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Presence and Characterization of Prolactin Receptors in Human Benign Breast Tumours

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Abstract—*Prolactin receptors have been determined in 64 benign breast tumours. A specific binding of 0.5% or more (with a range of 0.5–3.3%) was found in 34.4% of the cases and was considered prolactin-receptor-positive. The binding was found to be specific only for lactogenic hormones. By Scatchard analysis the dissociation constant was* 2.55×10^{-10} *mol/l and the binding capacity was 4.6 fmol/mg protein.*

INTRODUCTION

THERE are several lines of evidence indicating that prolactin is involved in the development of some experimental mammary tumours and the presence of specific prolactin binding sites have been demonstrated in human breast cancer by different authors [1–6]. However, reports on the presence of prolactin receptors in benign human mammary tumour are very few and generally negative. For instance, de Souza *et al.* [7], studying 8 benign tumours by the immunoperoxidase method, were not able to find any prolactin-positive reaction. Also, Holdaway and Friesen [1], in 3 fibroadenomas and 6 cystic hyperplasia using as radioactive ligand both hPRL and hGH, found that specific binding was always less than 1%. In preliminary studies [8] determining prolactin receptors in 20 benign mammary tumours we found in 6 tumours (30%) a specific binding of prolactin of 0.5% or more, and considered these tumours as prolactin-positive [9].

In the present study we report our results obtained in a larger number of benign breast tumours, in which we determined prolactin specific binding sites and also their hormonal specificity and affinity constant.

MATERIALS AND METHODS

Patients

The presence of prolactin receptors was studied in 64 patients undergoing surgery for benign breast affections: 24 fibroadenomas, 34 cystic disease of the breast and 6 gynaecomastias. Tumour specimens were obtained immediately after surgery and kept at −20°C for up to 2–3 days until processing. The age of patients was between 15 and 77 yr (mean 43.2 ± 17.1 yr).

Prolactin iodination

^{125}I-labelled human prolactin ([^{125}I]-hPRL, Lot Nos N26253 and N260016) was purchased from New England Nuclear (Boston, MA, U.S.A.). The specific activity of the labelled hormone varied from 37 to 48 μCi/μg as calculated by isotope recovery. The integrity of the [^{125}I]-hPRL was verified on membranes using a laboratory standard prolactin receptor from lactating rabbit mammary gland. At the concentration of mammary gland receptor used (0.5 mg) at least 10–13% of the iodinated prolactin was specifically bound.

Preparation of tumour membranes

Tumour samples were homogenized at 4°C with a Polytron PT-10 homogenizer in 0.3 M sucrose. The homogenate was then centrifuged at 15,000 *g* for 30 min and the resulting supernatant fluid recentrifuged at 105,000 *g* for 60 min. The crude microsomal pellet was resuspended in 25 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4, with a Teflon-glass homogenizer and stored frozen until assayed for protein measurement [10] and for binding assay.

Assay of prolactin receptors

Binding assay for prolactin was carried out according to Shiu *et al.* [11], with some modifications. In the binding assay approximately 100,000 counts/min (1.3–1.6 ng) of [^{125}I]-hPRL were added to each tube containing 0.5 mg of membrane protein in a final volume of 0.5 ml

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assay buffer (25 mM Tris–HCl, 10 mM $MgCl_2$, 0.1% bovine serum albumin, pH 7.4). After 16 hr of incubation at 20°C bound and free [^{125}I]-hPRL were separated by low-speed centrifugation (1500 *g*) for 20 min at 4°C. The supernatant was decanted and the membrane pellet was counted for radioactivity in a Packard auto-gamma counter. The specific binding was calculated as the difference between binding in the absence and the presence of excess unlabelled ovine prolactin (2 μg/ml) expressed as a percentage of the total counts added to incubation medium.

In addition to these 'single-point assays', Scatchard analysis [12] was also performed in a pool of five prolactin-receptor-positive tumours by transformation of binding data from the competition studies with increasing concentrations of unlabelled ovine prolactin, mixed with a fixed amount of tracer. The dissociation constant (K_d) and binding capacity were determined for prolactin concentrations between 1.6 and 60 ng/ml. All determinations for single-point assays and individual Scatchard points were performed in triplicate and the results were expressed as mean values. The deviation from the mean was always less than 12% of the mean value.

The specificity of binding was tested with a single concentration (2 μg/ml) of the following hormones: human prolactin (NIAMDD-hPRL-I-6), human growth hormone (NIAMDD-rGH-I-4), rat growth hormone (NIAMDD-pGH-I-4), rat luteinizing hormone (NIAMDD-rLH-I-5) and porcine insulin (Schwarz/Mann).

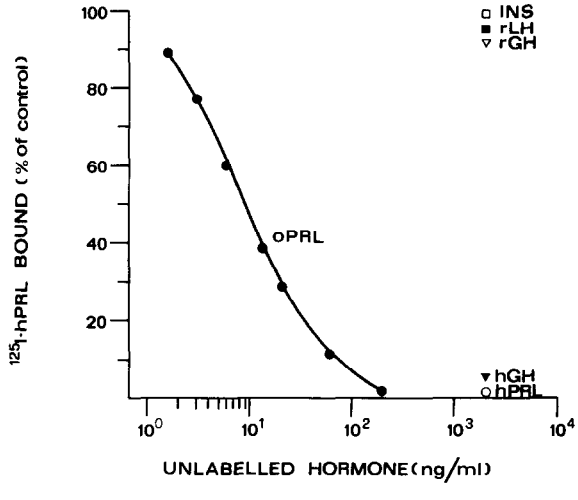

Fig. 1. Competitive inhibition of the binding of [^{125}I]-*hPRL to membranes from a pool of five prolactin-receptor-positive tumours by unlabelled ovine prolactin (●), human prolactin (○), human GH (▼), rat GH (▽), rat LH (■) and porcine insulin (□). The ordinate represents the binding as a percentage of the control (specific binding in the absence of unlabelled hormone) and the abscissa the final concentration of unlabelled hormones in the incubation medium. Non-specific binding represented 47% of the total radioactivity bound.*

Degradation studies of [^{125}I]-hPRL by tumour membranes were performed by a modification of the method of Posner *et al.* [13].

RESULTS

Prolactin receptors were measured in 64 benign breast tumours. In 22 tumours (34.4%) a specific binding of prolactin of 0.5% or more was observed. These tumours were considered receptor-positive [5, 9].

The specific binding values in the positive tumour group averaged 1.07 ± 0.63, with a range of 0.5–3.3%. Nine of these tumours (40.9%) showed more than 1.0% specific binding. Specific binding was less than 0.5% in all specimens of normal human mammary tissues studied. In one case of mammary tissue (normal epithelium) obtained from a lactating young woman a specific binding of 0.7% was found.

Non-specific binding calculated as a percentage of the total binding was 48 ± 10%, with a range of 38–63%, and was therefore relatively high but similar to that previously seen by us and by other authors in human breast cancer [1, 3, 4] or in the mammary gland membranes of a lactating rat [14].

The specificity of binding of ^{125}I-labelled human prolactin to membranes from a pool of five prolactin-receptor-positive tumours is reported in Fig. 1. The binding was inhibited by unlabelled ovine prolactin, human prolactin and human growth hormone, but not by rat luteinizing hormone, rat growth hormone or porcine insulin. The binding-specific capacity calculated by Scatchard analysis was 4.6 fmol/mg protein, and the K_d (dissociation constant) was 2.55×10^{-10} mol/l (Fig. 2).

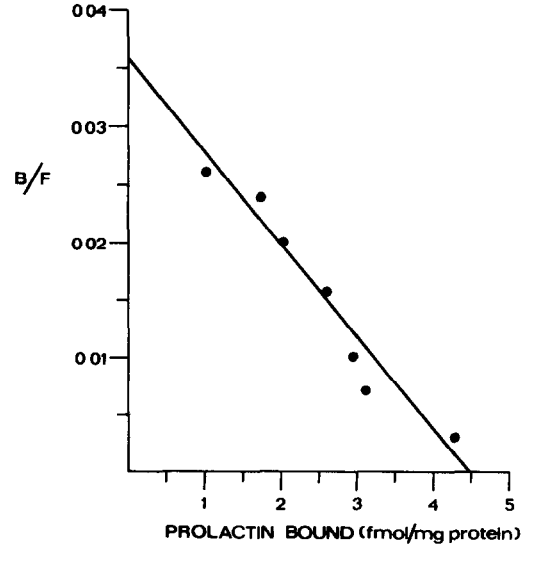

Fig. 2. Scatchard analysis performed by transformation of binding data from the competition curve with increasing concentrations of unlabelled ovine prolactin.

The degradation of [^{125}I]-hPRL (as measured by the ability of the hormone to bind to fresh rat liver membranes) after incubation for 16 hr at 20°C in the presence of tumour membranes was about 4%.

As reported in Table 1, no differences in positivity distribution was found between fibroadenomas and cystic diseases of the breast. All cases of gynaecomastia were prolactin-receptor-negative.

Table 1. *Distribution of prolactin receptors in* 64 *human breast tissues*

	No. of cases	PRL R−	PRL R+
Fibroadenoma	24	15 (62.5%)	9 (37.5%)
Cystic disease of the breast	34	21 (61.8%)	13 (38.2%)
Gynaecomastia	6	6 (100%)	0 (0%)
Total	64	42 (65.6%)	22 (34.4%)

Prolactin-negative (PRLR−): specific binding <0.5%.
Prolactin-positive (PRLR+): specific binding ≥0.5%.

DISCUSSION

The present results confirm those we already obtained in a small number of tumours [8]. Prolactin receptors are present not only in some human breast cancers but also in some mammary benign tumours. The apparent disagreement between our results and those reported by de Souza *et al.* [7] and by Holdaway and Friesen [1] could be explained by the very reduced number of tumours studied by these authors and by the different cut-off considered by Holdaway and Friesen for positivity (>1% of specific binding).

The role of prolactin in the genesis and maintenance of benign breast tumours is questionable. As reported by us [8] and by other authors [15, 16], no significant modifications of prolactin serum levels were found in patients affected by benign lesions of the breast.

However, the detection of some prolactin-receptor-positive benign breast tumours could indirectly confirm the importance of prolactin as a growth factor for these tumours and give further support to the use of anti-prolactinaemic drugs (like bromocriptine) in the therapy of some benign breast diseases.

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