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Breast cancer cell-associated endopeptidase EC 24.11 modulates proliferative response to bombesin

DM Burns[1], B Walker[2], J Gray[2] and J Nelson[2]

[1]School of Biomedical Sciences, University of Ulster at Jordanstown, Shore Rd, Newtownabbey, Co. Antrim, BT37 0QB, Northern Ireland; [2]Division of Biochemistry, School of Biology and Biochemistry, Queen's University, Medical Biology Centre, 97 Lisburn Road, Belfast BT7 1NL, Northern Ireland

Summary We have investigated the production, growth and inactivation of gastrin-releasing peptide (GRP)-like peptides in human breast cancer cell lines. Radioimmunoassay detected GRP-like immunoreactivity (GRP-LI) in T47D breast cancer cells but not in the conditioned medium, indicating rapid clearance. No GRP-LI was found in the ZR-75-1 or MDA-MB-436 cells or their conditioned medium. High-performance liquid chromatography (HPLC) analysis of the GRP-LI in the T47D cells revealed a major peak, which co-eluted with $GRP_{18–27}$, and a minor more hydrophilic peak. In vitro stimulation of T47D cell growth by bombesin (BN) was enhanced to 138% of control levels (bombesin alone) by the addition of the selective endopeptidase EC 3.4.24.11 inhibitor phosphoramidon (0.1 ng ml^{-1}). Fluorogenic analysis using whole cells confirmed low levels of this phosphoramidon-sensitive enzyme on the T47D cells. This enzyme, previously unreported in human breast cancer cells, significantly modulates both T47D growth and its response to BN-induced growth.

Keywords: enkephalinase; bombesin; gastrin-releasing peptide; breast cancer cells; neutral endopeptidase 24.11

Bombesin (BN), a tetradecapeptide originally isolated from amphibian skin in 1970, has potent hypertensive and gastrin-releasing effects in mammals (Erspamer et al, 1970; Erspamer et al, 1972*a*,*b*). Its homologue, gastrin-releasing peptide (GRP), is a 27-amino-acid peptide (McDonald et al, 1979) that is highly conserved in amphibian, avian, canine, rat and human species (McDonald et al, 1980; Reeve et al, 1983; Orloff et al, 1984; Zoeller et al, 1989; Nagalla et al, 1992). A fully active decapeptide identical to the C-terminus of GRP ($GRP_{18–27}$) has also been isolated from porcine and human tissue (Minamino et al, 1984; Orloff et al, 1984). GRP-like immunoreactivity (GRP-LI) and receptor binding is distributed predominantly in the brain, spinal cord, gut and lung, where it is thought to act both as a growth factor and as a neuroregulatory hormone (Moody et al, 1980; Sunday et al, 1988).

GRP has been detected in 60–70% of small-cell lung cancers (SCLC) (Wood et al, 1981; Yamaguchi et al, 1983; Bostwick et al, 1984), and GRP receptors have been demonstrated in numerous SCLC cell lines (Moody et al, 1983). In addition, the involvement of GRP as an autocrine growth factor in SCLC is well established (Cuttitta et al, 1985). This prompted a vast research effort to develop GRP receptor antagonists as a potential treatment for SCLC. More recently, evidence has been accumulating to suggest that GRP may play a role in the growth of breast cancer cells. GRP-LI has been detected in a proportion of breast tumours (Foster and Tan, 1984; Gaudino et al, 1986; McKillop et al, 1988) but not in normal tissues (Bostwick and Bensch, 1985; Gaudino et al, 1986). Using Northern blotting, GRP mRNA has been detected in 25% of unselected primary breast tumours (Pagini et al, 1991). More recently, Vangsted et al (1991) detected GRP-LI in 39% of primary breast tumours from post-menopausal women. GRP-LI has been detected in a number of established breast cancer cell lines accompanied by much higher levels of the C-terminal extension peptide (CTEP) (Weber et al, 1989; Vangsted et al, 1991; Yano et al, 1992). GRP receptors have been described in breast cancer cell lines T47D and MDA-MB-436 (Giacchetti et al, 1990). Previously, we have shown that both BN and GRP stimulate proliferation of breast cancer cell lines when culture serum has been stripped of endogenous GRP-like peptides (Nelson et al, 1991); other workers have confirmed these findings (Yano et al, 1992).

The cell-surface enzyme neutral endopeptidase EC 24.11 (EC 3.4.24.11), known to be involved in the catabolism and inactivation of a number of neuropeptides in the brain and GI tract (Relton et al, 1983; Matsas et al, 1984), has also been shown to hydrolyse and inactivate GRP (Bunnett et al, 1988). This enzyme inactivates GRP and $GRP_{18–27}$ by cleaving the His25–Leu26 bond; the His20–Trp21 bond is also cleaved, but to a much lesser extent (Bunnett et al, 1988). Other work suggests that high levels of neutral endopeptidase EC 24.11 regulate GRP-mediated growth of fetal lungs (King et al, 1993), and there is also considerable evidence that this enzyme regulates extracellular GRP levels in SCLC cell lines. Colony formation in SCLC cell lines that secrete GRP-like peptides is potentiated when neutral endopeptidase EC 24.11 is inhibited using phosphoramidon. This increase in colony formation is reduced in the presence of GRP receptor antagonists, indicating that a major role of neutral endopeptidase EC 24.11 is to reduce the activity of secreted GRP-like peptides (Shipp et al, 1991). Decreased EC 24.11 expression in prostatic cancer cell lines has been correlated with increased growth response to bombesin, as well as progression to an androgen-independent phenotype (Papandreou et al, 1998).

Given the reports that GRP is degraded locally in both normal and neoplastic tissues and the mounting evidence that GRP-like peptides may act as growth factors in human breast cancer, we

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Correspondence to: D Burns, School of Biomedical Sciences, University of Ulster at Jordanstown, Shore Rd, Newtownabbey, Co. Antrim BT37 0QB, Northern Ireland

have investigated the possibility that these cells also possess neutral endopeptidase on their cell surface. Here, we report the presence of an EC 3.4.24.11-like enzyme on the surface of breast cancer cells. The growth of these cells and their response to bombesin was also greatly enhanced in the presence of the EC 3.4.24.11 selective inhibitor phosphoramidon.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle medium (DMEM), RPMI-1640 and L-15 media were obtained from Flow Laboratories, Irvin, UK; fetal calf serum (FCS) and trypsin were purchased from Gibco, Paisley, UK. Phosphoramidon, anti-rabbit immunoprecipitation reagent, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), leucine aminopeptidase and the enzyme inhibitors isopropyl difluorophosphate, iodoacetamide and pepstatin were obtained from Sigma Chemical Co, Poole, Dorset, UK. The fluorogenic substrate *N*-succinyl-Ala-Ala-Phe-AMC (AAP-AMC) and bombesin were obtained from Novabiochem, Nottingham, UK. The specific elastase inhibitor was synthesized according to the method previously published (Oleksyszyn and Powers, 1991).

Cell culture

The T47D (Keydar et al, 1979), ZR-75-1 and MDA-MB-436 breast cancer cell lines were obtained from the European Collection of Animal Cell Cultures, Porton Down, UK. The SCLC H345 cell line was obtained from Imperial Cancer Research Fund Laboratories, London, UK. The T47D and H345 cell lines were maintained in DMEM supplemented with 10% FCS and 10% heat-inactivated FCS respectively. The ZR-75-1 cells were maintained in RPMI-1640 medium supplemented with 5% FCS, and the MDA-MB-436 cells were maintained in L-15 medium supplemented with 10% FCS. Cells were routinely passaged when subconfluent using 2 ml of 2.5 mg ml^{-1} trypsin in calcium-free saline (0.14 M sodium chloride, 5 mM potassium chloride, 4 mM sodium hydrogen carbonate, 5 mM glucose).

Growth studies

After trypsinization, cells were inoculated into 96-well plates at 1×10^4 cells per well in medium used for routine passaging.

After 24 h, the medium was removed and the treatments added in medium containing heat and dextran-coated charcoal-treated FCS (HT and DCC FCS) prepared as detailed previously (Nelson et al, 1987, 1991). Treatments were either control (medium only), 0.001–100 nM bombesin or 0.001–100 nM bombesin in the presence of phosphoramidon (0.1–100 ng ml^{-1}). For cell enumeration, a modification of the MTT assay (Twentyman and Luscombe, 1987) was used as follows; 10 µl of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT 20 mg ml^{-1}) was added to each well and incubated for 2 h at 37°C, the medium was removed and the formazan crystals were solubilized in 0.2 ml of DMSO. The colour intensity was then measured at 510 nm using a Titertek Multiscan plate reader.

Preparation of cells and conditioned media for radioimmunoassay (RIA)

Preconfluent flasks (90%) were incubated in serum-free media for 24 h, the conditioned medium was removed and centrifuged at 2500 ***g*** for 10 min and stored at –20°C before Sep-Pak extraction. Cells were removed from monolayer by scraping and washed in serum-free medium and frozen at –20°C. The extraction procedure used for the cell pellets is a modification of that described previously by Gaudino et al (1986). Cells were washed in ice-cold extraction buffer (50 mM Hepes, 5 mM magnesium chloride, 1 mg ml^{-1} bacitracin and 10 µg ml^{-1} phosphoramidon) and boiled for 10 min in 10% formic acid. The extracts were neutralized and clarified by centrifugation at 3800 ***g*** for 15 min. The supernatants and conditioned media were desalted and concentrated using Sep-Pak C18 elution as follows; cartridges were pre-wet with 10 ml each of water, ethanol and 0.05% trifluoroacetic acid (TFA), the sample was passed through the cartridge three times. After washing with 0.05% TFA, the peptides were eluted with 80% acetonitrile containing 0.05% TFA. Positive controls in which [^{125}I]GRP was added to cell pellets showed a 79% recovery of radioligand from the cartridge. The eluent was lyophilized and frozen. Immediately before measurement, the residue was dissolved in RIA assay buffer.

HPLC analysis of cell extracts

Reverse phase high-performance liquid chromatography (HPLC) analysis was carried out using Delta-Prep System (Waters Associates) fitted with a µBondpak C_{18} analytical column. Buffer A was deionized water containing 0.05% trifluoroacetic acid, initially at 98% decreased to 0% after 25 min. Buffer B comprised acetonitrile containing 0.05% trifluoroacetic acid, started at 2% and increased to 65% after 25 min. The solvent flow rate was 1 ml min^{-1} and samples were collected over a period of 1 min. Three runs were performed and eluents for corresponding intervals were pooled and lyophilized and stored at –20°C before radioimmunoassay.

Radioimmunoassay

This assay utilized a rabbit $GRP_{18–27}$ antiserum raised by this group and described previously (Walker et al, 1995). Five thousand c.p.m. of [^{125}I]GRP at a 1:1000 dilution of the antisera and standards or unknown samples were added in duplicate to siliconized Eppendorf tubes in a final volume of 0.2 ml of radio-immunoassay buffer (50 mM sodium phosphate, 10 mM EDTA and 0.3% RIA-grade bovine serum albumin, pH 7.4). A blank (no antiserum) and a standard curve with 0–1000 fmol per tube $GRP_{18–27}$ was set up for each test. After 24 h incubation at 4°C, bound radioligand was immunoprecipitated by anti-rabbit immunoprecipation reagent for 2 h at room temperature and centrifuged at 7500 ***g*** for 15 min. The supernatant was removed and bound radioligand was counted for 1 min in a LBK gamma-counter.

Fluorogenic enzyme assay

EC 24.11 activity was measured in whole-cell suspensions in a coupled assay using *N*-succinyl-Ala-Ala-Phe 7-amido-4-methyl-coumarin (AAP-AMC) as substrate. Cells were removed from flasks by scraping, washed in phosphate-buffered saline (PBS) and suspended in fluorogenic assay buffer (0.1 M sodium phosphate buffer, pH 7.5, with 0.05% Brijj) at 1×10^{-4} cells ml^{-1} before experiment. Thirty-six units (140 U mg^{-1} protein) of leucine aminopeptidase (EC 3.4.11.1) from porcine kidney and *N*-succinyl-Ala-Ala-Phe-AMC (AAP-AMC) were added to the

cuvette to give a final concentration of 10 μM in a final volume of 1.0 ml. The reaction was initiated by the addition of an appropriate number of whole cells in fluorogenic assay buffer. Samples were excited at 383 nm and emission was measured at 455 nm. Production of fluorogenic product at room temperature was measured for 10 min using a Perkin-Elmer fluorimeter model LS50B. Specificity of the assay was tested by performing the following negative controls before each experiment: AAP-AMC alone; AAP-AMC and leucine aminopeptidase; and AAP-AMC with leucine aminopeptidase and cells suspension. Inhibitors were incubated in the presence of cells for 30 min before addition of AAP-AMC and leucine aminopeptidase and the assay.

Statistical analysis

Control and treatment data from the growth studies were compared using an unpaired Student's *t*-test.

RESULTS

Measurement of GRP-LI in breast cancer cell lines

RIA was performed on the breast cancer cell lines T47D, ZR-75-1 and MDA-MB-436 cell pellets and their conditioned medium. The T47D cells were found to contain 1.09 pmol GRP-LI per 10^7 cells and dilutions paralleled the standard curve (Figure 1). Conditioned medium from the T47D cells, however, did not contain any GRP-LI. Immunoreactivity was not detected in ZR-75-1, MDA-MB-436, Cos-7 or A431 cell lines or their conditioned medium.

The immunoreactivity detected in the T47D cells was further investigated using reversed-phase HPLC. A minor peak eluted after 6 min, however the vast majority eluted in a sharp peak after 20 min. This corresponds very closely with the elution time of 19.5 min for $GRP_{18–27}$ (Figure 2).

Effect of phosphoramidon on BN-induced cell growth

MTT assays were set up to examine the effect of 0.1–100 ng ml^{-1} phosphoramidon alone and in combination with 0.1 nM BN on the T47D cells in HT and DCC FCS (Figure 3). After 3 days, 1–100 ng ml^{-1} of the inhibitor gave a marked inhibition (19–35%) of cell growth alone. This dose range of phosphoramidon also dramatically potentiated the response of these cells to BN. Whereas BN alone showed a small increase in cell number after 3 days, in this instance it was not statistically significant. However, in combination with 0.1–100 ng ml^{-1} phosphoramidon the cell growth was markedly increased. Phosphoramidon (0.1 ng ml^{-1} and 1 ng ml^{-1}) gave a 38% and 30% increase in BN-induced growth respectively. Phosphoramidon (0.1 ng ml^{-1}) appeared to be a threshold dose above which higher concentrations of inhibitor produced no increased effect. When compared with cell numbers in the presence of 1 ng ml^{-1} phosphoramidon alone, 0.1 nM BN in combination with phosphoramidon produced an increased growth of 132% ($P < 0.01$).

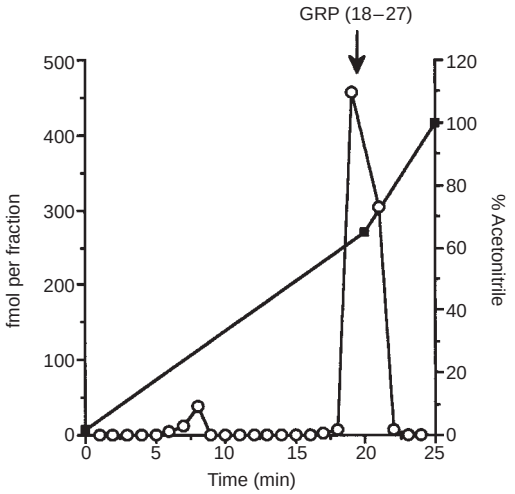

Figure 2 Reverse-phase HPLC separation of T47D cell extract followed by GRP radioimmunoassay of fractions. Elution buffer was acetonitrile containing 0.05% trifluoroacetic acid starting at 2% and increasing to 65% after 25 min. Eluents from corresponding fractions were pooled from three runs and lyophilized before radioimmunoassay. Elution time of $GRP_{18–27}$ is indicated by an arrow

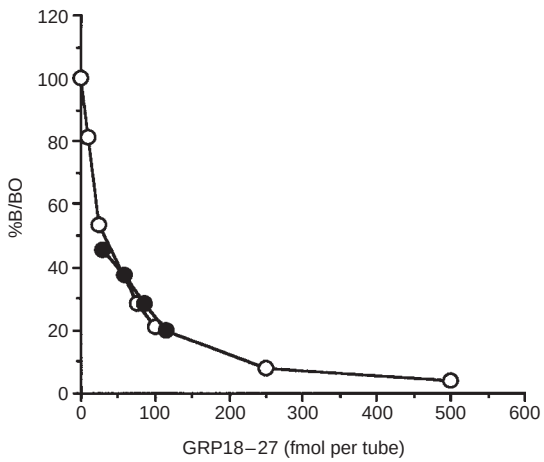

Figure 1 Radioimmunoassay standard curve for $GRP_{18–27}$ –○– and T47D cell extract –●–. [^{125}I]GRP (5000 c.p.m.) and antiserum at final dilution of 1:1000 per tube were used and incubations were performed at 4°C for 24 h. Data points represent the means of duplicate tubes

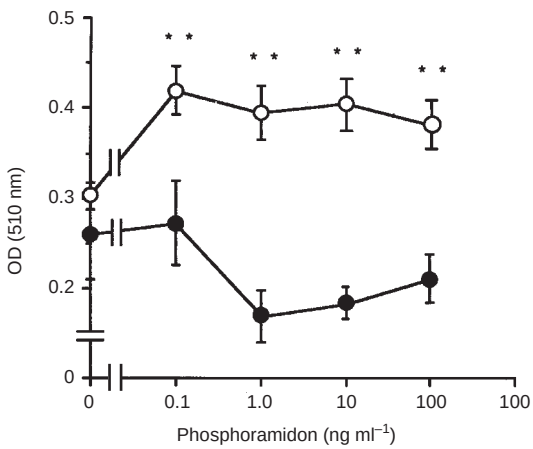

Figure 3 The effect of phosphoramidon –●–, and phosphoramidon in the presence of bombesin (0.1 nM) –○– on the growth of T47D cells after 3 days in DMEM +10% HT and DCC-treated FCS. Results are the mean optical density + s.d. for eight wells. **$P < 0.01$ vs phosphoramidon only in Student's *t*-test

Fluorogenic measurement of EC 3.4.24.11

For all experiments, the substrate alone produced baseline fluorescence indicating that there was no autohydrolysis. Similarly, the substrate in the presence of leucine aminopeptidase (LAP) produced baseline fluorescence eliminating the possibility of enkephalinase contamination of the LAP. The SCLC cell line H345 was found to produce a turnover of substrate of 303.6 pmol per 10^6 cells h^{-1}. This activity was reduced by 76.6% in the presence of phosphoramidon. The T47D cells exhibited a much lower turnover of substrate with 4.6 pmol per 10^6 cells h^{-1} being recorded alone with 56% of this activity being phosphoramidon sensitive. The inhibitors DFP (0.5 mM), iodoacetamide (10 μM) and pepstatin (10 μM) did not inhibit substrate turnover. In the presence of the elastase inhibitor Z-Val-diphenyl phosphonate (43 μM), a small inhibition was recorded (data not shown).

DISCUSSION

It is widely accepted that SCLC tumours arise from endocrine cells because they display characteristics such as amine precursor uptake and decarboxylation (APUD) staining and contain several neuropeptides (Baylin et al, 1980; Gazdar et al, 1980; Marangos et al, 1982). Some of these neuropeptides, including bombesin, have been shown to act as autocrine growth factors in numerous SCLC cell lines (Cuttitta et al, 1985; Bepler et al, 1988; Sethi and Rozengurt, 1991). Unlike SCLC, the vast majority of breast cancers are epithelium-derived tumours, with only 5% being estimated to be carcinoids with a neuroendocrine origin (Papotti et al, 1989). These tumours are reported to be strongly immunoreactive for GRP-LI (Nesland et al, 1985; Pagini et al, 1991). The presence of neuropeptides in a large proportion of breast tumours would, therefore, be unexpected, however, in at least one study mRNA for GRP was detected in 25% of unselected breast tumours and it had poor correlation with neuroendocrine differentiation (Pagini et al, 1991). Interestingly, GRP-LI has also been reported in breast milk in a number of studies (Ekman et al, 1985; Westrom et al, 1987). Thus, the presence of GRP in breast cancer cells may instead be due to lactational differentiation, a phenomenon which is known to occur in breast carcinomas (Clayton et al, 1982; Lee et al, 1984; Baildam et al, 1988).

The occurrence of GRP-LI in breast cancer cell lines is not without controversy. Although it has been detected in T47D cells in this study and by Vangsted et al (1991), Northern blot analysis for prepro-GRP mRNA has been negative (Giachetti et al, 1990; Pagini et al, 1991). This lack of correlation between GRP immunoreactivity and mRNA detection is observed in other tumour types (Hamid et al, 1987, 1989), and has been attributed to antisera cross-reactivity with related neuropeptides (Pagini et al, 1991). Alternatively, detection of a low-abundance mRNA for this peptide may require reverse transcription polymerase chain reaction (RT-PCR) and this was not carried out in the studies on breast cancer cell lines (Giachetti et al, 1990; Pagini et al, 1991). Hence, it is important to further characterize this immunoreactivity. In this study, HPLC analysis strongly suggests the major peak is $GRP_{18–27}$ because it co-eluted with this standard. This is in agreement with similar chromatographic analysis performed by Vangsted et al (1991). The minor, more hydrophilic peak may represent a larger peptide, perhaps GRP. Failure to detect GRP-LI in conditioned medium from T47D cell-conditioned medium here and by Vangsted et al (1991) suggests that it is rapidly cleared from the extracellular surface. Of importance is the fact that the C-terminal extension peptide (CTEP), which is cleaved from prepro-GRP during processing, has been found in the T47D-conditioned medium in the absence of the mature peptide (Vangsted et al, 1991). Accumulation of a similar stable CTEP has also been described in SCLC cell lines (Vangsted and Schwartz, 1990). The processing and subsequent degradation of the prepro-GRP moiety clearly happens extracellularly in both cancer cell types.

Inhibition of fluorogenic substrate hydrolysis by phosphoramidon is a well-documented specific test for EC 3.4.24.11 activity (Hudgin et al, 1981; Shipp et al, 1991; Frame et al, 1996). The demonstration that T47D whole cell-mediated turnover of AAP-AMC is sensitive to phosphoramidon strongly suggests the presence of the membrane-bound endopeptidase EC 3.4.24.11 on these cells. In the growth studies, after 3 days all concentrations of phosphoramidon tested (0.1–100 ng ml^{-1}) enhanced BN-induced growth. At this time, 1–100 ng ml^{-1} phosphoramidon alone inhibited growth, however the reason for this is unclear at present. In other tissues, EC 3.4.24.11 has been shown to degrade a range of peptides including enkephalins, substance P, bradykinin, endothelin and angiotensin I and II (Matsas et al, 1984; Vijayaraghavan et al, 1990). Although only endothelin (which is not mitogenic) has been shown to be produced by the T47D cells (Schrey et al, 1992), the possibility of other as yet unidentified EC 3.4.24.11-labile growth factors being expressed by the T47D cells cannot be ruled out. Previous work in this laboratory found BN to be mitogenic for breast cancer cell lines in vitro, with 0.001–0.1 nM giving significant growth stimulation in the T47D cells (Nelson et al, 1991). The potentiation of BN-induced growth by phosphoramidon after 3 days is likely to be due to a prolonged half-life of the peptide in the medium.

The fluorogenic assay found that there was approximately 100-fold more EC 3.4.24.11 activity on the H345 SCLC cell line compared with the T47D cells. This may be an explanation for the difference in BN dose required for mitogenesis in cell lines from these two cancer types. GRP/BN-stimulated colony formation is maximal at 50 nM in SCLC cells, but in the breast cancer cells significant stimulation is observed in the picomolar range in this laboratory (Carney et al, 1981; Nelson et al, 1991). However, this variation in mitogenicity may also be due to differing numbers of BN/GRP receptors on these cell lines. Variations in the mitogenic response to BN/GRP have been also observed between the classical and variant SCLC cell lines, this may be due to differing numbers of GRP receptors reported on these cell lines (Kado-Fong and Malfoy, 1989; Cardona et al, 1991). Stimulation of growth of breast cancer cells by BN has also been confirmed by other workers under serum-free or HT and DCC FCS conditions. In all these studies, no effect was found in medium supplemented with untreated FCS. A soluble form of EC 3.4.24.11 has been described in urine, plasma and breast cyst fluid, but routine culture FCS to date has not been investigated. Although the lack of response to BN in untreated FCS has been attributed to the presence of endogenous GRP-LI which is removed by HT and DCC treatment, the role of a soluble EC 3.4.24.11 in FCS is yet to be studied.

These findings have practical implications for experimental research in bombesin-like peptides and breast cancer. Firstly, failure to inhibit EC 24.11-like activity in the past may have compromised RIA or immunocytochemistry sensitivity, however all the buffers used for cell homogenization for RIA and HPLC in this study contained phosphoramidon. Secondly, binding of ligands in radioreceptor studies on breast cancer cell lines could be signifi-

cantly reduced because phosphoramidon was found to greatly increase binding of [^{125}I]GRP to SCLC cells (Cardona et al, 1992).

Extensive research efforts have been employed in the development and evaluation of GRP receptor antagonists as potential treatments for SCLC and, more recently, pancreatic, prostatic, gastric, colorectal and breast cancer (Jungwirth et al, 1997; Nagy et al, 1997). The presence of EC 24.11-like activity on breast cancer cells is a crucial consideration in the design of GRP receptor antagonists. Whereas the sissile bonds and the relative rates of hydrolysis have been identified for GRP and its homologues, to date none of these antagonists have been evaluated for resistance to cleavage by the enzyme.

EC 24.11 has been described on human endometrium, where levels vary through the menstrual cycle but activity correlates with plasma progesterone levels (Casey et al, 1991; Head et al, 1993). More recently, reduced EC.24.11 mRNA expression has been demonstrated on endometrial cancer cells compared with normal endometrial tissue (Pekonen et al, 1995). Levels of this enzyme are also reported to be 70–90% reduced on SCLC cell lines (Shipp et al, 1991). Additionally, EC 24.11 loss appears to play a role in the development of androgen-independent cells by allowing neuropeptides such as GRP to enhance growth (Papandreou et al, 1998). Thus, reduced clearance of peptides may be the aberrant factor which results in increased growth response of cancer cells. Therefore, the concept of measuring peptidases in tumours may be complementary to assessing growth factor levels as indicators of tumour growth or progression.

This is the first indication of the presence of such an enzyme on human breast cancer cells and supports the finding that GRP is an autocrine growth factor in the T47D cells. It is possible that reduced activity of this enzyme accompanies breast tumorigenesis because levels in breast cysts decreased in those women who carry a higher risk of future breast cancer when compared with those who are not associated with any subsequent increase in incidence (Frame et al, 1996).

In normal tissues, EC 3.4.24.11 has a diverse distribution, but is usually found on cells bearing specific receptors for the multiple possible target peptides. In the brain, it is responsible for the inactivation of the enkephalins (Malfroy et al, 1978), whereas in lung it down-regulates neurogenic inflammation by reducing local levels of tachykinins (Martins et al, 1990; Nadel, 1990; Nadel and Borson, 1991). It is also responsible for control of GRP-induced growth in fetal lung, where alterations in levels of GRP throughout maturation are accompanied by corresponding changes in EC 3.4.24.11 levels (King et al, 1993).

Given that GRP-like peptides are present in high amounts in breast milk, it is probable that an enzyme would also control these levels locally. EC 3.4.24.11 may also be present on normal breast cells in a similar manner to normal lung tissue as Gusterson et al (1985) reported specific staining for the CALLA antigen on normal human breast sections. This antigen has subsequently been shown to be identical to EC 3.4.24.11 (Ship et al, 1989). It, therefore, would be of interest to determine whether EC 3.4.24.11 or GRP-like peptides played a modulatory role in normal breast development.

In conclusion, this study demonstrates the production of a $GRP_{18–27}$ peptide by T47D breast cancer cells, but not by ZR-75-1 or MDA-MB-436. This peptide is rapidly processed, degraded and inactivated in the extracellular medium. A previously unreported enzyme with an EC 3.4.24.11 profile on the human breast cancer cell line T47D is also detected. Although this enzyme is present at low levels, it significantly modulates the growth of the T47D cells both alone and in response to BN in vitro.

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