonists both as tools for biomedical research and as potential therapeutic agents. Antagonists to classical monoamine transmitters, such as acetylcholine, α- and β-adrenergic agonists, and histamines, have had a large impact on clinical medicine, and attention is now focused on producing peptide hormone antagonists that are effective *in vivo* (31). An antagonist must bind to the specific receptor without producing the conformational changes that initiate the biological response. Synthetic peptide antagonists, such as those that are active against parathyroid hormone (32) and glucagon (33), are already providing useful structure–activity information. Many such peptide and nonpeptide antagonists have been developed empirically (17, 34–36), but designing antagonists remains problematic. Unexpectedly, the substance P antagonist peptide A, which has minimal structural homology with bombesin, was found to be an antagonist for bombesin-stimulated enzyme secretion from dispersed pancreatic acini (17) and to block its growth-stimulatory effects (3, 18). To identify a more potent antagonist, we have systematically screened substance P antagonists for activity against bombesin-like peptides.

Peptide D has been described (37) as a substance P antagonist that blocked the hyoscine-resistant opiate withdrawal contracture in guinea pig ileum. The results presented here demonstrate that peptide D is a potent GRP antagonist in mouse 3T3 cells. During the course of these studies we also found that peptide D blocks mitogenesis induced by vasopressin, but not that induced by phorbol

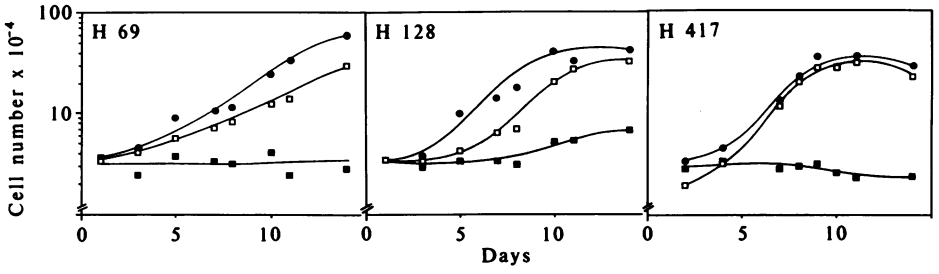

FIG. 6. Peptide A reversibly inhibits the growth of SCLC cell lines. Stock cultures of cell lines H69, H128, and H417 were maintained in RPMI 1640 medium with 10% (vol/vol) fetal bovine serum (heat inactivated) in a humidified atmosphere of 10% CO_2/90% air at 37°C. They were passaged every 7 days. Identical growth was obtained in the serum-free medium of RPMI 1640 supplemented with HITES (30) (10 nM hydrocortisone, insulin at 5 μg/ml, transferrin at 100 μg/ml, 10 nM 17β-estradiol, 30 nM sodium selenite) and 0.25% bovine serum albumin. Cells were washed twice with RPMI 1640 medium then incubated in the serum-free medium in the absence (●) or presence (□, ■) of 150 μM peptide A. After 4 days they were again washed twice with RPMI 1640 medium then resuspended at a density of 5×10^4 cells per ml in the absence (●, □) or presence (■) of 150 μM peptide A (day 0). Cell number was determined at intervals over 14 days by using a Coulter Counter after disaggregation of cell clumps by passing through 19-gauge and 21-gauge needles. Each point represents the mean of three determinations.

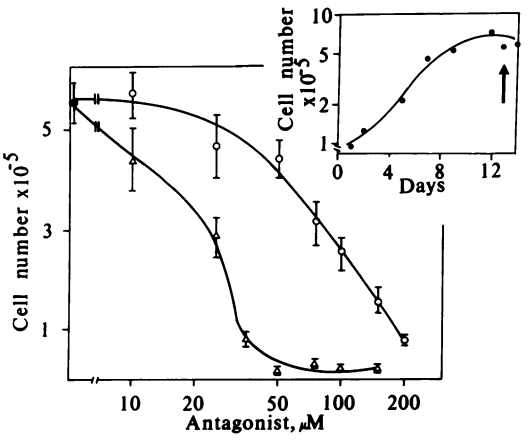

FIG. 7. Inhibition of SCLC cell growth *in vitro* by peptides A and D is concentration-dependent. Cultures of H69 cells were washed twice with RPMI 1640 medium, then incubated in serum-free medium (as in Fig. 6) in the absence (●) or presence of various concentrations of peptides A (○) and D (△). Cell number was determined in a Coulter Counter after disaggregation of cell clumps by passing through 19-gauge and 21-gauge syringe needles. Samples were incubated for 13 days, when the controls (*Inset*) had achieved 10-fold increase in number, indicated by the arrow. Each point represents the mean (±SD) of five determinations.

12,13-dibutyrate, cholera toxin with isobutylmethylxanthine, EGF, or PDGF. The observation that both peptides A and D prevent the actions of three unrelated peptides (GRP, substance P, and vasopressin) through their specific receptors is intriguing and warrants further investigation. Remarkably, peptide D is consistently ≈5-fold more potent than peptide A (and both are more potent by far than the closely related peptide B, spantide) suggesting that the substitution of D-phenylalanine for glutamine at position 5 is critical to its activity.

Lung cancer is the commonest fatal malignancy in the developed world, and its incidence is increasing rapidly (38). SCLC constitutes 25% of lung cancers and is, therefore, of major clinical and economic importance. Evidence that bombesin-like peptides, which are secreted by these tumors (20–22), can act as autocrine growth factors (25) has prompted the search for clinically useful GRP antagonists. We have presented evidence that two peptides that are antagonists of GRP and vasopressin profoundly inhibit the growth of SCLC cells *in vitro* although the participation of other growth-promoting peptides cannot be excluded. More potent and specific antagonists could, therefore, be useful as therapeutic agents with potentially high tissue penetration.

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