Advances in Brief

Stimulation by Bombesin and Inhibition by Bombesin/Gastrin-releasing Peptide Antagonist RC-3095 of Growth of Human Breast Cancer Cell Lines

Tetsu Yano, Jacek Pinski, Kate Groot, and Andrew V. Schally

Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center, New Orleans, Louisiana 70146, and Section of Experimental Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112

Abstract

Recently, it was reported that bombesin/gastrin-releasing peptide (GRP) have mitogenic effects on some human breast cancer cell lines. In this study, we investigated the effects of bombesin/GRP and its receptor antagonist (RC-3095) on the proliferation of breast cancer cell lines, MDA-MB-231, MCF-7 MIII, and MCF-7. Stimulation by bombesin and inhibition by RC-3095 of cell growth were found in MDA-MB-231 and MCF-7 MIII cells cultured in phenol red-free medium with 5% heat-inactivated and dextran-coated charcoal-treated fetal bovine serum (DCC-FBS). A stimulatory effect by bombesin was not observed in the presence of untreated FBS. [3H]Thymidine incorporation into DNA in these cells was suppressed by RC-3095. MCF-7 cells failed to respond to bombesin and RC-3095 in the presence of either FBS or DCC-FBS. GRP-like immunoreactivity was found in the cell extracts and FBS, but it was undetectable in DCC-FBS. It appears that the stimulatory effect of bombesin on cell proliferation of MCF-7 MIII and MDA-MB-231 cell lines could be obtained because of reduction in the levels of some serum factors in DCC-FBS. These results suggest that bombesin/GRP may act as growth factors through bombesin/GRP receptors in some human breast cancers.

Introduction

BN3 originally isolated from the skin of the frog Bombina bombina (1) and its mammalian counterpart, GRP, have been reported to be mitogenic for several cells such as 3T3 mouse fibroblasts (2), human normal bronchial epithelial cells (3), and SCLC cells (4, 5). It has been shown that SCLC cells produce BN/GRP-like peptides which can act as autocrine growth factors for these cells (4, 5). There is some evidence that BN/GRP may be involved in the function and growth of human breast cancer (6-11).

Powerful BN/GRP receptor antagonists have been developed in view of their possible clinical application for the treatment of SCLC (12, 13). Modern BN/GRP antagonists are based on the tetradecapeptide sequence of bombesin, the carboxy-terminal amino acid sequence of GRP, or on shorter sequences thereof (12, 13). Recently, we synthesized [n-TpiGlu-Leu-His-Leu] bombesin (6-14) (RC-3095), a new short chain pseudononapeptide BN/GRP antagonist (13). Antagonist RC-3095 inhibits GRP (14-27) stimulated amylase release from superfused rat pancreatic acini at nanomolar concentrations and has strong binding affinity to Swiss 3T3 and SCLC H-345 cells (13).

MCF-7 MIII is an estrogen-independent but estrogen-sensitive subline of MCF-7 human breast cancer cell line (14, 15). Recently, we found that RC-3095 inhibits growth of MCF-7 MIII human breast cancer xenografts in nude mice (16) and MXT estrogen-independent mammary cancer in mice (17). While MCF-7 MIII cells are fully estrogen-independent in vitro, these cells still retain the same level of estrogen receptor expression and the sensitivity to the inhibitory effects to antiestrogens. MCF-7 MIII cells appear to have acquired an intermediate phenotype which is estrogen independent in vitro but estrogen responsive in vivo. In this study, we investigated the effects of bombesin and RC-3095 on the proliferation of 3 human breast cancer cell lines, namely, MCF-7, MCF-7 MIII, and estrogen-independent MDA-MB-231. In addition, GRP-like immunoreactivity in these cell lines was measured.

Materials and Methods

Bombesin receptor antagonist RC-3095 [n-TpiGlu-TrpAla-Val-Gly-His-Leu-NH2] was synthesized in our laboratory by solid-phase methods (13), was kindly provided by Dr. Robert Clarke and Dr. Philip Ptasinski (Georgetown University Medical Center, Washington, DC.). MDA-MB-231 cells were obtained from the American Type Culture Collection (Rockville, MD). MCF-7 cells were maintained in DMEM containing phenol red and supplemented with 5% FBS. MCF-7 MIII and MDA-MB-231 cells were grown in phenol red-free medium with 5% FBS or DCC-FBS which was prepared as described below. FBS was sterilized with 0.22 μm nylon membrane filter. All media also contained 15 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, 2 mm glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml). Cells were cultured in Costar T-75 flasks in a humidified atmosphere of 5% CO2 and 95% air at 37°C and were passaged every 4-6 days with the use of trypsin-EDTA.

Cell Growth Studies. Cells from 70-80% confluent cultures were seeded into Costar 24-multiwell plates at a density of 104 cells/well in the growth medium. A portion of MCF-7 cells was washed with phenol red-free DMEM twice and seeded in this medium containing 5% DCC-FBS. After 48 h (day 0), the medium was replaced with fresh medium containing bombesin or RC-3095. These peptides were dissolved in dimethyl sulfoxide and diluted with the medium. The final concentration of dimethyl sulfoxide was always less than 0.05%. The medium was changed again on day 2. The cell number for each well was determined in a Coulter Counter after detachment of the cells by trypsinization on
day 4. The cell viability, assessed by trypan blue dye exclusion, was more than 90%. All cell growth data are representative of three independent experiments.

[3H]Thymidine Incorporation. Cells were seeded into Falcon 96-multiwell plates at a density of 5000 cells/well in the growth medium. In the case of MDA-MB-231 and MCF-7 MIII cell lines, cells, maintained in the presence of untreated FBS, were used. After 48 h, the medium was replaced with fresh medium containing 0.5% FBS and various concentrations of RC-3095. After a further 48 h, the medium was changed again and 0.5 μCi [methyl-3H]thymidine/well was added. After incubation for 4 h, the cells were washed twice with phosphate-buffered saline and 3 times with 10% trichloroacetic acid at 4°C. The precipitate was solubilized in 1 N NaOH and neutralized with 10% acetic acid. The radioactivity was measured in a liquid scintillation counter.

Radioimmunoassay of GRP-like Peptides. The method described by Korman et al. was followed (18). MDA-MB-231 and MCF-7 MIII cells, maintained in the presence of DCC-FBS, and MCF-7 cells, cultured in the presence of FBS, were used. Cells from confluent cultures in three T-75 flasks were harvested by trypsinization, washed three times in phosphate-buffered saline, and then extracted with boiling 2 N acetic acid. The supernatant was quickly frozen and lyophilized prior to radioimmunoassay. For extraction of serum, 100 μl of FBS or DCC-FBS were added to 300 μl of a solution containing 5% formic acid, 15% trifluoroacetic acid, 9% HCl, and 1%, NaCl. The precipitated proteins were centrifuged at 2000 x g for 30 min and the supernatant was reconstituted in assay buffer (10 mM sodium phosphate buffer, pH 7.6, containing 25 mM EDTA, 140 mM NaCl, 0.1% gelatin, and 0.1% sodium azide). Synthetic GRP (14-27) was used as an unlabeled standard and was also iodinated by the standard chloramine-T method (19). Specific rabbit antiserum to GRP(14-27), which showed total cross-reactivity with the active carboxy-terminal sequence of GRP, was kindly provided by Dr. T. W. Moody (George Washington University Medical Center) and was used at a final dilution of 1:700,000. The detection limit of assay was 5 fmol/tube.

Statistical Analyses. All data are expressed as the mean ± SEM, and statistical analyses were performed with Student’s t test.

Results

As shown in Figs. 1 and 2, bombesin stimulated proliferation of MDA-MB-231 and MCF-7 MIII cells to a similar extent at concentrations between 10^-9 and 10^-6 M in the presence of 5% DCC-FBS. The maximal increase in cell numbers was about 40% at 10^-6 M bombesin for MDA-MB-231 cells and about 60% at 10^-5 M bombesin for MCF-7 MIII cells. The growth-stimulatory effects of bombesin were not found in the presence of untreated FBS. Antagonist RC-3095 inhibited the growth of these cells dose dependently at concentrations between 10^-8 and 10^-5 M in the presence of 5% DCC-FBS (Figs. 1 and 2). At the 10^-5 M concentration of RC-3095, the final cell number decreased by 50% for both cell lines. In another experiment, MCF-7 MIII cell growth stimulated by 10^-7 M bombesin, was inhibited by RC-3095 (10^-8-10^-5 M) in a dose-dependent manner to control levels (data not shown). [3H]Thymidine incorporation into DNA in these cells was suppressed by RC-3095 at a concentration of 10^-7-10^-5 M, although the extent of inhibition was relatively small (Table 1). In contrast, growth of MCF-7 cells was not affected by bombesin or RC-3095 at concentrations between 10^-10 and 10^-5 M in the presence of either untreated FBS or DCC-FBS (data not shown). Similarly, [3H]-thymidine incorporation into DNA was not inhibited by RC-3095 in MCF-7 cells (Table 1).

Low levels of GRP-like immunoreactivity could be detected in the cell extracts or FBS, but not in DCC-FBS (Table 2). Among 3 breast cancer cell lines, MDA-MB-231 cell extracts showed the highest GRP-like immunoreactivity (4.0 fmol/10^6 cells).

Discussion

The present study shows that bombesin stimulates the proliferation of MDA-MB-231 and MCF-7 MIII human breast cancer cells in vitro under certain culture conditions, that is, in the presence of DCC-FBS. The stimulatory effect of bombesin could not be obtained in the presence of untreated FBS.

Table 1 Effect of RC-3095 on incorporation of [3H]thymidine by MDA-MB-231, MCF-7 MIII, and MCF-7 MII human breast cancer cell lines

<table>
<thead>
<tr>
<th>Concentration of RC-3095 (M)</th>
<th>[3H]Thymidine incorporation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231</td>
<td>MCF-7 MIII</td>
</tr>
<tr>
<td>10^-7</td>
<td>89.3 ± 1.6*</td>
</tr>
<tr>
<td>10^-6</td>
<td>81.8 ± 4.1*</td>
</tr>
<tr>
<td>10^-5</td>
<td>75.2 ± 3.2*</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001 versus control by Student’s t test.
This finding is similar to that in the other studies using T47D and ZR-75-1 human breast cancer cell lines (9). The lack of stimulatory effect of bombesin in untreated FBS may be due to degradation of BN/GRP by peptidases (5, 11, 20). Serum factors may interfere with BN/GRP receptors (5). It is also possible that cells are already fully stimulated by growth factors included in the medium containing phenol red and FBS or in the phenol red-free medium with DCC-FBS. In our study, BN/GRP binding sites were previously found in T47D and MDA-MB-231 cell lines by Giacchetti et al. (7), GRP had no stimulatory effect on growth of these cell lines in the presence of DCC-FBS, which contains reduced levels of estrogen. BN/GRP binding sites were not detected in MCF-7 cells by Giacchetti et al. (7) or by us (data not shown). Patel and Schrey (8, 10) found BN/GRP binding sites in MCF-7 cells and showed that bombesin stimulated inositol lipid hydrolysis and Ca\(^{2+}\) efflux in human breast cancer cells by bombesin. Cancer Res., 50: 235–239, 1990.

References

Stimulation by Bombesin and Inhibition by Bombesin/Gastrin-releasing Peptide Antagonist RC-3095 of Growth of Human Breast Cancer Cell Lines

Tetsu Yano, Jacek Pinski, Kate Groot, et al.