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Effect of the gastrin-releasing peptide antagonist BIM 26226 and lanreotide on an acinar pancreatic carcinoma

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Abstract

The effects of a potent specific gastrin-releasing peptide receptor antagonist, BIM 26226 ([D-F_5 Phe^6, D-Ala^{11}] bombesin (6–13) OMe), and the long-acting somatostatin analogue, lanreotide (BIM 23014), on the growth of an acinar pancreatic adenocarcinoma growing in the rat or cultured in vitro were investigated. Lewis rats bearing a pancreatic carcinoma transplanted s.c. in the scapular region, were treated with gastrin-releasing peptide (30 μg/kg per day), BIM 26226 (30 and 100 μg/kg per day) and lanreotide (100 μg/kg per day) alone or in combination for 14 successive days. Chronic administration of BIM 26226 and lanreotide significantly inhibited the growth of pancreatic tumours stimulated or not by gastrin-releasing peptide (GRP), as shown by a reduction in tumour volume, protein, ribonucleic acid, amylase and chymotrypsin contents. This effect was more pronounced with 100 μg/kg per day BIM 26226 than with 30 μg/kg per day. However, BIM 26226 and lanreotide, given together, did not exert any additive effect on GRP-treated and -untreated tumours. In cell cultures, both BIM 26226 and lanreotide (10^{-6} M) inhibited [^{3}H]thymidine incorporation in tumour cells induced or not by GRP, but no increased effect was observed after combined treatment with both agents. Binding studies showed that BIM 26226 had a high affinity for GRP receptors in tumour cell membranes (IC_{50} = 6 nM). These results from in vivo and in vitro experiments suggest that BIM 26226 and lanreotide are able to reduce the growth of an experimental acinar pancreatic tumour. Thus, these agents represent interesting steps toward the development of new approaches for treatment of pancreatic carcinomas. © 1998 Elsevier Science B.V.

Keywords: Gastrin-releasing peptide; Bombesin/GRP receptor antagonist; Somatostatin analogue; Acinar pancreatic cancer; Exocrine pancreas; Receptor

1. Introduction

Pancreatic growth is affected by a number of gastrointestinal hormones and neuropeptides that also regulate its secretory function. Among them, the amphibian tetradecapeptide, bombesin, and its mammalian homologue, gastrin-releasing peptide (GRP), have been shown to stimulate pancreatic growth in suckling and adult animals (Lhoste et al., 1985, 1989; Lehy et al., 1986; Alexander et al., 1988; Damgé et al., 1988; Borysewicz et al., 1992) while somatostatin and its structural analogue, SMS 201–995, reduced these effects (Morisset, 1984; Hajri et al., 1991a).

These peptides have also been implicated in the growth of human or experimental pancreatic tumours transplanted in animals. Generally, experimental tumours induced in animals can be of acinar cell origin (induced by azaserine in the rat) or ductular cell origin (induced by nitrosamine in the hamster) like the major human pancreatic tumours. Peptides from the bombesin/GRP family exert various effects on pancreatic tumours. They promote the development of preneoplastic lesions induced by azaserine and the growth of an acinar carcinoma transplanted in the rat (Lhoste and Longnecker, 1987; Douglas et al., 1989; Hajri et al., 1992; Meijers et al., 1992a). However, they exert contradictory effects on ductular pancreatic carcinoma. Bombesin-like peptides inhibit the development of preneoplastic ductular pancreatic lesions in hamsters (Meijers et al., 1991) and the growth of human ductular pancreatic adenocarcinoma xenografted in nude mice (Alexander et al., 1988); but they have little or no inhibitory effect on nitrosamine-induced ductular pancreatic adenocarcinoma in hamsters (Szepeshazi et al., 1993). Analogues of somatostatin such as SMS 201–995 (octreotide, Sandostatin) and RC-160 have been shown to inhibit the development of preneoplastic ductular lesions in hamsters and the growth

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of ductular and acinar pancreatic adenocarcinoma growing in hamsters, nude mice and rats (Upp et al., 1988; Hajri et al., 1991b; Szepeshazi et al., 1991; Meijers et al., 1992b). In contrast, SMS 201–995 enhances the growth of acidophilic atypical acinar cell nodules induced by azaserine in rats (Meijers et al., 1992b).

Thus, because experimental and human pancreatic cancer cell lines posses receptors for bombesin/GRP peptides and somatostatin (Hajri et al., 1991b, 1992, 1996; Szepeshazi et al., 1991; Dietrich et al., 1994) and because cancer development and growth can be influenced by hormonal therapy, several laboratories have synthesized specific bombesin/GRP receptor antagonists and somatostatin analogues for hormonal therapy of these cancers. Among them, BIM 26226 ([D-F_5 Phe^6, D-Ala^{11}] bombesin (6–13) OMe) is a potent and highly specific GRP receptor antagonist with a longer half-life in vivo (Coy et al., 1992). It has been shown to depress amylase secretion in the pancreas and in the pancreatic acinar cell carcinoma cell line, AR4-2J (Coy et al., 1992; Dietrich et al., 1994). The octapeptide, lanreotide (BIM 23014, somatuline), is a somatostatin analogue with an increased biological half-life because of its resistance to enzymatic degradation (Parmar et al., 1989). It has been shown to be endocrinologically potent but differs from other somatostatin analogues by having a more profound effect on growth hormone and insulin-like growth factor (IGF-I) (Parmar et al., 1989; Sassolas et al., 1989). It inhibits the growth of different tumours in vitro and in vivo such as prostate cancer, breast cancer and human small cell lung carcinoma (Parmar et al., 1989; Bogden et al., 1990a,b; Prévost et al., 1991, 1994). Thus, it was of great interest to investigate, in vivo and in vitro, the effects of the bombesin/GRP receptor antagonist, BIM 26226, and the somatostatin analogue, lanreotide, alone or in combination, on a pancreatic acinar carcinoma.

2. Materials and methods

2.1. Materials

Gastrin-releasing peptide [GRP-(1–27)], GRP-(1–16), GRP-(1–10) (neuromedin C), neuromedin B and bombesin were obtained from Bachem (Bubendorf, Switzerland). RC-3095 was a generous gift from Dr A.V. Schally (Tulane University Medical Center; New Orleans, USA). The GRP receptor antagonist, BIM 26226, and the long-acting somatostatin analogue, lanreotide (BIM 23014, somatuline), were kindly given by Ipsen Biotech (Paris, France). EGF (epidermal growth factor), bovine serum albumin, leupeptin, aprotinin, phenylmethylsulfonyl fluoride, EGTA and soybean trypsin inhibitor were from Sigma (La Verpillière, France). [^{125}I]GRP (1800–2000 Ci/mmol) was purchased from Amersham (Les Ulis, France). Dulbecco's modified Eagle's medium (DMEM) supplemented with antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml), fungizone 0.25 μg/ml, 10% fetal calf serum and EDTA were obtained from Gibco/BRL (Cergy-Pontoise, France). HEPES and bacitracin were from Euromedex (Strasbourg, France). Other chemicals were of standard reagent grade.

The original pancreatic tumour was a kind gift from D.S. Longnecker (Lebanon, NH, USA) and was maintained in our laboratory by successive subcutaneous implantations in the interscapular region of Lewis rats (CNRS, Orléans, France). Originally, this tumour model was induced by azaserine in the rat and characterized as an acinar adenocarcinoma phenotype (Longnecker et al., 1981).

2.2. Animals, tumour dissociation, transplantation procedures and in vivo experimental procedure

2.2.1. Tumour cell preparations

The tumours were removed from donor rats, washed in ice-cold DMEM supplemented with antibiotics and chopped into small fragments which were passed through an 18-gauge needle. The resulting slurry was incubated for 10 min in Hank's solution containing 2.5 mmol/l EDTA (lacking Ca^{2+} and Mg^{2+}), centrifuged at $500 \times g$ for 5 min and washed twice with cold medium. The pellet was resuspended in DMEM supplemented with antibiotics and 10% fetal calf serum. Aliquots (500 μl), 2–3 mg, of tumour tissue were injected subcutaneously in the scapular region of male adult Lewis rats weighing approximately 200 g. Palpable tumours appeared in 95–100% of rats, after 10–15 days.

2.2.2. In vivo experiments

For in vivo studies, 80 Lewis rats bearing a palpable tumour were randomly divided into ten groups of eight rats each, subjected to following treatments: saline (control); BIM 26226 (30 or 100 μg/kg per day); lanreotide (100 μg/kg per day); BIM 26226 (100 μg/kg per day) + lanreotide (100 μg/kg per day); GRP (30 μg/kg per day); GRP (30 μg/kg per day) + BIM 26226 (30 μg/kg per day); GRP (30 μg/kg per day) + BIM 26226 (100 μg/kg per day); GRP (30 μg/kg per day) + lanreotide (100 μg/kg per day); GRP (30 μg/kg per day) + BIM 26226 (100 μg/kg per day) + lanreotide (100 μg/kg per day). Peptides were injected subcutaneously, three times daily, for 14 successive days, in the presence of 15% hydrolysed gelatin in order to prolong their absorption. During this period, tumours were measured three times per week with calipers and tumour volume was calculated from the formula: $\pi/6 \times$ maximal length $\times$ maximal height $\times$ maximal width assuming an ellipsoid shape. On day 15, the animals were killed after an overnight fast. The tumours and the pancreases were quickly removed and carefully trimmed, weighed, and stored at -20°C.

2.3. Growth of acinar pancreatic tumour cells in primary cell cultures

The effects of GRP, BIM 26226 and lanreotide on the growth of acinar pancreatic tumour cells in primary cell cultures were estimated by [^{3}H]thymidine incorporation assay.

Tumour cells prepared as described above were dispersed in an enriched medium which consisted of Dulbecco's modified Eagle's and Waymouth media (v/v)

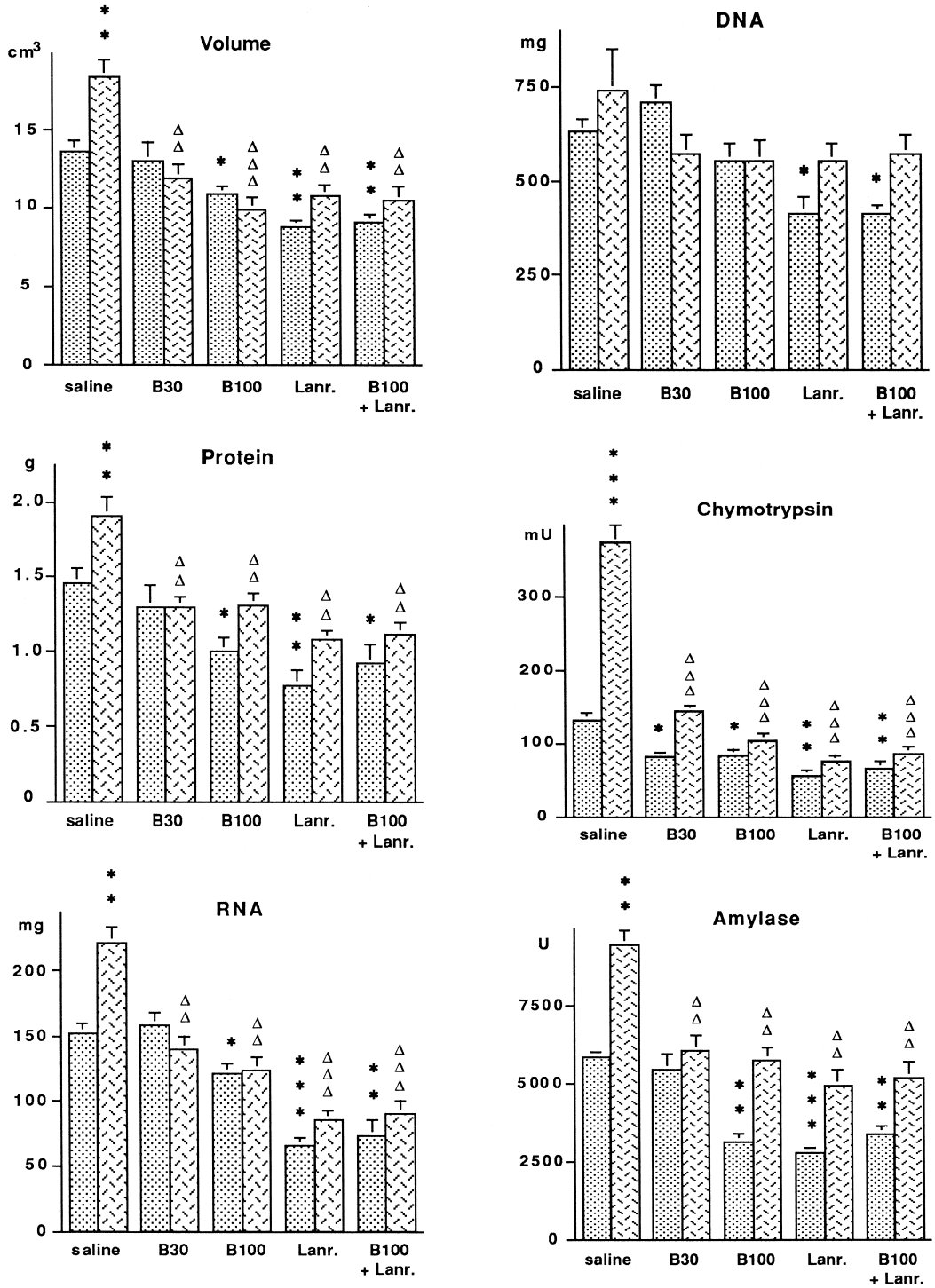

Fig. 1. Tumour volume and protein, RNA, DNA, chymotrypsin and amylase contents in animals treated for 14 days with saline (control group), BIM 26226 (30 and 100 μg/kg per day), lanreotide (100 μg/kg per day) or BIM 26226 (100 μg/kg per day) and lanreotide (100 μg/kg per day), alone (dotted columns) or in association with GRP (30 μg/kg per day) (squared columns). Results, expressed as means $\pm$ S.E.M. for eight animals, were compared with those for the control group receiving saline alone ($*$ $P < 0.05$, $**$ $P < 0.01$, $***$ $P < 0.001$) or for the GRP-treated group ($\triangle\triangle$ $P < 0.01$, $\triangle\triangle\triangle$ $P < 0.001$).

supplemented with 15% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 10 μg/ml streptomycin, 0.25 μg/ml fungizone, 0.5 mM isobutyl-1-methylxanthine, 0.25 mg/ml soybean trypsin inhibitor (SBTI), 5 μM ascorbic acid, 10 μg/ml transferrin, 0.35 ng/ml sodium selenite, 1% (v/v) MEM essential vitamins, 1 μM carbachol, 1 μg/ml dexamethasone, 5 μg/ml insulin and 10 ng/ml EGF. For experimental purposes, $2–5 \times 10^5$ cells in 2 ml of enriched medium were seeded into individual collagen-coated wells of 6-cell plates, and allowed to attach overnight at 37°C. The culture was maintained in a humidified atmosphere of 5% CO_2/95% air at 37°C, and the medium was changed every day.

After cell attachment, the enriched medium was aspirated and the cultures were washed twice with fetal calf serum free-DME medium to remove residual serum. After 24 h of serum starvation, the culture medium was supplemented with 2.5% fetal calf serum alone or with various concentrations of GRP, BIM 26226 or lanreotide alone or associated. These peptides (200 μl/well) were added three times daily, from a concentrated stock solution in DMEM/Waymouth (v/v) for 24 h. A total of 0.5 μCi/ml [^{3}H]thymidine was added to each dish during the last 12 h of culture.

2.4. Receptor binding studies

Receptor binding studies were performed on plasma membranes isolated from freshly prepared tumour cells.

2.4.1. Preparation of isolated tumour cell membranes

Tumour pancreatic cells were resuspended in five volumes of a lysis sucrose HEPES buffer (SHB) containing 0.25 M sucrose, 50 mM HEPES pH 7.4, 10 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 0.1% bacitracin and

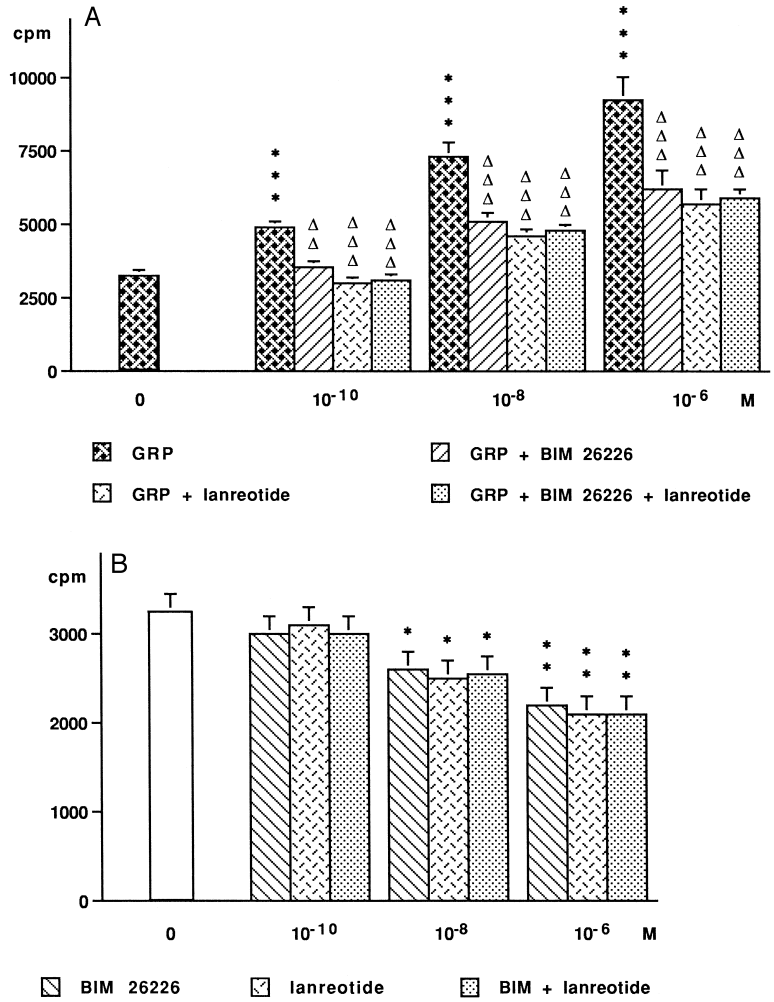

Fig. 2. [^{3}H]thymidine incorporation into pancreatic tumour cells cultured in presence of GRP, BIM 26226 and lanreotide. (A) Treatment with increasing concentrations of GRP (10^{-10} to 10^{-6} M) alone or associated with 10^{-6} M BIM 26226, 10^{-6} M lanreotide or 10^{-6} M BIM 26226 and 10^{-6} M lanreotide together. (B) Treatment with increasing concentrations (10^{-10} to 10^{-6} M) of BIM 26226, lanreotide or BIM 26226 and lanreotide together. Results, expressed as means ± S.E.M. of six independent observations from three separate experiments, were compared with those for the untreated control group ($* P < 0.05$, $** P < 0.01$, $*** P < 0.001$) or for the GRP-treated group ($\triangle\triangle P < 0.01$, $\triangle\triangle\triangle P < 0.001$).

0.01% soybean trypsin inhibitor. Cells were first homogenized with a Polytron for 30–45 s at setting 6, and then with a Dounce homogenizer using 10 strokes with a tight-fitting pestle. This homogenate was centrifuged at $600 \times g$, for 10 min at 4°C (S1). The pellet was resuspended in 2.5 volume SHB buffer, homogenized again and centrifuged (S2). The pooled supernatants (S1 + S2) were centrifuged at $10\,000 \times g$ for 30 min at 4°C. The supernatant solution was then made up to 0.1 M NaCl and 0.2 mM $MgSO_4$ by the addition of concentrated solutions of these salts and then centrifuged at $48\,000 \times g$ for 45 min at 4°C. The resulting pellet was washed in HEMGI buffer (50 mM HEPES pH 7.2, 2 mM EGTA, 5 mM $MgCl_2$, 120 mM NaCl, 4.7 mM KCl, 10% (v/v) glycerol, 40 μg/ml bacitracin, 1 mM PMSF, 0.01% SBTI, 10 μg/ml leupeptin and 5 μg/ml aprotinin), then centrifuged for 30 min at 4°C at $48\,000 \times g$. The pellet was resuspended in HEMGI (30% glycerol final concentration) and stored at −80°C.

2.4.2. Binding studies

Specific binding of [^{125}I]GRP-(1–27) to plasma membranes was assayed in HEMI buffer, pH 6.8, containing 0.5% bovine serum albumin. Approximately 250–350 μg of membrane proteins was incubated with 25 pM [^{125}I]GRP-(1–27) in the presence or absence of 0.1 μM unlabeled GRP to determine the non-specific and total binding, respectively. The pharmacological specificity of the [^{125}I]GRP-(1–27) binding to tumour cell membranes was tested in the presence of varying concentrations of GRP analogues, GRP receptor antagonists (BIM 26226 and RC-3095), EGF and lanreotide. The binding reaction was terminated after 60 min at 22°C by the addition of 0.5 ml of ice-cold HEMI buffer, centrifuged at $14\,000 \times g$ for 10 min at 4°C, then washed twice with ice-cold phosphate buffered saline (PBS). The radioactivity was counted with a γ-counter.

2.5. Biochemical and radiochemical analysis

After in vivo experiments, tumours and pancreases were homogenized in ice-cold distilled water (100 mg/ml) in a Polytron set (medium speed). Protein content was determined by the method of Lowry et al. (1951). Amylase content was measured according to the method of Danielsson (1974), using maltose as a standard. Chymotrypsin was assayed as indicated by Nagel et al. (1965) after activation of chymotrypsin by trypsin. After extraction, DNA was determined by the diphenylamine method using calf thymus DNA as standard (Richards, 1974) and RNA by the orcinol method using yeast RNA as a standard (Schneider, 1957).

Cells cultures were rinsed with PBS at 4°C, harvested and centrifuged for 3 min at $300 \times g$ at 4°C. The cells were then washed twice with ice-cold PBS, and sonicated in 1 ml of water with a probe-type sonicator. Aliquots were removed for determination of protein content and 0.5 ml of each sonicated sample was precipitated with trichloroacetic acid (final concentration 10%) at 4°C for 20 min. The precipitates were washed twice with cold 5% trichloroacetic acid and dissolved in 1 ml of 0.2 M NaOH/0.1% SDS, neutralized with 1 M HCl and counted in a beta liquid scintillation counter.

2.6. Statistical analysis

The mean and standard error of the mean were calculated for the values for each parameter. A one-way analysis of variance followed by the Newmann–Keuls test was applied for group comparisons.

3. Results

3.1. Effect of GRP, BIM 26226 and lanreotide on tumour growth in vivo

The effects of GRP, BIM 26226 and lanreotide administered three times daily for 14 successive days on pancreatic tumour volume, protein and RNA contents are presented in Fig. 1. GRP treatment (30 μg/kg per day) significantly increased the values for these parameters, by 36, 32 and 45%, respectively ($P < 0.01$). Chymotrypsin and amylase contents were increased, respectively, by 183 and 63% ($P < 0.001$ and 0.01), but the DNA content was increased less (17%, NS) (Fig. 1).

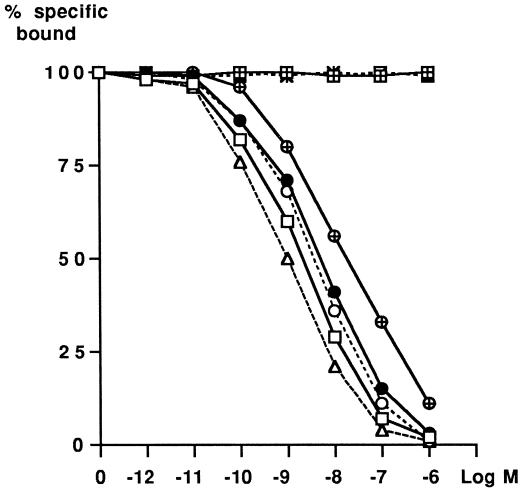

Fig. 3. Inhibition of [^{125}I]GRP binding to pancreatic tumour cell membrane preparations by GRP analogues, antagonists, lanreotide and EGF. Preparations were incubated with 25 pM [^{125}I]GRP for 60 min at 22°C in the presence of varying concentrations of GRP analogues, antagonists, lanreotide and EGF. Specific saturable binding is expressed as the percentage of maximal specific binding. Each value represents the mean of six independent observations from three separate experiments. GRP—(1–27) (-□-); BIM 26226 (-•-); RC-3095 (–◦–); neuromedin B (-⊕-); neuromedin C (–△–); lanreotide (-cross inside the square-); GRP—(1–16) (–■–); EGF (–∗–).

BIM 26226 treatment completely inhibited these GRP-induced effects on growth parameters, but this inhibition was slightly more pronounced with the highest concentration of BIM 26226 (100 μg/kg per day) than with the lowest concentration (30 μg/kg per day). However, non-stimulated pancreatic tumour cells were less sensitive to the GRP receptor antagonist since only the 100 μg/kg per day concentration significantly reduced the volume of the

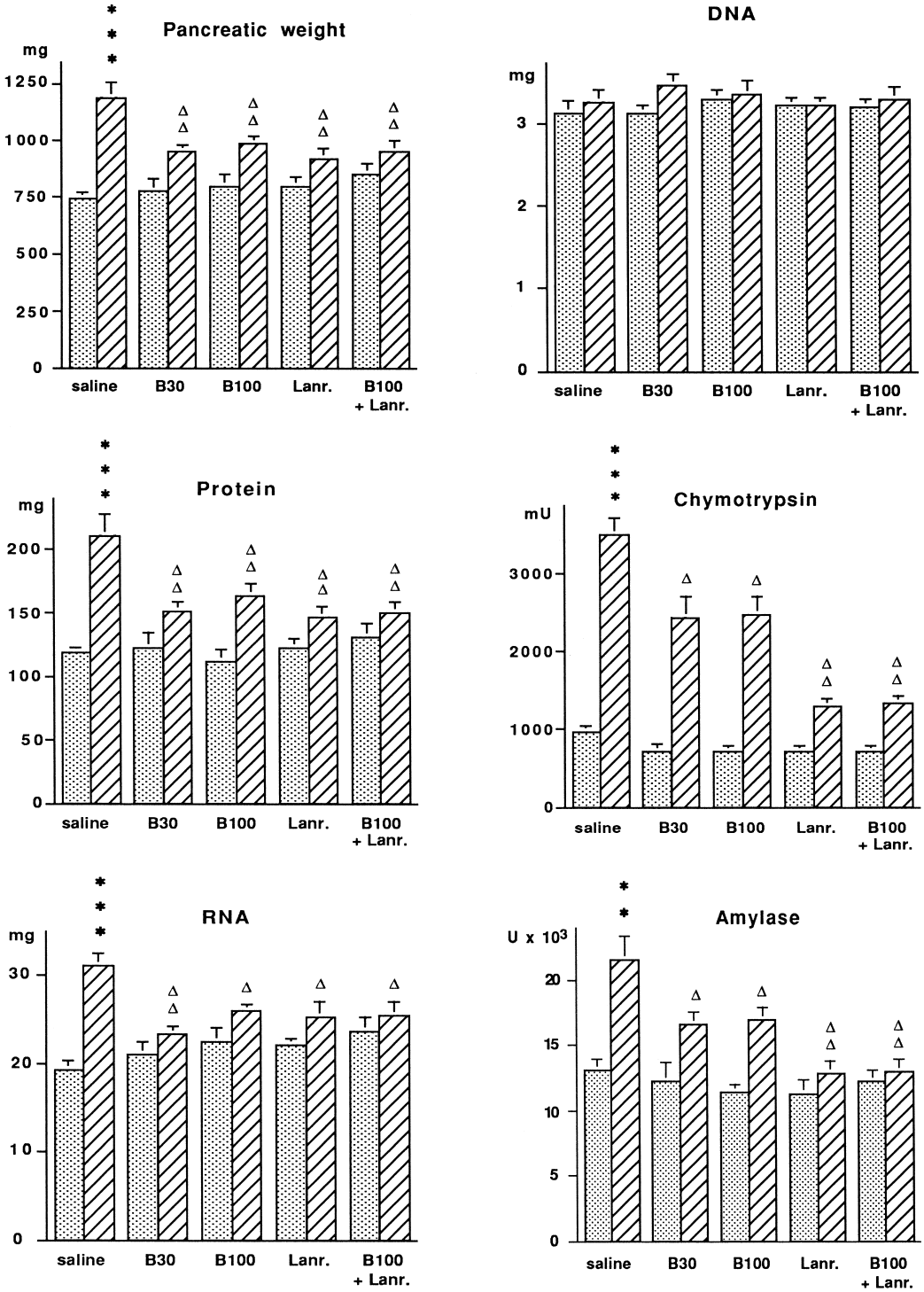

Fig. 4. Pancreatic weight and protein, RNA, DNA, chymotrypsin and amylase contents in animals treated for 14 days with saline (control group), BIM 26226 (30 and 100 μg/kg per day), lanreotide (100 μg/kg per day) or BIM 26226 (100 μg/kg per day) and lanreotide (100 μg/kg per day), alone (dotted columns) or in association with GRP (30 μg/kg per day) (hatched columns). Results, expressed as means ± S.E.M. for eight animals, were compared with those for the control group receiving saline alone (∗∗ $P < 0.01$, ∗∗∗ $P < 0.001$) or for the GRP-treated group (△ $P < 0.05$, △△ $P < 0.01$).

tumour and its content of protein, RNA, amylase and chymotrypsin.

Lanreotide treatment (100 μg/kg per day) showed effects on pancreatic tumour growth similar to those of BIM 26226 (Fig. 1). It completely suppressed GRP-induced growth of the tumour and, itself, significantly reduced tumour growth. However, BIM 26226 and lanreotide administered together at the doses of 100 μg/kg per day did not show any additive effect on tumour growth induced or not by GRP.

3.2. Effect of GRP, BIM 26226 and lanreotide on [^{3}H]thymidine incorporation in cultured pancreatic tumour cells

DNA synthesis was estimated from the [^{3}H]thymidine incorporation in primary cultured pancreatic tumour cells during the last 24 h of culture.

As represented in Fig. 2A, GRP added to the culture medium at different concentrations (10^{-10}, 10^{-8} and 10^{-6} M) significantly increased [^{3}H]thymidine incorporation by 51, 125 and 185% ($P < 0.001$), respectively.

BIM 26226, at the concentration of 10^{-6} M, reduced the stimulating effects of increasing concentrations of GRP by 28% ($P < 0.01$) to 33% ($P < 0.001$) (Fig. 2A).

BIM 26226 by itself significantly decreased [^{3}H]thymidine incorporation in tumour cells at the concentrations of 10^{-8} M (-20%, $P < 0.05$) and 10^{-6} M (-32%, $P < 0.01$) (Fig. 2B).

Lanreotide, at the concentration of 10^{-6} M, significantly decreased the mitogenic effect of GRP by approximately 38% ($P < 0.001$) (Fig. 2A). In addition, by itself, it reduced significantly the [^{3}H]thymidine incorporation at 10^{-8} M (-23%, $P < 0.05$), and more so at 10^{-6} M (-35%, $P < 0.01$) (Fig. 2B).

Lanreotide and BIM 26226, when added together to the medium, did not exert a greater inhibitory effect on [^{3}H]thymidine incorporation, than did the single peptides (Fig. 2A,B).

3.3. Effect of BIM 26226, lanreotide and other peptides on [^{125}I]GRP binding to tumour cell membranes

To examine the pharmacological specificity of BIM 26226 binding to tumour cell membranes, the latter and a variety of peptides were tested for their ability to competitively displace the binding of [^{125}I]GRP. Fig. 3 showed that BIM 26226 and RC-3095, two GRP receptor antagonists, as well as GRP-(1–27), neuromedin B and neuromedin C were able to displace [^{125}I]GRP binding in a concentration-dependent manner. BIM 26226 and RC-3095 had a high affinity for GRP receptors, the IC_{50} being, respectively, 6 and 5 nM. However, all peptides unrelated to GRP such as GRP-(1–16), EGF, and lanreotide failed to inhibit [^{125}I]GRP binding.

3.4. Effect of GRP, BIM 26226 and lanreotide on normal pancreatic growth in vivo

As represented in Fig. 4, GRP (30 μg/kg per day) administered for 14 successive days markedly increased pancreatic weight, protein, RNA, chymotrypsin and amylase contents by 61, 76, 61, 265, and 65% ($P < 0.001$); GRP, however, did not alter significantly the DNA content. BIM 26226 at both concentrations used (30 and 100 μg/kg per day) and lanreotide (100 μg/kg per day) significantly reduced the effects of GRP. However, BIM 26226 and lanreotide administered together at the concentrations of 100 μg/kg per day, did not exert any greater effect on GRP-induced pancreatic growth.

BIM 26226 and lanreotide, alone or in combination, did not significantly affect pancreatic growth parameters (Fig. 4).

4. Discussion

Our results, in agreement with our previous observations (Hajri et al., 1992), indicated that GRP stimulates the growth of an acinar pancreatic carcinoma transplanted in the scapular region of rats. This tumour was originally induced by azaserine in Lewis rats (Longnecker et al., 1981). It is a well differentiated carcinoma in which the cells are arranged in acini filled with numerous zymogen granules and possess secretory enzyme activities such as amylase, lipase and chymotrypsin. Previously (Hajri et al., 1992, 1996), we had demonstrated an overexpression of bombesin/GRP receptors with high affinity ($K_d = 0.35$ nmol/l, $B_{max} = 189$ fmol/mg protein) in these cells, as compared to normal pancreatic cells ($K_d = 0.42$ nmol/l, $B_{max} = 31$ fmol/mg protein). The present study confirmed these observations and demonstrated a direct mitogenic effect of GRP in primary cultured acinar pancreatic tumour cells.

BIM 26226, a specific bombesin/GRP receptor antagonist, has been shown to exert an antimitogenic activity on 3T3 cells (Jensen and Coy, 1991). However, it stimulated the growth of human gastrinoma implanted in nude mice (Chu et al., 1996) and exerted no effect on the growth of the human gastric cancer cell line SIIA (Bold et al., 1994). Thus, the effects of BIM 26226 on cancers are contradictory and remain controversial.

Since BIM 26226 inhibits the release of amylase induced by GRP in both pancreas and AR4-2J cells, an acinar cancer cell line originally induced by azaserine in the rat (Coy et al., 1992; Dietrich et al., 1994), we investigated its in vivo and in vitro effects on an acinar pancreatic carcinoma.

BIM 26226, administered chronically to rats bearing the pancreatic tumour, inhibited the growth of the tumour stimulated or not by GRP. This effect was more marked

with the highest concentration of BIM 26226 (100 μg/kg per day) than with the lowest concentration (30 μg/kg per day). In addition, secretory enzyme activities stimulated by GRP or not were also markedly reduced.

BIM 26226 could act indirectly on tumour cells by inhibiting the GRP-induced release of a number of gastrointestinal hormones and growth factors that may be involved in the growth of the tumour, such as cholecystokinin, gastrin or secretin (Miyata et al., 1980).

Though this latter hypothesis cannot be ruled out, our in vitro studies clearly demonstrate a direct effect of BIM 26226 on tumour cells. Indeed, BIM 26226 inhibited [^{3}H]thymidine incorporation into GRP-treated acinar pancreatic carcinoma cells. A dose-dependent inhibition of DNA synthesis was also observed under basal (non-stimulated) conditions. Thus, it can be hypothesized that tumour cells may be able to release a GRP/bombesin like peptide which could act as an autocrine growth factor as reported for human ductal pancreatic cell lines and human small cell lung carcinoma (Cuttita et al., 1985; Wang et al., 1996).

Our in vitro binding assays confirm the direct effect of BIM 26226 on pancreatic tumour cells. BIM 26226 displaced the binding of [^{125}I] GRP to acinar tumour plasma membranes with an efficacy similar to that of native GRP and RC-3095, another GRP-receptor antagonist developed by Szepeshazi et al. (1991, 1993, 1994), Schally et al. (1994), Qin et al. (1994, 1995a). These results indicate that BIM 26226 binds specifically to GRP receptors present on tumour plasma membranes. Thus, the inhibitory effect of BIM 26226 on in vitro and in vivo growth of an acinar pancreatic carcinoma can be explained by the competitive occupancy of specific binding sites for GRP on the cells.

Other reports have shown similar antitumoral effects of another GRP antagonist, RC-3095, on pancreatic ductular carcinoma induced by nitrosamines in hamsters or human cancer cell lines (SW 1990, CFPAC-1) growing in nude mice (Szepeshazi et al., 1991, 1993, 1994; Schally et al., 1994; Qin et al., 1994, 1995a).

The antimitogenic activity of BIM 26226 could be explained in the context of its specific interaction with GRP receptors. Thus, all intracellular steps activated by GRP will be blocked by BIM 26226. It is now accepted that binding of GRP to specific G-protein-associated receptors stimulates phosphoinositide turnover, mobilizes intracellular Ca^{2+}, activates protein kinase C, and induces arachidonic acid release, leading to the mitogenic effect. In addition, GRP can induce transmodulation of other receptors, especially those with tyrosine kinase activity such as EGF receptors (Liebow et al., 1992). Indeed, bombesin and GRP have been shown to enhance the phosphorylation of EGF receptors, stimulating growth of tissues and tumours (Liebow et al., 1994). The GRP receptor antagonist, RC-3095, was able to induce down-regulation of EGF receptors in ductal pancreatic cancers, leading to an antitumoral effect (Szepeshazi et al., 1993, 1994; Qin et al., 1994). A similar mechanism of action is not excluded for BIM 26226. However, the latter also depressed the intracellular accumulation of Ca^{2+} induced by bombesin in a gastric tumour cell line without affecting its growth (Bold et al., 1994). Thus, the post-receptor events induced by BIM 26226 seem to be more complex.

The present investigation also indicated that pancreatic tumour growth can be inhibited by an analogue of somatostatin, lanreotide, which also inhibits the growth of the normal pancreas induced by GRP but not that under basal conditions. Thus, lanreotide, which differs from native somatostatin with respect to relative potency, duration of action and tissue selectivity (Moreau et al., 1991), exerts effects on the acinar pancreatic carcinoma similar to those of SMS 201–995, another somatostatin analogue (Hajri et al., 1991b).

Since there exist different receptors for somatostatin and bombesin/GRP on tumour cell membranes, it would be expected that the administration of lanreotide and BIM 26226 together would potentiate the antitumoral effect of each peptide alone. Surprisingly, our experiments did not confirm this hypothesis. Indeed, we found no additive inhibitory effect, either on pancreatic tumour growth or on the normal pancreas when lanreotide and BIM 26226 were administered together in rats treated or not with GRP.

Lanreotide could affect the growth of acinar pancreatic carcinoma in different ways. It could act indirectly by inhibiting the release of a number of gastrointestinal hormones, peptides and growth factors involved in neoplastic processes such as cholecystokinin, bombesin/GRP, gastrin, EGF and IGF I (Sassolas et al., 1989; Reubi and Laissue, 1995; Frystyk et al., 1996). Lanreotide and two other somatostatin analogues, SMS 201–995 and RC-160, have also been shown to inhibit vascularization of the tumour, explaining in part the antitumoral action (Barrie et al., 1993; Reubi and Laissue, 1995; Woltering et al., 1997).

Whatever is the case, our in vitro studies demonstrate a direct effect of lanreotide on cultured tumour cells. Lanreotide inhibited DNA synthesis both in the presence and absence of GRP. Thus, lanreotide seems to be directly implicated in the growth of acinar tumour cells. Similar results have been previously reported for SMS 201–995 in the acinar pancreatic carcinoma growing in the rat and in cultured AR4-2J cells (Viguerie et al., 1989; Hajri et al., 1991b). The effect of lanreotide may be explained by a direct interaction with specific receptors of somatostatin on acinar tumour plasma membranes. As previously reported by us (Hajri et al., 1991b), these receptors were overexpressed and were of high affinity (K_d = 0.16 nmol/l and B_{max} = 100 fmol/mg protein). In the last years, five different somatostatin receptors (sst_{1-5}) have been identified. They form a distinct group in the superfamily of G-protein coupled receptors (Bruns et al., 1996). In the AR4-2J cell line, which is close to our azaserine-induced carcinoma, the somatostatin analogues, lanreotide, RC 160 and SMS

201–995, primarily interact with $sstr_2$ (Delesque et al., 1995; Coy and Taylor, 1996).

Lanreotide could block cellular proliferation by inhibiting centrosomal separation (Mascardo and Sherline, 1982) or by opposing the growth stimulation induced by EGF (Hierowski et al., 1985). In human ductular pancreatic cell lines (Mia Paca-2 cells), RC-160 has been shown to cause the dephosphorylation of EGF receptors and inhibition of EGF-induced growth by stimulating protein tyrosine phosphatase activity (Hierowski et al., 1985; Liebow et al., 1989; Delesque et al., 1995). Since bombesin/GRP receptor antagonists induce the down-regulation of EGF-receptors (Szepeshazi et al., 1993, 1994; Qin et al., 1994), it is conceivable that the tumour inhibitory effect induced by the combined administration of lanreotide and BIM 26226 is not greater than that of single agents. The antitumoral effect could also be explained through the inhibition of intracellular cAMP pathway induced by somatostatin analogues (Qin et al., 1995b).

5. Conclusion

Thus, our results indicate that BIM 26226 and lanreotide are able to inhibit the growth of an acinar pancreatic carcinoma. This effect may be mediated, at least in part, by the occupation of specific receptors overexpressed in these tumour cells as previously reported by us (Hajri et al., 1991b, 1996). Whether or not BIM 26226 and/or lanreotide exert similar effects on ductular pancreatic tumours needs to be investigated. In conclusion, these findings provide evidence that the development of peptide agonists and antagonists is of great interest in the treatment of pancreatic carcinoma.

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