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# Epidermal Growth Factor Receptors in Human Hyperplastic Prostate Tissue and Their Modulation by Chronic Treatment With a Gonadotropin-Releasing Hormone Analog*

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**ABSTRACT.** We characterized the epidermal growth factor (EGF) receptor in the membrane fraction of prostatic tissue from men with benign prostatic hyperplasia (BPH). The maximum specific binding of [$^{125}$I]EGF to the BPH membrane fraction was achieved after 30-min incubation at 35 C. Analysis of the binding data revealed two classes of binding sites, one of high affinity [$K_d$, 2.5 ± 0.5 (±SE) × $10^{-11}$ mol/L] and one of lower affinity (2.2 ± 0.3 × $10^{-9}$ mol/L). [$^{125}$I]EGF binding was inhibited by excess EGF, but not by insulin, proinsulin, fibroblast growth factor, or insulin-like growth factors I and II. In prostatic tissue of men with BPH treated for 3 months with the GnRH agonist analog Goserelin (Zoladex, depot formulation), the binding capacities of both sites were significantly higher than those of BPH tissue from untreated men ($P < 0.001$). These results demonstrate that prostatic tissue from men with BPH contains two classes of specific binding sites for EGF, and their levels are modulated by chronic GnRH agonists treatment. (*J Clin Endocrinol Metab* **68:** 740, 1989)

THE PATHOGENESIS of human benign prostatic hyperplasia (BPH) is not clear (1). While it is generally accepted that androgens are required to promote and maintain the growth of prostatic tissue, the mitogenic activity of androgens has yet to be unequivocally demonstrated *in vitro*. In fact, proliferation of prostatic epithelial cells in culture requires glucocorticoid, insulin, epidermal growth factor (EGF), PRL, and other pituitary factors, but not androgens (2, 3). Thus, it may be that the *in vivo* mitogenic action of androgens may be mediated through other hormones and/or growth factors.

The biological effects of EGF are thought to be mediated by interaction with specific plasma membrane receptors. While specific binding sites for EGF and their regulation by androgens have been demonstrated in rat prostatic tissue (4, 5), whether human prostatic tissue contains EGF-binding sites is controversial (6–8). Thus, in the absence of unequivocal evidence that prostatic tissue contains EGF receptors, the attractive hypothesis that EGF and other growth factors might be involved in the pathogenesis of BPH is far from established.

This study was undertaken to study the possible presence of specific EGF-binding sites in prostatic tissue from men with BPH using biochemical techniques and to study the possible variations of EGF receptor levels in such men chronically treated with a GnRH agonist analog.

## Materials and Methods

### *Subjects*

We studied 17 men (age range, 60–75 yr) with BPH. Informed written consent was obtained from all men, and the study was approved by the local institutional review committee. One group of men (n = 12) had received no therapy before undergoing suprapubic adenomectomy, whereas the other 5 men had been treated for 3 months with monthly sc injections of the GnRH agonist analog Goserelin (Zoladex, ICI Pharma, Milan, Italy; a 3.6 mg/cyclindrical rod depot formulation) (9, 10). In these men suprapubic adenomectomy was performed at the end of the third month of treatment, 21–30 days after the last injection. Plasma samples for testosterone (T) and dihydrotestosterone (DHT) measurements were obtained from these men before treatment and on the day of surgery.

### *Growth factors*

Human EGF (receptor grade), human [$^{125}$I]EGF (27.7 GBq/μmol), and insulin-like growth factor-I (IGF-I) were obtained

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from Amersham (Amersham, Buckinghamshire, United Kingdom). Fibroblast growth factor (FGF) was obtained from Collaborative Research (Waltham, MA), IGF-II (sequence 69–84) was purchased from Peninsula Laboratories (Belmont, CA), human insulin (batch 66/304) was kindly provided by the International Laboratory for Biological Standards (Mill-Hill, London, United Kingdom), and bovine proinsulin was a gift from Novo Industri (Copenhagen, Denmark).

*Preparation of prostatic membranes*

Prostatic tissue was either used fresh or snap-frozen in liquid nitrogen. All subsequent procedures were carried out at 4 C. About 2–3 g tissue were washed in 10 mmol/L Tris-HCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 0.25 mol/L sucrose, and 1 mmol/L phenylmethylsulfonylfluoride, pH 7.4 (buffer 1); cut into small pieces; and manually homogenized in 4 vol (wt/vol) buffer 1. The homogenate then was filtered through nylon gauze (110 mesh), and the filtrate was centrifuged for 40 min at 105,000 × *g*. The washed pellet was resuspended in 10 mmol/L Tris-HCl and 150 mmol/L NaCl and gently rehomogenized using a Teflon-glass homogenizer. An aliquot was used to measure protein concentration by the method of Bradford (11), using BSA as standard. To the suspended pellet 1 g/L BSA was added, and the sample was kept in liquid nitrogen until RRA.

*EGF binding assay*

The [$^{125}$I]EGF binding assay was carried out in buffer 2 (10 mmol/L Tris-HCl, 150 mmol/L NaCl, and 1 g/L BSA, pH 7.4). Duplicate or triplicate aliquots (25–75 μg) of the membrane suspension were incubated with increasing concentrations of [$^{125}$I]EGF (0.025–0.2 nmol/l) in tubes without unlabeled EGF and with a fixed concentration of labeled EGF (0.2 nmol/L) in tubes with increasing concentrations of unlabeled EGF (1–50 nmol/L) at 35 C for 30 min in a final volume of 200 μL. Nonspecific binding was assessed in the presence of 50 nmol/L unlabeled EGF. The total binding of 0.2 nmol/L [$^{125}$I]EGF to 25–75 μg membrane protein ranged from 1.3–3.4%, and nonspecific binding ranged from 0.2–0.6% of the total added radioactivity. The incubations were stopped by the addition of 1 mL cold buffer 2 and rapidly filtered under vacuum using Whatman GF/B filters (Whatman, Clifton, NJ). Each filter was washed four times with 2 mL ice-cold buffer 2, and its radioactivity was counted in a γ-counter at 70% efficiency.

*Plasma T and DHT measurement*

Plasma T and DHT concentrations were measured simultaneously by isotopic dilution mass spectrometry, as previously reported (10).

*Statistical analysis*

The significance of differences between the receptor concentrations in the two groups of men (untreated and treated) was assessed using Wilcoxon's test for unpaired data. $P < 0.001$ was considered significant.

## Results

Preliminary experiments were carried out to determine the length of time required for maximal EGF binding to BPH prostatic membranes at different temperatures (4, 22, and 35 C). The maximal specific [$^{125}$I]EGF binding to four different membrane preparations occurred after 30 min at 35 C, and the binding was stable for an additional 15 min. The binding at 25 C was slightly lower than that at 35 C, although the binding profiles for both temperatures were similar. The binding at 4 C was much lower than that at 35 C. Therefore, all subsequent binding assays were performed at 35 C for 30 min (Fig. 1). The effect of protein concentration on EGF binding to four different membrane preparations then was studied. The binding was linear between protein concentrations of 0.2–1.5 mg/mL. Therefore, all subsequent assays were carried out using membrane preparations with protein concentrations of about 1 mg/mL.

Analysis of the binding data from experiments with the 17 individual samples of prostatic tissue by Scatchard analysis using the LIGAND program (12) demonstrated the presence of 2 different classes of binding sites for EGF, 1 with high affinity [$K_d$, 2.5 ± 0.5 (±SE) × $10^{-11}$ mol/L] and the other with lower affinity (2.2 ± 0.3 × $10^{-9}$ mol/L; Fig. 2). The values were similar in the tissues from the 12 untreated and the 5 treated men. The mean binding capacities for the 2 classes of binding sites in the

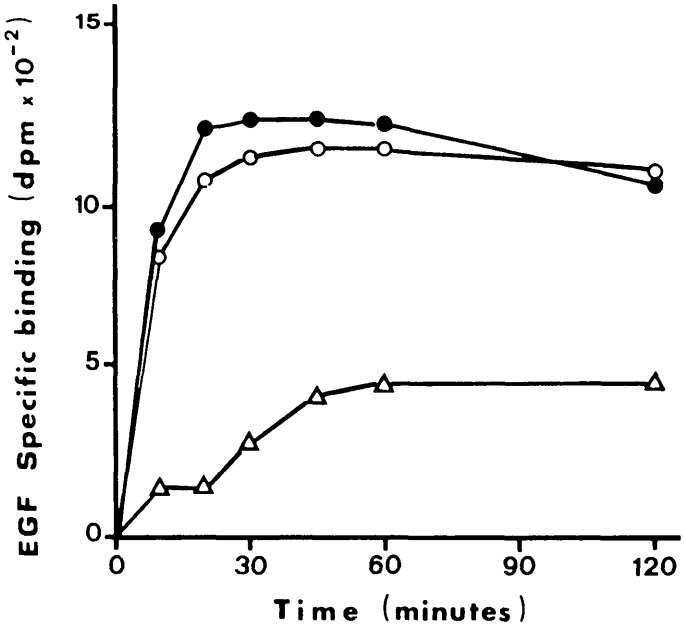


FIG. 1. Effect of incubation time and temperature on EGF binding. BPH tissue membranes from one untreated man were incubated at 4 C (△), 25 C (○), and 35 C (●) with 0.2 nmol/L [$^{125}$I]EGF for various times (10–120 min). Values were corrected for nonspecific binding measured in the presence of 50 nmol/L unlabeled EGF. Each *point* is the mean of triplicate analyses from one of four experiments which gave similar results.

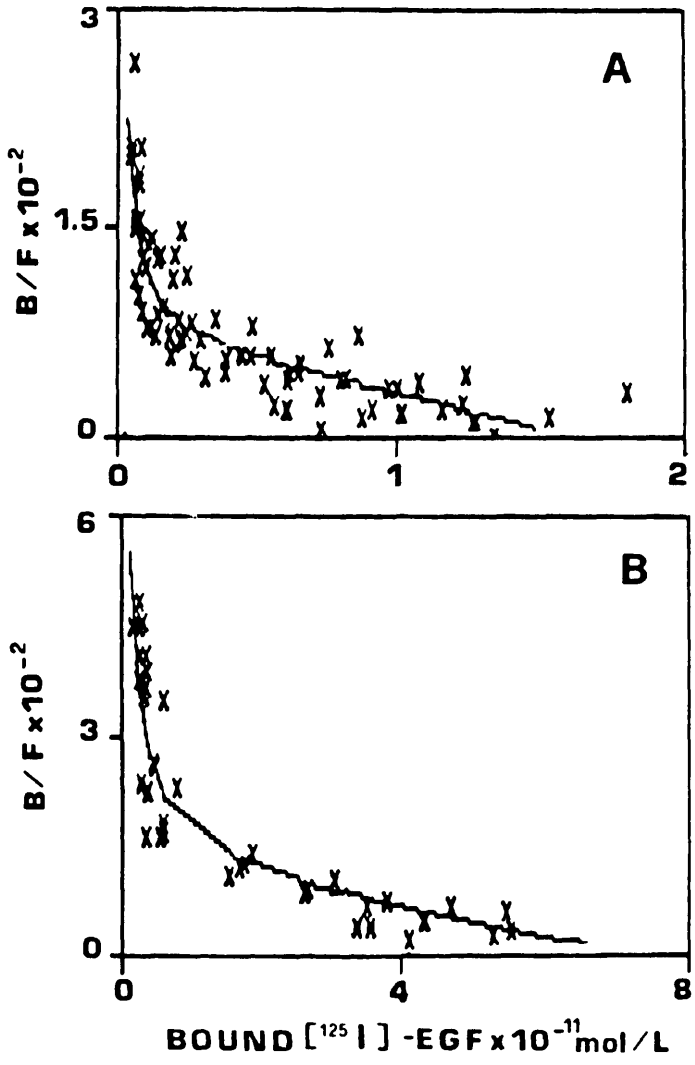


FIG. 2. Scatchard analysis of [$^{125}$I]EGF binding to membrane preparations of BPH tissue from 12 untreated men (A) and 5 men chronically treated with a GnRH analog (B). The data were processed by the program LIGAND (SCAFIT) for a two-class binding site model, since that model gave the best fit in terms of least squares analysis. Each *point* is the mean of duplicate analyses. B/F, Bound to free ratio.

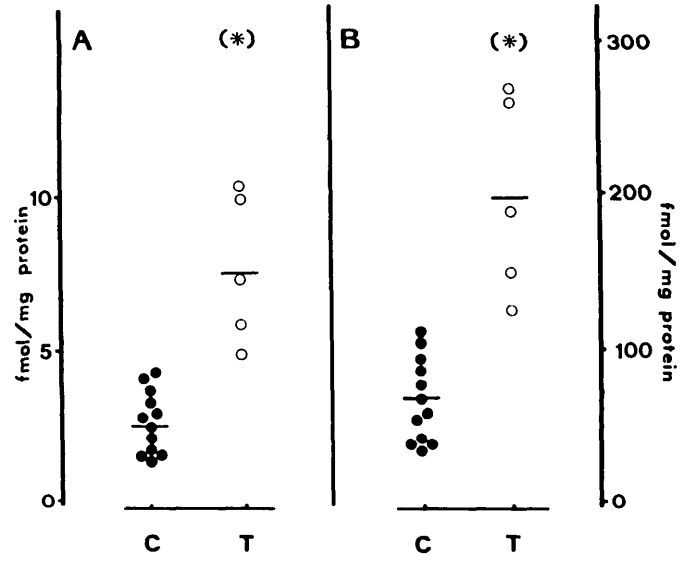


FIG. 3. Binding capacities of the two classes of [$^{125}$I]EGF-binding sites (A, sites with high affinity; B, sites with affinity lower than A) of membrane preparations of BPH tissue from 12 untreated men (C) and 5 treated men (T). *Horizontal lines* indicate the mean values. *, $P < 0.001$ compared to value in untreated men.

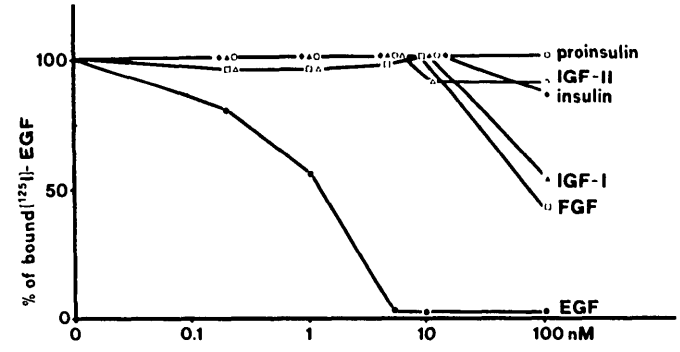


FIG. 4. Specificity of EGF binding. BPH tissue membranes from one untreated man were incubated in triplicate at 35 C with 0.2 nmol/L [$^{125}$I]EGF and increasing concentrations of unlabeled polypeptides.

BPH tissues from the 12 untreated men were 2.5 ± 0.2 (±SE) and 68.0 ± 7.8 fmol/mg protein, respectively. The mean binding capacities of the 2 components in the BPH tissues from the 5 treated men were 7.5 ± 1.0 and 198 ± 28 fmol/mg protein, respectively (Fig. 3). The values in the treated men were significantly higher than those in the untreated men. The specificity of the binding is illustrated in Fig. 4. Incubation of prostatic membranes with [$^{125}$I]EGF (0.2 nmol/L) and excess unlabeled insulin, proinsulin, FGF, IGF-I, and IGF-II did not result in significant inhibition of [$^{125}$I]EGF binding.

The mean plasma T concentrations (nanomoles per L) measured before and after 3 months of treatment with the GnRH agonist were 19.44 ± 2.29 (±SE) and 0.62 ± 0.34, respectively, while the mean plasma DHT concentrations were 1.37 ± 0.31 and 0.10 ± 0.03, respectively. These results are in agreement with previously reported data (13).

## Discussion

Our results demonstrate the presence of specific EGF receptors in BPH tissue. The binding data from 17 separate prostates were analyzed using the computer program LIGAND (12) for one- and two-class binding site models. The fitting of a two-site binding model to the data provided the best estimates (in the least squares sense) of binding affinities and capacities, confirming previously reported studies on EGF receptors in BPH tissue (7, 14). The $K_d$ values of the two binding sites reported previously (7, 14) are higher than those we found (10- and 3-fold, respectively), as are the mean of

the two binding site concentrations (5- and 2-fold, respectively). These differences may be due to the different experimental conditions and data processing.

The BPH tissue from the 5 men treated for 3 months with a long-acting GnRH agonist analog had significantly ($P < 0.001$) higher concentrations of both classes of binding sites for [$^{125}$I]EGF than those in the BPH tissue from the 12 untreated men. On the other hand, plasma T and DHT levels were very low in the treated men, as previously reported (13). This effect of pharmacological castration on EGF receptor concentrations is not in agreement with the results of a study in normal rats in which administration of a GnRH agonist for 14 days did not alter EGF receptor concentrations in prostatic tissue, whereas combined agonist and antiandrogen administration caused a significant rise of EGF receptor concentrations (5). Since GnRH agonist administration alone decreased prostate weight by only 23%, the failure of the GnRH analog to alter prostatic EGF receptor concentrations could have been due to incomplete blockade of testicular androgen secretion in these animals.

Androgen-induced increases in EGF receptor concentrations in the androgen-sensitive LNCaP cell line (established from a metastatic lesion of human prostatic adenocarcinoma) have been studied (15). Our data provide evidence for the opposite conclusion, probably due to the different experimental models. Moreover, our results are in agreement with recent *in vivo* findings on androgen down-regulation of EGF receptors in the rat prostate; castration of mature rats resulted in a 3- to 6-fold increase in [$^{125}$I]EGF binding, while treatment of castrated rats with DHT decreased EGF-binding capacity (4, 5). In conclusion, it seems likely that androgens down-regulate EGF receptors in rat and human prostate, although the mechanism of action is not known.

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