Characterization of Bombesin/Gastrin-releasing Peptide Receptors in Human Breast Cancer and Their Relationship to Steroid Receptor Expression

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ABSTRACT

Bombesin (BN) and its mammalian counterpart, gastrin-releasing peptide (GRP), are hormonally active peptides which appear to function as autocrine or paracrine growth factors in a variety of cells. As part of a long-term investigation of the relationship of peptide and steroid hormone receptors to breast cancer progression and treatment, we examined the binding of [125I-Tyr4]BN to membranes isolated from 100 human breast carcinomas. Thirty-three of these tumors expressed BN/GRP receptor levels of >10 fmol/mg membrane protein. Two classes of [Tyr4]BN-binding sites were detected using Scatchard analyses of radioligand association data from hormone displacement curves. The high-affinity binding sites exhibited a mean dissociation constant (Kd) of 2.1 nM and a mean specific binding capacity (Bmax) of 237 fmol/mg membrane protein. The low affinity binding sites had a mean dissociation constant (Kd) of 0.3 μM and a mean binding capacity (Bmax) of 5.9 pmol/mg membrane protein. BN/GRP receptor expression in a breast carcinoma was unrelated to patient age. When the levels of BN/GRP receptors were compared to the content of the sex steroid receptors, a highly significant positive correlation (P < 0.005) was observed between the binding capacities of high-affinity [Tyr4]BN-binding sites and estrogen receptor levels and between the concentrations of low affinity [Tyr4]BN-binding sites and progesterin receptor levels (P < 0.05). This represents the first report of these labile, regulatory proteins in biopsies of human breast carcinomas. Expression of specific receptor proteins for BN/GRP, potent mitogens, in a large number of human breast cancers suggests that they may be involved in tumor cell progression. The approach based on determination of BN/GRP receptors might be useful to guide a hormonal therapy with BN/GRP antagonists in some women with breast cancer.

INTRODUCTION

The differentiation and development of the human breast including its function during pregnancy and lactation are regulated by a variety of hormones including 17β-estradiol and progesterone, cortisol, the thyroid hormones, and certain peptide hormones, such as prolactin, growth hormone, and oxytocin (1). There is increasing evidence that growth factors, particularly epidermal growth factor, are involved in development and function of normal and neoplastic breast (2–4). Estrogens and EGF3 may influence the mitotic state of mammary epithelial cells and appear to be involved in ductal development, while progesterone promotes differentiation of these cells resulting in lobular development. Prolactin acts synergistically with estrogen and progesterin to bring about ductal and lobuloalveolar cell development. Prolactin is also the principal hormone that stimulates lactogenesis. The exact roles of the other hormones are less well understood, and most of our knowledge is derived from studies in experimental animals (5).

Breast carcinoma, a disease of the mammary epithelium, is the second leading cause of cancer-related deaths among American women (6). In 1993, more than 180,000 new cases of breast carcinoma were diagnosed in women in the United States alone. Approximately 48,000 women in the United States will die from this disease in 1994 (6). More than 1 million women in America are currently living with stage I, II, or III breast cancer, which indicates the magnitude of this health problem.

Approximately 30% of women who present with node-negative primary breast cancer are at high risk for early recurrence of metastatic disease and decreased overall survival (7). Although most breast cancer patients with node-positive disease are at high risk for decreased disease-free survival and overall survival, 30% appear to be at low risk, considering their clinical course. One of the objectives in the application of molecular endocrinology to the problem of breast cancer is to distinguish these high risk patients from those with low risk using clinical laboratory tests (7, 8).

The presence of estrogen and progesterin receptors in a breast tumor biopsy serves as a predictive indicator of therapeutic response to endocrine treatments, such as tamoxifen and medroxyprogesterone acetate (9, 10). These receptor proteins are also prognostic indices of a patient's clinical course (7, 8). Recently, overexpression of EGF receptors in breast cancer (2, 4) has been correlated with decreased disease-free interval and overall survival (3, 11). Thus, these receptors and other cellular expression products appear to be useful as prognostic factors in clinical management of breast cancer.

As part of a long-term investigation to define the molecular mechanisms of endocrine control of breast cancer, we have systematically evaluated human tumor biopsies for hormone receptors. In addition to extensive evaluation of sex steroid receptors (8, 12), we detected prolactin receptors in human breast cancer (13) although no clinical utility of this finding could be demonstrated thus far. We have likewise characterized EGF receptors in human breast (4), endometrial (14), and ovarian carcinomas.4 Specific, high-affinity binding sites for luteinizing hormone-releasing hormone and somatostatin were also identified in human mammary tumor cell lines and in biopsy specimens of breast cancer by our group (4, 14) and by others (15–17). Our long-term goal is to exploit the presence of hormone binding mechanisms for new therapeutic approaches in breast and other endocrine-responsive cancers.

Recent evidence suggests that tetradecapeptide bombesin, originally isolated from amphibia, and its mammalian counterpart gastrin-releasing peptide can function as growth factors, and through an autocrine or paracrine mechanism it may modulate the growth of some benign and neoplastic tissues (18, 19). BN-like peptides function as potent mitogens in vitro and have been implicated as autocrine growth factors in the pathogenesis and progression of some human small cell lung carcinomas (18, 20, 21). Various studies also demonstrated that bombesin/GRP may be involved in the function and...
growth of human breast cancer (22—27). BN-like immunoreactivity has been reported in rat mammary tumors (28), in a small proportion of human breast cancer and breast carcinoid specimens (25), in human and animal milk (29), and in normal bovine mammary epithelium (19). It has also been shown that the BN/GRP antagonist RC-3095 inhibits growth of estrogen-dependent- and-independent MXT mammary cancer in mice (30) and MCF-7 MIII human breast cancer in nude mice (31). In addition, specific receptors for bombesin/GRP have been demonstrated in human breast tumor cell lines, MDA-MB-231, MCF-7 MIII, and T47D, but were not detected in MCF-7 and BT-20 cell lines or in normal breast epithelial cells (23, 31, 32).

Three receptor subtypes associated with the bombesin-like peptides have been described and cloned. The cloned receptors have been distinguished by their relative affinity for binding a series of specific ligands. They are classified as: the GRP-preferring subtype (GRP receptor), found in the intestine from the esophagus to the rectum (19); the neuromedin B-prefering subtype, present in the esophageal muscularis mucosa (33); and the bombesin receptor subtype 3, present in the testis and lung cancer cell lines (21), the natural ligand of which is not yet known. GRP receptor subtypes bind GRP and bombesin with high affinity and neuromedin B with lower affinity. In contrast, the neuromedin B receptor subtype has a higher affinity for neuromedin B than for GRP or bombesin (34). All three subtypes belong to the serpentine receptor superfamily and are coupled through G proteins to effectors including phospholipase C.

Collectively these observations strongly suggest the need for evaluation and characterization of bombesin/GRP receptors in human breast cancer biopsies to enhance our understanding of the mechanisms involved in these responses. In this study, we report for the first time the presence of specific high-affinity binding sites for [Tyr][BN] in human breast cancer specimens. Biopsy specimens from 100 human breast cancers were analyzed simultaneously for [Tyr][BN]-binding sites on membrane preparations and estrogen and progesterone receptors in cytosol in order to correlate the levels of expression of these receptors.

MATERIALS AND METHODS

Chemicals. [Tyr][BN] was purchased from Bachem (Torrance, CA) while [125I-Tyr][BN] was prepared in our laboratory using radiisotope 125I-labeled sodium from Amersham (Arlington Heights, IL). 17β-[H]estradiol and [H]R5020 were purchased from NEN Products/DuPont (Wilmington, DE). Other peptides and chemicals were obtained from Sigma (St. Louis, MO), Pierce (Rockford, IL), Baxter (Muskegon, MI), Debiopharm SA (Lausanne, Switzerland), or American Peptide Company, Inc. (Sunnyvale, CA).

Preparation of Breast Cancer Specimens. Biopsies of human breast cancer were submitted for clinical determinations of sex hormone receptors. Patient age ranged from 29 to 94 years.

Preparation of Estrone and Progesterone Receptors. Estrone and progesterone receptors were determined by radioiodination binding using a titration assay (35). Briefly, cytosol was prepared as described using 10 mM Tris-HCl buffer (pH 7.4), containing 1.5 mM EDTA, 10 mM monooctylglycerol, 10% glycerol, and 10 mM sodium molybdate. Protein concentrations of the cytosol were determined by the method of Bradford (39) with some modifications. The samples were thawed and fat debris was removed using cotton swabs. Immediately after this, tumor specimens were homogenized (1:5, w/v) in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM EDTA-5 mM MgCl2-30 μM/ml bacitracin on ice using an Ultra-Turrax (IKA-Werk-Tekmar, Cincinnati, OH) homogenizer at maximal speed (stirred 3 times for 5 s at 15-s intervals). The homogenate was centrifuged at 50,000 x g for 10 min at 4°C, and the lipid layer was removed carefully. The supernatant containing the crude membrane fraction was centrifuged again at 70,000 x g for 50 min at 4°C in a Beckman L8-80 M ultracentrifuge. The pellet was washed once by first resuspending it in an ice-cold homogenization buffer and then sedimenting it as before. The final pellet was resuspended in homogenization buffer and stored at −80°C until used for the bombesin receptor binding studies. Limited results are available regarding the stability of these receptors to freezing since they have been described only recently (35). During our study, bombesin binding was performed immediately after membrane preparation and again 3–4 weeks later as a control in biopsies with sufficient tissue. No significant changes in either Kd or receptor levels were observed. The storage of human breast tumor biopsies at −86°C is well accepted for retarding the degradation of steroid and peptide hormone receptors and certain enzymes (4, 8). Protein concentration was determined by the method of Bradford (39) using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). For receptor studies, the membrane fractions were diluted with homogenization buffer to a protein concentration of 20–30 μg/50 μl.

Radioiodination of [Tyr][BN] and Bombesin Receptor Assay. [Tyr][BN] was radiolabeled with 125I-labeled sodium using an Enzymobead iodination kit (Bio-Rad, South Richmond, CA) as described (40). Mono-[125I-Tyr][BN] was purified by reverse-phase HPLC on a SynChropak RP-8 column utilizing a gradient elution with 0.1% trifluoroacetic acid- and acetonitrile-containing eluents. Radioiodination with this method generally yielded 100–200 μCi of purified [125I-Tyr][BN]. Since the mono- and diiodinated [Tyr][BN] were isolated in pure form, separated from each other and from the noniodinated peptide and oxidation products, a theoretical specific activity of 1800–2000 Ci/mmol was calculated. The adsorption of mono-[125I-Tyr][BN] to different types of surfaces such as borosilicate glass, polystyrene, polypropylene, Aquasil-coated glass, Sigma coated glass, and siliconized polypropylene was evaluated. Since essentially no surface adsorption occurred using borosilicate glass tubes, these tubes offered the most favorable conditions.

Ligand competition experiments were carried out in 12 x 75 mm borosilicate glass tubes in a total volume of 150 μl binding buffer [25 mM Tris-HCl, 5 mM EDTA, 5 mM MgCl2, 0.25 mM phenylmethylsulfonyl fluoride, 10 mM monooctylglycerol, 30 μg/ml bacitracin, and 0.1% BSA (pH 7.4)] for 60 min at 24°C. Membrane homogenates were incubated in duplicate or in triplicate with approximately 0.15 nm [125I-Tyr][BN] as radioligand and with increasing concentrations (10−12—10−6 M) of unlabeled [Tyr][BN] or structurally unrelated peptides as competitors. The reaction was terminated by adding 250 μl ice-cold binding buffer to the tubes, and the bound ligand was separated from free ligand by centrifugation at 4500 x g for 10 min at 4°C. The pellet was washed twice with 500 μl ice-cold buffer, and the radioactivity in the pellet of each tube was counted in a gamma counter (Micromedic Systems, Inc., Huntsville, AL). Some experiments were performed with membrane protein concentrations ranging from 10 to 100 μg/tube in order to determine the minimal amount of protein required to assess specific binding at a satisfactory level.

Mathematical Analysis of the Binding Data. Estimates of specific ligand binding capacities and affinities were calculated by the Ligand-PC computerized curve-fitting program of Munson and Rodbard (41) as modified by...
RESULTS

Expression of Sex Hormone Receptors in Tumor Biopsies. To ensure that the population of human breast carcinomas utilized in this investigation of bombesin receptor expression was representative of both potentially endocrine-responsive and -unresponsive tumors, sex hormone receptors were examined in the cytosol preparations. Examination of estrogen receptor levels in biopsies as a function of patient age is presented in Fig. 1. Utilizing 50 years of age or younger for premenopausal status, the median estrogen receptor level was 45 fmol/mg cytosol protein (n = 29). The median estrogen receptor level was 98 fmol/mg for biopsy specimens from postmenopausal patients (n = 71).

Fig. 2 examines the relationship of patient age and progestin receptor levels in breast carcinomas. The median progestin receptor level was 17 fmol/mg for premenopausal patients (n = 29), while that for postmenopausal patients (n = 71) was 44 fmol/mg.

In general, progestin receptor level rises as a function of an increase in estrogen receptor level (e.g., 12). When this relationship was examined in the tumor population studied (Fig. 3), the equation of the line expressing the correlation was

\[ y = 0.97x + 7.28 \]  

\( r = 0.64 \). Utilizing a value of \( \leq 10 \) fmol/mg for estrogen receptor negativity, the median progestin receptor level was 5 fmol/mg (n = 36); estrogen receptor-positive tumors had a median progestin receptor level of 145 fmol/mg (n = 64). Utilizing a value of \( \leq 10 \) fmol/mg for progestin receptor negativity, the median estrogen receptor level was 10 fmol/mg (n = 47); progestin receptor-positive tumors exhibited a median value of 172 fmol/mg estrogen receptors (n = 53).

Characterization of [Tyr<sup>4</sup>]<i>B</i>N Receptors in Biopsies. Of the 100 tumor preparations examined, 27 contained sufficient membranes of high integrity resulting in Scatchard plots satisfactory for detailed analyses of specific [<sup>125</sup>I-Tyr<sup>4</sup>]<i>B</i>N binding. In six additional cases, limited quantities of tumor membrane fractions indicated the presence of [Tyr<sup>4</sup>]<i>B</i>N receptors. Using a single saturating dose of ligand, 67 biopsies did not exhibit [Tyr<sup>4</sup>]<i>B</i>N receptors regardless of the procedure applied. Using a computerized curve-fitting program, typical displacement curves were obtained. The nonlinear curve fitting and Scatchard plot analyses suggested that the labeled [Tyr<sup>4</sup>]<i>B</i>N was associated with two classes of receptor sites (Fig. 4). In 27 tumors analyzed by complete displacement, the high-affinity binding sites exhibited a mean dissociation constant (\( K_{d1} \)) of 2.1 ± 1.8 (SD) nM (range, 0.1–7.8 nM) and a mean maximal binding capacity (\( B_{max1} \)) of 237 ± 222 fmol/mg membrane protein (range, 12–1050 fmol/mg protein). The low affinity sites bound bombesin with a mean dissociation constant (\( K_{d2} \)) of 0.3 ± 0.2 μM (range, 0.01–1.3 μM) and a mean maximal binding capacity (\( B_{max2} \)) of 5.9 ± 5.5 pmol/mg membrane protein (range, 0.6–25 pmol/mg protein). In addition, more than 24% of BNR-positive samples displayed relatively elevated values (>100 fmol/mg membrane protein) of high-affinity [Tyr<sup>4</sup>]<i>B</i>N-binding sites. From our experience with the expression of other peptide hormone receptors (4, 8, 14), levels greater than 100 fmol/mg membrane protein are “elevated” relative to those observed in nonmalignant breast tissue.
Table 1 Distribution of bombesin receptors in human breast carcinomas

<table>
<thead>
<tr>
<th>Sex hormone receptor status</th>
<th>BOMBESIN/GRP RECEPTORS IN HUMAN BREAST CANCER</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR+</td>
<td>50</td>
</tr>
<tr>
<td>ER+</td>
<td>10</td>
</tr>
<tr>
<td>ER-</td>
<td>5</td>
</tr>
<tr>
<td>ER-PR+</td>
<td>15</td>
</tr>
<tr>
<td>ER-PR-</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 1 Distribution of bombesin receptors in human breast carcinomas according to sex hormone receptor status

The binding of $[^{125}]$Tyr$^4$BN was completely displaced by increasing concentrations ($10^{-12}$–$10^{-6}$ M) of nonlabeled [Tyr$^4$]BN and GRP(14–27), none of the structurally or functionally unrelated peptides tested inhibited binding of labeled [Tyr$^4$]BN at $10^{-6}$ M. [Tyr$^4$]BN and GRP(14–27) exhibited equally high affinity for the receptor subtype while neuromedin B was less effective by two orders of magnitude in displacing $[^{125}]$Tyr$^4$BN binding (Fig. 5). Displacement by unlabeled [Tyr$^4$]BN (Fig. 5) gave an apparent dissociation constant virtually identical to that obtained for the high-affinity receptor determined by radioligand binding (Fig. 4).

Influence of Membrane Protein Concentration on $[^{125}]$Tyr$^4$BN Binding in Human Breast Cancer. A common error in receptor measurements arises from the instability of receptor binding at low protein concentrations. This point was assessed in two experiments in which human breast cancer membrane fractions prepared in homogenization buffer were serially diluted in the same buffer and assayed for $[^{125}]$Tyr$^4$BN binding. This work indicates that accurate results can be obtained over a wide range (20–100 µg/tube) of membrane protein concentrations. For most assays, protein concentration in the incubates was maintained at approximately 20–50 µg/50 µl.

Correlation of Bombesin Receptors with Expression of Estrogen and Progestin Receptors. Table 1 and Figs. 6 and 7 show the

![Graph](graph.png)

Fig. 3. Interrelationship of estrogen and progestin receptor expression in human breast carcinomas. The results of analyses of two carcinoma biopsies do not appear on the figure; one biopsy contained 1654 fmol/mg protein estrogen receptors and 3654 fmol/mg protein progestin receptors, while the second biopsy contained 1277 fmol/mg protein estrogen receptors and 512 fmol/mg protein progestin receptors.

![Graph](graph.png)

Fig. 4. Representative example of ligand competition assay of [Tyr$^4$]bombesin binding. A constant amount of $[^{125}]$Tyr$^4$ bombesin was incubated with increasing amounts of unlabeled [Tyr$^4$]bombesin using a membrane preparation of human breast cancer. Specific binding was determined at each concentration as described in "Materials and Methods." Each point represents the mean of triplicate determinations. Inset, Scatchard plot of specific $[^{125}]$Tyr$^4$ bombesin-binding data analyzed by a two-site model.

![Graph](graph.png)

Fig. 5. Ligand-binding specificity of [Tyr$^4$]bombesin binding. Competition for binding of labeled [Tyr$^4$]bombesin to human breast cancer membranes was determined in the presence of increasing concentrations of epidermal growth factor (v), human growth hormone releasing hormone (v), somatostatin (v), [D-Trp$^6$] luteinizing hormone-releasing hormone (v), [Tyr$^4$]bombesin (v), gastrin-releasing peptide(14–27) (v), and neuromedin B (v). One hundred % specific binding is defined as the difference between binding in the absence and in the presence of $10^{-6}$ M [Tyr$^4$]BN. Points, mean of at least three experiments, each performed in duplicate.
Table 2 Relationship between the binding capacities of [Tyr^4]BN and those of ER and PR receptors in human breast cancer specimens

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Type of receptor</th>
<th>ER</th>
<th>PR</th>
<th>High affinity</th>
<th>Low affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Tyr^4]BN</td>
<td>ER</td>
<td>0.578*</td>
<td>0.671</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 50</td>
<td>n = 27</td>
<td>n = 23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>[Tyr^4]BN (high affinity)</td>
<td>PR</td>
<td>0.578</td>
<td>0.311</td>
<td>0.541</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 50</td>
<td>n = 21</td>
<td>n = 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>[Tyr^4]BN (low affinity)</td>
<td>PR</td>
<td>0.385</td>
<td>0.541</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 23</td>
<td>n = 18</td>
<td>n = 27</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* Correlation coefficient (r).

Table 2 Relationship between the binding capacities of [Tyr^4]BN and those of ER and PR receptors in human breast cancer specimens.
Table 3: \([^{125}\text{I-Tyr}^4]\)bombesin-binding as a function of patient age

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Positive/total</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;29)</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>30—39</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>40—49</td>
<td>9/21</td>
<td>43</td>
</tr>
<tr>
<td>50—59</td>
<td>4/20</td>
<td>20</td>
</tr>
<tr>
<td>60—69</td>
<td>6/17</td>
<td>35</td>
</tr>
<tr>
<td>70—79</td>
<td>8/21</td>
<td>38</td>
</tr>
<tr>
<td>80—89</td>
<td>5/14</td>
<td>36</td>
</tr>
<tr>
<td>(&gt;90)</td>
<td>1/3</td>
<td>33</td>
</tr>
</tbody>
</table>

The data confirm the analyses shown in Table 2 that shows there was no correlation between the presence of high-affinity binding sites for \([\text{Tyr}^4]\)BN and PR levels. In addition, no correlation was observed between binding capacities of either high or low affinity \([\text{Tyr}^4]\)BN-binding sites (Table 2). Finally, no statistically significant correlation was found between the dissociation constants \((K_a, K_d)\) of the two classes of \([\text{Tyr}^4]\)BN-binding sites in human breast cancer specimens examined \((r = 0.115; n = 27)\).

Relationship of Patient Age and \([^{125}\text{I-Tyr}^4]\)BN Binding. The menopausal status of a patient is related to age. Most studies assume that 50 years of age is a useful time to evaluate marker expression as a function of premenopausal and postmenopausal status. There was no significant correlation of BNR levels with age of patients either when high-affinity \([\text{Tyr}^4]\)BN-binding sites were considered \((r = 0.291; n = 27)\) or when low affinity \([\text{Tyr}^4]\)BN-binding sites were evaluated \((r = 0.211; n = 27)\). As shown in Fig. 8 and Table 3, the presence of BNR in breast carcinomas was not related to patient age, with the exception that specific \([\text{Tyr}^4]\)BN-binding was not detected in biopsies from patients under 40 years of age. However, only 4 specimens were examined.

**DISCUSSION**

Bombesin, GRP, and related peptides influence the release of gastrointestinal hormones, stimulate gastric and pancreatic secretions, and gastrointestinal motility (19—21, 27, 29, 32). Bombesin-like immunoreactivity has been demonstrated in the brain, gastrointestinal tract, lungs, and mammary glands of various animals (19, 22). Bombesin and gastrin-releasing peptide also appear to function as autocrine or paracrine growth factors in a variety of cells (18, 19—23, 25, 26). These studies indicate that bombesin/GRP receptors are involved in the regulation of cell proliferation and differentiation. Our understanding of the biological effects of bombesin and GRP suggests that these peptides utilize membrane-bound receptors as part of their mechanism of signal transduction (20, 21, 24, 32).

As part of a long-term investigation of the relationship of peptide and steroid hormone receptors to human breast cancer progression and treatment, we characterized \([\text{Tyr}^4]\BN) receptors in biopsy samples of breast carcinomas. Several studies by our group (27, 31, 43, 44) using bombesin antagonists demonstrated the presence of bombesin/GRP receptors in human breast, pancreatic, and gastric cancer cells grown in tissue culture. Giacchetti et al. (23) also described BNR/GRP receptors in certain breast cancer cell lines that were absent in normal breast epithelium. To our knowledge, this study is the first to identify and characterize these receptor proteins in biopsies of human breast carcinomas.

Examination of the binding of \([^{125}\text{I-Tyr}^4]\)BN was performed using membranes isolated from 100 human breast carcinomas. The analysis of complete displacement curves of \([^{125}\text{I-Tyr}^4]\)BN by unlabeled \([\text{Tyr}^4]\)BN and the Scatchard plots of these data indicate two classes of binding sites, one with high affinity \((\text{mean } K_a = 2.1 \text{ nm})\) and low binding capacity \((\text{mean } B_{\text{max}} = 237 \text{ fmol/mg membrane protein})\) and the other with low affinity \((\text{mean } K_d = 0.3 \mu \text{m})\) and high binding capacity \((\text{mean } B_{\text{max}} = 5.9 \text{ pmol/mg membrane protein})\). The specificity of the receptors for \([\text{Tyr}^4]\BN) binding was studied using competitive ligand-binding experiments. These receptor proteins recognized bombesin and GRP(14—27) peptides in a specific manner with high affinity as ascertained by evaluation of a variety of unlabeled compounds. Since the affinity of \([\text{Tyr}^4]\BN) and GRP(14—27) was 100-fold higher than that of neuromedin B, we concluded that human breast carcinomas contain primarily the BNR/GRP receptor subtype. In addition, these data suggest that high-affinity binding represents association with bombesin/GRP receptors while low affinity binding represents association with neuromedin B receptors. Further investigation using different ligands will distinguish the receptor subtypes. In contrast to human breast cancer cell lines, MDA-MB-231, MCF-7, MII, and T-47D, in which a single class of high-affinity BNR/GRP-binding sites were detected (23, 31), both high and low affinity binding sites were detected. All human breast cancer specimens, in our experience, demonstrated both high and low affinity binding sites, but the significance of these two types of sites is unclear at present. Thirty-three of 100 human breast carcinomas expressed \([\text{Tyr}^4]\)BN receptor levels of \(>10 \text{ fmol/mg membrane protein}\). The relatively wide variation in the dissociation constants and the maximal binding capacity of BNR/GRP receptors is not clear at present. The presence of BNR/GRP receptors of different affinity subtypes suggests that polymorphism may be related to biological response. Usually, the receptor isoform exhibiting the higher affinity has been correlated with clinical response to therapy as in the cases of estrogen and progesterin receptors (7—9, 12) in breast cancer.

It is interesting to note that the expression of BNR/GRP receptors appears to be related to clonal origin. Some human breast tumor cell
A statistically significant correlation was also noted between the bombesin/GRP antagonists (45, 46). Since short-chain bombesin (6—-of high-affinity [Tyr4]BN-binding sites and estrogen receptor levels. The presence of these sex hormone receptors in a tumor biopsy is related concentrations of low affinity [Tyr4]BN-binding sites and progestin receptors in breast carcinoma biopsies is correlated with a greater affinity [Tyr4]BN-binding sites and progestin receptor levels.

Progression. Measurement of bombesin/GRP receptors may provide nografts in athymic nude mice were also reported (31).

Reported this peptide on growth of MCF-7 MIII human breast cancer xc measured by the decrease in cell number and the reduction in [3H]thy... synthesized and tested for inhibition in our laboratory (27, 30, 31, 43, 14) antagonists with a reduced peptide bond between positions 13 and 14 were reported to be 100 times more potent than earlier antagonists (45), many modified pseudonapeptide-bombesin antagonists were synthesized and tested for inhibition in our laboratory (27, 30, 31, 43, 46). RC-3095 was one of the most powerful of these antagonists (46).

Recently, we reported that bombesin stimulated the proliferation of MCF-7 MIII and estrogen-independent MDA-MB-231 human breast cancer cell lines in vitro and that RC-3095 inhibited this growth as measured by the decrease in cell number and the reduction in [3H]thy-midine incorporation into DNA (27). The growth inhibition of estrogen-dependent and -independent MXT mammary cancer in mice by the BN/GRP antagonist RC-3095 (30) and the suppressive effect of this peptide on growth of MCF-7 MIII human breast cancer xenografts in athymic nude mice were also reported (31).

A highly significant positive correlation (P < 0.005) was observed between the binding capacity of high-affinity [Tyr4]BN-binding sites and estrogen receptor levels and between the concentrations of low affinity [Tyr4]BN-binding sites and progestin receptor levels (P < 0.05). It is accepted that the expression of estrogen and progesterin receptors in breast carcinoma biopsies is correlated with a greater incidence of response to endocrine therapy (8—10). Furthermore, the presence of these sex hormone receptors in a tumor biopsy is related to improved prognosis (7). Because sex hormone receptor expression is associated with a potential endocrine response in breast cancers, we analyzed the presence of BN/GRP receptors in different tumor subtypes. When the levels of BN/GRP receptors were compared to the content of the sex steroid receptors, a highly significant positive correlation (P < 0.005) was observed between the binding capacities of high-affinity [Tyr4]BN-binding sites and estrogen receptor levels. A statistically significant correlation was also noted between the concentrations of low affinity [Tyr4]BN-binding sites and progestin receptor levels (P < 0.05), although at a lower level of confidence.

To our knowledge, this is the first report of these regulatory proteins in human breast carcinomas. Expression of specific receptor proteins for BN/GRP in a large number of human breast cancers suggests that these potent mitogens may be involved in tumor cell progression. Measurement of bombesin/GRP receptors may provide an additional means of characterizing human breast cancers with respect to their metastatic potential, as has been suggested for EGF receptors (3, 4, 11). Furthermore, the identification of these receptors directly on membranes of human breast carcinomas provides a greater stimulus for the development of BN/GRP antagonists with therapeutic potential. It is also possible that, on the basis of such receptor studies, highly active bombesin/GRP antagonists could be used for the treatment of selected breast cancers in women.

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Characterization of Bombesin/Gastrin-releasing Peptide Receptors in Human Breast Cancer and Their Relationship to Steroid Receptor Expression

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