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Insulin-Like Growth Factor-I Receptors in Human Hyperplastic Prostate Tissue: Characterization, Tissue Localization, and Their Modulation by Chronic Treatment with a Gonadotropin-Releasing Hormone Analog*

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ABSTRACT. Insulin-like growth factor I (IGF-I) receptors were characterized in membranes obtained from prostate tissue of patients affected by benign prostatic hyperplasia (BPH) before and after treatment with a GnRH agonist analog. Binding of [^{125}I]IGF-I to membranes obtained from untreated patients was specific and time and temperature dependent. Analysis of the binding data yielded two classes of binding sites, one of high affinity (K_d, 10^{-11} mol/L) and one of lower affinity (K_d, 10^{-9} mol/L). BPH membrane preparations were affinity-cross linked to labeled IGF-I, and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Analysis by autoradiography revealed one labeled protein with an apparent M_r = 300K under nonreducing conditions and two labeled proteins with M_r = 270K and M_r = 130K under reducing conditions. Excess unlabeled IGF-II reduced both of them, whereas the same excess of IGF-I completely abolished them.

In membrane preparations of prostatic tissues from patients affected by BPH and treated for 2 months with a GnRH agonist analog, the binding capacities of both binding sites were significantly higher than those of BPH tissue from untreated patients, whereas binding affinities were unchanged.

The IGF-I receptor in BPH prostate tissue of untreated patients was mainly localized in the basal layer of the epithelium, as demonstrated by immunohistochemical staining, whereas in the tissue from treated patients positive staining was found also in the glandular epithelium.

These results demonstrate that: 1) specific binding sites for IGF-I are present in prostatic tissue from patients with BPH, 2) androgen deprivation increases their binding capacities and seems to modify their epithelial localization. (*J Clin Endocrinol Metab* **72:** 740–746, 1991)

BENIGN prostatic hyperplasia (BPH) is a common pathology in elderly subjects. Both epithelial and stromal components contribute in varying grade to the typical nodular transformation of the prostate tissue in this disorder (1). The etiopathogenesis of BPH is still debated. Although the prostate is a known target organ for androgens, their effect alone appears to be insufficient to explain prostatic diseases, either BPH and carcinoma. It has been postulated that their effect is mediated through the production of local factors acting with paracrine and/or autocrine mechanisms. *In vivo* and *in vitro* studies support this hypothesis. In fact, specific receptors for epidermal growth factor (EGF) are present in human normal, hyperplastic, and adenomatous prostate (2–4), and are clearly influenced by androgens (3, 4). Moreover, growth-promoting polypeptides related to EGF and fibroblast growth factors (5, 6), or apparently novel peptides (7), are present in extracts of normal, hyperplastic and adenocarcinoma prostatic tissues. *In vitro* studies have demonstrated that human prostate cancer cell lines express and produce growth factor-like proteins (8, 9), are sensitive to exogenous EGF, and androgens increase the growth response to EGF (10). In addition, it has been demonstrated that polypeptide pituitary factors are mitogens for prostate epithelial cells (11, 12). However, although it appears that polypeptide growth factors may be involved in prostatic hyperplasia

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and carcinoma, the role of each of them, as well as their interactions with androgens, are still to be elucidated.

Insulin-like growth factor-I (IGF-I), known as a growth-promoting factor in different tissues and normal and transformed cell lines, is one of the potential mediators of androgen actions in the human prostate. In fact, it has been demonstrated that IGF-I is expressed in hormone-dependent tissues such as uterine and breast tissues (13, 14), and it may function as mediator of estrogen action (15). Moreover, in a preliminary study we demonstrated the presence of IGF-I specific receptors in human hyperplastic prostate tissues (16). In the present study besides to confirm the presence of specific IGF-I binding sites in prostate tissue from subjects affected by BPH, we investigated both their tissue localization, and the effect of androgen deprivation on IGF-I receptor content and their tissue distribution.

Materials and Methods

Tissue samples

Prostatic tissue was obtained from 18 patients (age range 60–81 yr) undergoing suprapubic adenomectomy for BPH. One group of six men was treated for 2 months with monthly sc injections of the GnRH agonist analog Goserelin (Zoladex, ICI Pharma, Milan, Italy; a 3.6 mg/cylindrical rod depot formulation) before suprapubic adenomectomy. Plasma samples for testosterone (T) measurements were obtained from patients before treatment and on the day of surgery. Tissues were immediately frozen and were kept in liquid nitrogen. The patients gave written consent, and the study was approved by the local institutional review.

Materials

The recombinant analog of human IGF-I and [^{125}I]IGF-I (74 GBq/μmol) were purchased from Amersham International (Amersham, U.K.). CR-recombinant human IGF-II was purchased from Collaborative Research (Bedford, MA). Bovine insulin was purchased from Sigma (St. Louis, MO). Bovine proinsulin was a gift from the Novo Industry (Copenhagen, Denmark). Monoclonal antibody specific to human IGF-I receptor (α IR-3) was a gift from Dr. S. Jacobs, The Wellcome Research Laboratories (Research Triangle Park, NC). Anti-mouse immunoglobulin G (IgG) peroxidase-conjugate antibody (A-2028) was purchased from Sigma (St. Louis, MO). 3,4,3′,4′ tetra-aminodiphenilhydrochloride (diaminobenzidine) was purchased from BDH Chemical Ltd. (Poole, UK). Commercial RIA for T measurement was supplied by Mallinckrodt Diagnostica (Dietzenbach, Germany). All chemicals used were of the highest purity available.

Preparation of prostatic membranes

About 2–3 g frozen tissue were cut in small pieces and homogenized in a Teflon-glass homogenizer in 4 vol (wt/vol) 10 mmol/L Tris-HCl, 0.25 mol/L sucrose, 1 mmol/L phenylmethylsulfonyl fluoride, pH 7.4, at 4 C. The homogenate was then filtered through nylon gauze (110 mesh) and the filtrate centrifugated for 10 min at 1000 × *g*, at 4 C. The supernatant was adjusted to 0.1 mol/L NaCl and 0.2 mmol/L $MgCl_2$ and centrifugated for 40 min at 105,000 × *g*, at 4 C. The washed pellet was resuspended in 50 mmol/L Tris-HCL, pH 7.4, and gently rehomogenized in a Teflon-glass homogenizer. An aliquot was used to measure protein concentration by the method of Bradford (17). To the suspended pellet 5 g/L BSA were added and the sample was kept in liquid nitrogen until radioreceptor assay.

IGF-I binding assay

The [^{125}I]IGF-I binding assay was carried out in 50 mmol/L Tris-HCl buffer, containing 5 g/L BSA, pH 7.4. Duplicate or triplicate aliquots where indicated (50 to 100 μg protein) of the membrane preparations were incubated with increasing concentrations of [^{125}I]IGF-I (0.005–0.03 nmol/L) in tubes without unlabeled IGF-I and with fixed concentration of labeled IGF-I (0.03 nmol/L) in tubes with increasing concentrations of unlabeled IGF-I (0.05–30 nmol/L), at 4 C for 20 h in a final volume of 200 μl. Nonspecific binding was assessed in the presence of 30 nmol/L unlabeled IGF-I. Incubation was stopped by the addition of 0.8 mL cold buffer 50 mmol/L Tris-HCL, 10 g/L BSA (pH 7.4), and centrifugation at 6000 × *g* for 30 min at 4 C. The pellet was counted in a γ-counter at 70% efficiency. Total binding of 0.03 nmol/L [^{125}I]IGF-I to 50–100 μg membrane protein ranged from 11–21% and nonspecific binding ranged from 6–9% of total binding. Scatchard analysis of binding data was performed using the computer program LIGAND (18).

Affinity cross-linking

Crude prostatic membranes (1.4 mg protein/mL) in 50 mmol/L Tris-HCl buffer, pH 7.4, containing 5 g/L BSA, were incubated at 4 C for 20 h with [^{125}I]IGF-I (0.5 nmol/L) with or without unlabeled peptides (150 nmol/L). After incubation and centrifugation, the membranes were washed and then resuspended in the original incubation volume in binding buffer containing no albumin. Disuccinimidyl suberate, 50 mmol/L freshly dissolved in dimethylsulfoxide, was added to a final concentration of 0.5 mmol/L. After 30 min at 22 C the reaction was stopped by addition of cold 50 mmol/L Tris-HCl, pH 8.8. Membranes were pelletted at 15,000 × *g* for 15 min and suspended in 2% sodium dodecyl sulfate (SDS) electrophoresis sample buffer (19) with or without 5% 2-mercaptoethanol. Samples were boiled for 2 min and portions (20–30 μl) were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on linear gradient gels containing between 5 and 15% (wt/vol) acrylamide. A portion of crude membranes affinity cross-linked to IGF-I were solubilized by 1% Triton X-100 in 50 mmol/L Tris-HCl buffer, pH 7.4, at 22 C for 30 min and then centrifuged at 15,000 × *g* for 30 min. Solubilized receptors were subjected to SDS-PAGE as described before. The gels were stained with Coomassie blue, destained, dried and autoradiographed with Hyperfilm-MP (Amersham, Buckingamshire, UK). The mol wt standards (Bio-Rad, Richmond, CA) ranged from 14,000–200,000.

Immunohistochemistry

BPH prostate specimens from six untreated and four treated patients were processed for immunohistochemistry using immunoperoxidase technique as previously reported (20). The monoclonal antibody α IR-3 against human IGF-I receptor (primary antibody) was used at 1:400 dilution. The antimouse IgG peroxidase-conjugated antibody was used at 1:100 dilution. Sections incubated with nonimmune mouse serum and with high dilutions of primary antibody (1:1,000 to 1:10,000) were used as controls. The specificity of the antibody was tested by preincubating the sections with human IGF-I (1 μmol/L).

Statistical analysis

The significance of differences between the receptor concentrations in prostate tissue from untreated and treated subjects affected by BPH was assessed using Wilcoxon's test for unpaired data. The significance of correlation between binding capacities of high affinity sites with those of lower affinity sites was assessed using Spearman's test. P values less than 0.001 were considered significant.

Results

Patients

The mean (±SE) initial serum level of T (nmol/L) in six subjects affected by BPH was in the normal range (19.9 ± 1.1); after 2 months of treatment with Zoladex, the mean serum T level was reduced to below castrate values (0.6 ± 0.1).

IGF-I receptor in membranes preparations of BPH tissues from untreated and treated subjects

The specific [^{125}I]IGF-I binding to BPH prostatic membranes was maximal after 20 h at 4 C (data not shown). Therefore all subsequent binding assays were performed at 4 C for 20 h. The binding was linear between protein concentration of 0.5–2 mg/mL (data not shown). Therefore all subsequent assays were carried out using membrane preparations with protein concentration of 1–1.5 mg/mL. The α IR-3 antibody specifically inhibited binding of [^{125}I]IGF-I to BPH membranes (Fig. 1). Half-maximal inhibition of specific binding was observed at a concentration of 80 pmol/L. Figure 2 shows binding specificity of labeled IGF-I to prostatic membranes. Based on their ability to inhibit maximum binding by 50%, IGF-II, insulin, and proinsulin were, respectively 2.5%, and less than 0.001% as potent when compared to IGF-I. Scatchard analysis of the binding data obtained from 18 different samples of BPH prostatic tissue (12 untreated and 6 treated patients) gave similar results. Figure 3 depicts a typical experiment, showing two different classes of binding sites for IGF-I, one with high affinity and low capacity and the other with lower affinity and higher capacity. The mean K_d values for the high and low affinity binding sites in the BPH tissues from 12 untreated patients were 1.4 ± 0.1 (SE) × 10^{-11} mol/L and 0.7 ± 0.1 × 10^{-9} mol/L, respectively. K_d values for the two binding sites were not modified by the hormonal treatment (data not shown). The mean binding capacities for the high and low affinity binding sites in the BPH tissues from the 12 untreated patients were 9.2 ± 0.8 (±SE) fmol/mg protein and 117.5 ± 10.3 fmol/mg protein, respectively (Fig. 4). The mean binding capacities of the high and lower affinity binding sites in the BPH tissues from six treated patients were significantly ($P < 0.001$) higher than those of the untreated patients with values of 42.0 ± 2.9 and 369.9 ± 25.9 fmol/mg protein, respectively (Fig. 4). Correlation between binding capacities of the high affinity sites with those of the lower affinity sites in the BPH prostate tissues from untreated and treated subjects, gave a correlation coefficient of 0.97 and 0.99, respectively (Fig. 5); the slopes (±SD) of the two regression lines (12.5 ± 0.36 and 8.82

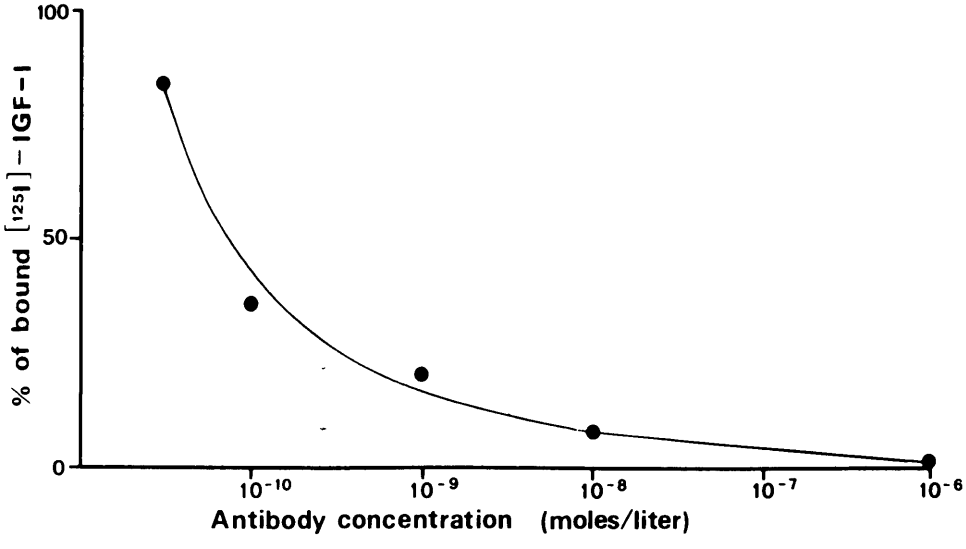

FIG. 1. Effect of a monoclonal antibody to type I receptor (αIR-3) on the BPH tissue membrane radioreceptor assay. BPH membranes from one subject were incubated in triplicate with 0.03 nmol/L [^{125}I]IGF-I and increasing amounts of the antibody. Nonspecific binding was assessed in the presence of 30 nmol/L unlabeled IGF-I. Each *point* is the mean of triplicate analyses.

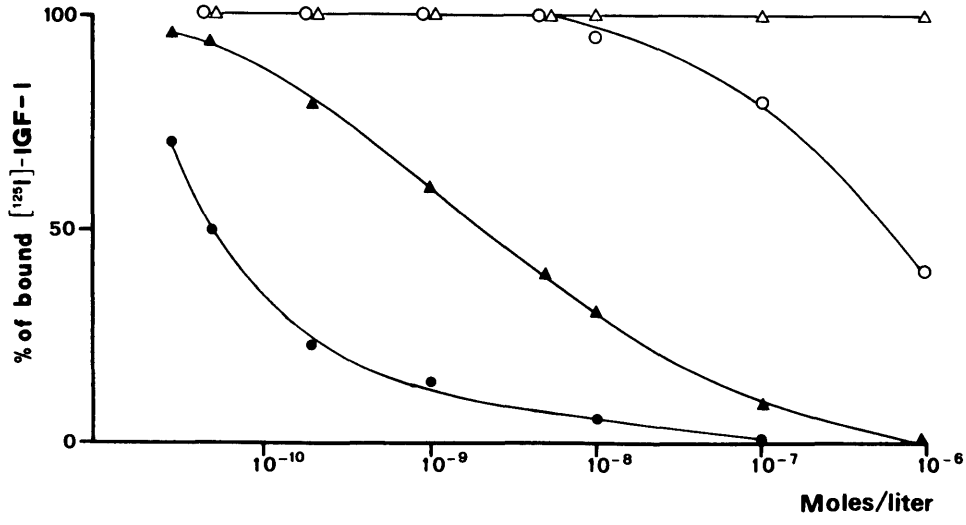

FIG. 2. Specificity of IGF-I binding. BPH tissue membranes from one subject were incubated with 0.03 nmol/L [^{125}I] IGF-I and increasing concentrations of unlabeled IGF-I (●), IGF-II (▲), insulin (○), and proinsulin (△). Each *point* is the mean of triplicate analyses.

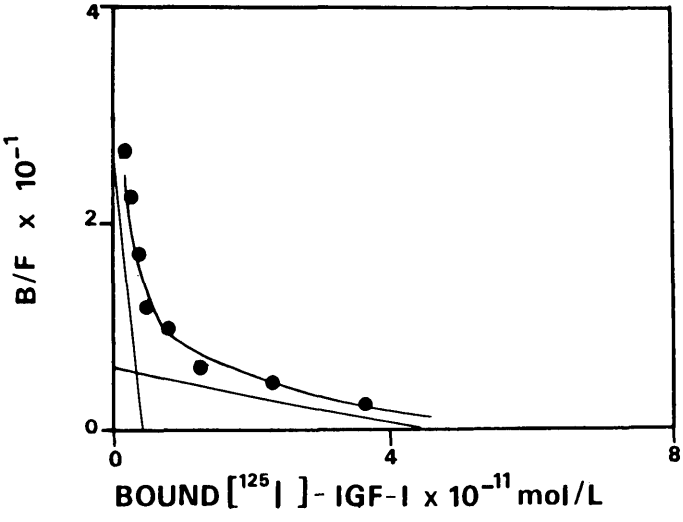

FIG. 3. Scatchard analysis of [^{125}I]IGF-I binding to one BPH membrane preparation. Each *point* is the mean of duplicate analyses.

± 0.03, respectively) were significantly ($P < 0.001$) different (Fig. 5).

Affinity cross-linking of IGF-I to prostatic membrane preparations

Cross-linking of labeled IGF-I (0.5 nmol/L) to BPH membranes either crude or solubilized by Triton X-100 obtained from one untreated (solubilized, Fig. 6A) and one treated subject (crude and solubilized, 6B), followed by linear gradient SDS-PAGE under nonreducing and reducing conditions, and autoradiography, identified patterns of labeled bands very similar. Under nonreducing conditions one protein band of approximately M_r 300K was reduced by 150 nmol/L unlabeled IGF-II and abolished by equal amount of IGF-I. The autoradiographic pattern observed under reducing conditions gave two protein bands of approximately 270K and 130K, respectively. The intensity of these bands was reduced by unlabeled IGF-II and abolished by IGF-I. The same pattern was observed using dithiothreitol (0.1 mol/L) as reducing agent (data not shown).

Immunohistochemical localization of IGF-I receptor

Representative localization of IGF-I receptor in BPH prostatic tissues obtained from six untreated and four treated subjects is illustrated in Fig. 7. Immunoperoxidase reactivity was confined mainly to the basal layer of the epithelial cells in prostatic tissue from one untreated subject (A). Positive staining was extended to glandular epithelium in the tissue specimen from one treated subject (C). Stroma was unstained in tissue specimens from both treated and untreated subjects. Preincubation of sections with 1 μmol/L IGF-I abolished immunostaining (B and D).

Discussion

Our data demonstrate that specific receptors for IGF-I are present in BPH and that they are localized in the epithelium. Moreover, IGF-I binding capacity in this tissue is influenced by androgens. Binding analysis showed two binding sites one with high affinity (K_d, 10^{-11} mol/L) and low capacity and one with lower affinity (K_d, 10^{-9} mol/L) and higher capacity. Molecular weights (M_r) of IGF-I receptors were estimated from their electrophoretic mobility under both nonreducing and reducing conditions. The autoradiographic patterns observed after cross-linking of labeled IGF-I to crude, unsolubilized (data not shown) and solubilized membranes from BPH prostatic tissue gave similar results. Under nonreducing conditions one labeled band at approximately 300k was present. Labeling of this band was reduced by 300-fold molar excess unlabeled IGF-II and was abolished by the

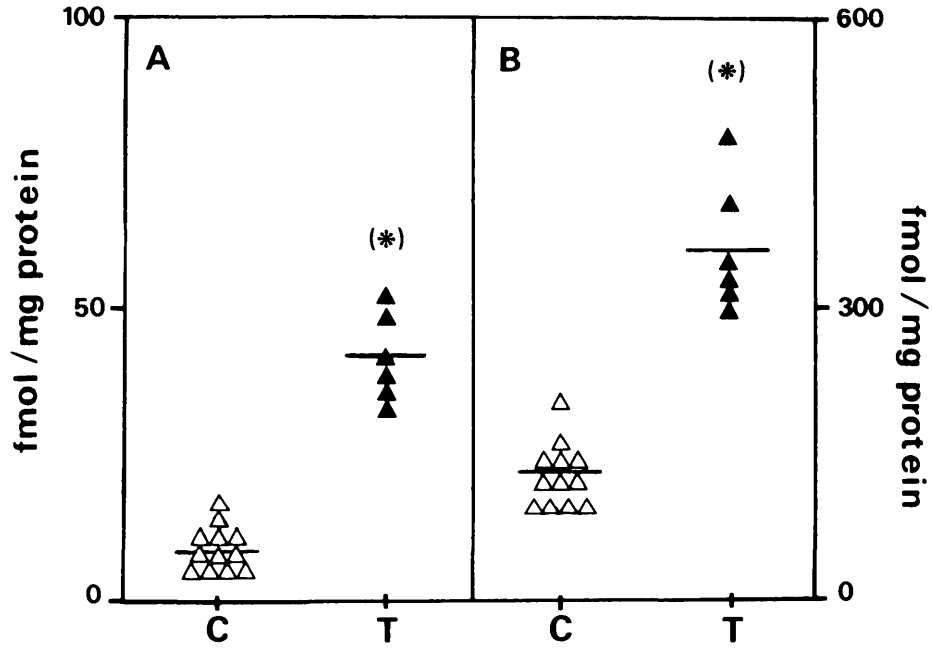

FIG. 4. Capacities of the two classes of IGF-I binding sites. A, Sites with high affinity; B, sites with low affinity in membrane preparations from BPH tissue of untreated (C) and treated subjects (T). *Horizontal lines* indicate the mean values. *, $P < 0.001$ compared to results obtained in untreated subjects.

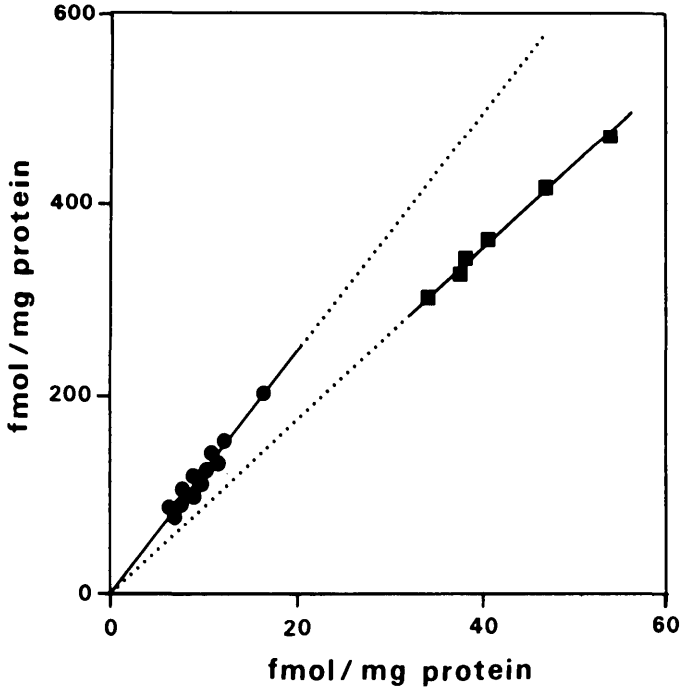

FIG. 5. Correlation between IGF-I binding capacity of the high affinity sites (abscissa) with those of the low affinity sites (ordinate) in BPH prostate tissues from untreated (●) and treated (■) subjects.

equal amount of unlabeled IGF-I. Under reducing conditions two main protein bands were observed, one at approximately 270K and one at approximately 130K. The latter one represents the α subunit of type I IGFs receptors, described in several tissues and cell lines (21, 22). The substantial amount of radioactivity migrating in the 270K region most probably represents cross-linking to type I subunit dimers.

In fact unlabeled IGF-II only reduced the intensity of this band. However, the same effect was observed for the 130K band as well as for the unreduced 300K protein, confirming our competitive binding studies that demonstrated the consistent interaction of IGF-II on the binding of IGF-I at concentrations 300-fold higher than that of the labeled ligand. On the contrary, IGF-I completely inhibited the labeled proteins observed either under nonreducing or reducing conditions. Moreover, a monoclonal antibody to type I receptor (α IR-3) completely inhibited the binding of labeled IGF-I to BPH membranes. Thus, it is likely that in this tissue specific IGF-I receptors are predominant, although IGF-II may interact to some extent with them.

Testicular suppression, induced by 2 months treatment with a long-acting GnRH agonist analog, significantly increased binding capacities of both binding sites (high and low affinity) in BPH prostate tissue, compared to those of untreated BPH. Moreover, an alteration in the proportion of lower and higher affinity receptor sites in favor of the latter was clear. If it may be assumed that higher affinity predisposes to a more effective ligand-receptor complex, then BPH prostatic tissue under androgen deprivation may become more responsive to IGF-I.

In a previous study (3) we demonstrated a significant increase of EGF receptor content in BPH prostatic tissue from patients subjected to the same pharmacological treatment employed in the present study. At present the possible role of IGF-I and EGF in prostatic normal tissue is unknown, but experimental evidences obtained under androgen deprivation induced either by castration (23)

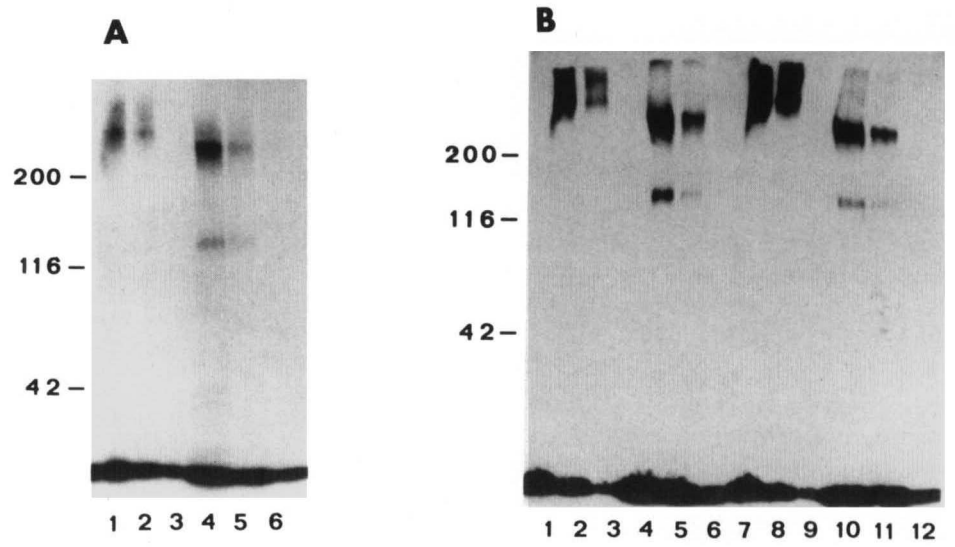

FIG. 6. Cross-linking of [^{125}I]IGF-I (0.5 nmol/L) to BPH membrane preparations. A, Solubilized membranes from one untreated subject under nonreducing (lines 1 to 3) and reducing conditions (4 to 6), in the absence (1, 4) and in the presence of 150 nmol/L IGF-II (2, 5) or 150 nmol/L IGF-I (3, 6). B, Crude (1 to 6) and solubilized (7 to 12) membranes from one treated subject under nonreducing (1 to 3; 7 to 9) and reducing conditions (4 to 6; 10 to 12) in the absence and presence of unlabeled ligands with the same sequence as reported for panel A. The migration of molecular weight standards ($\times 10^{-3}$) is indicated.

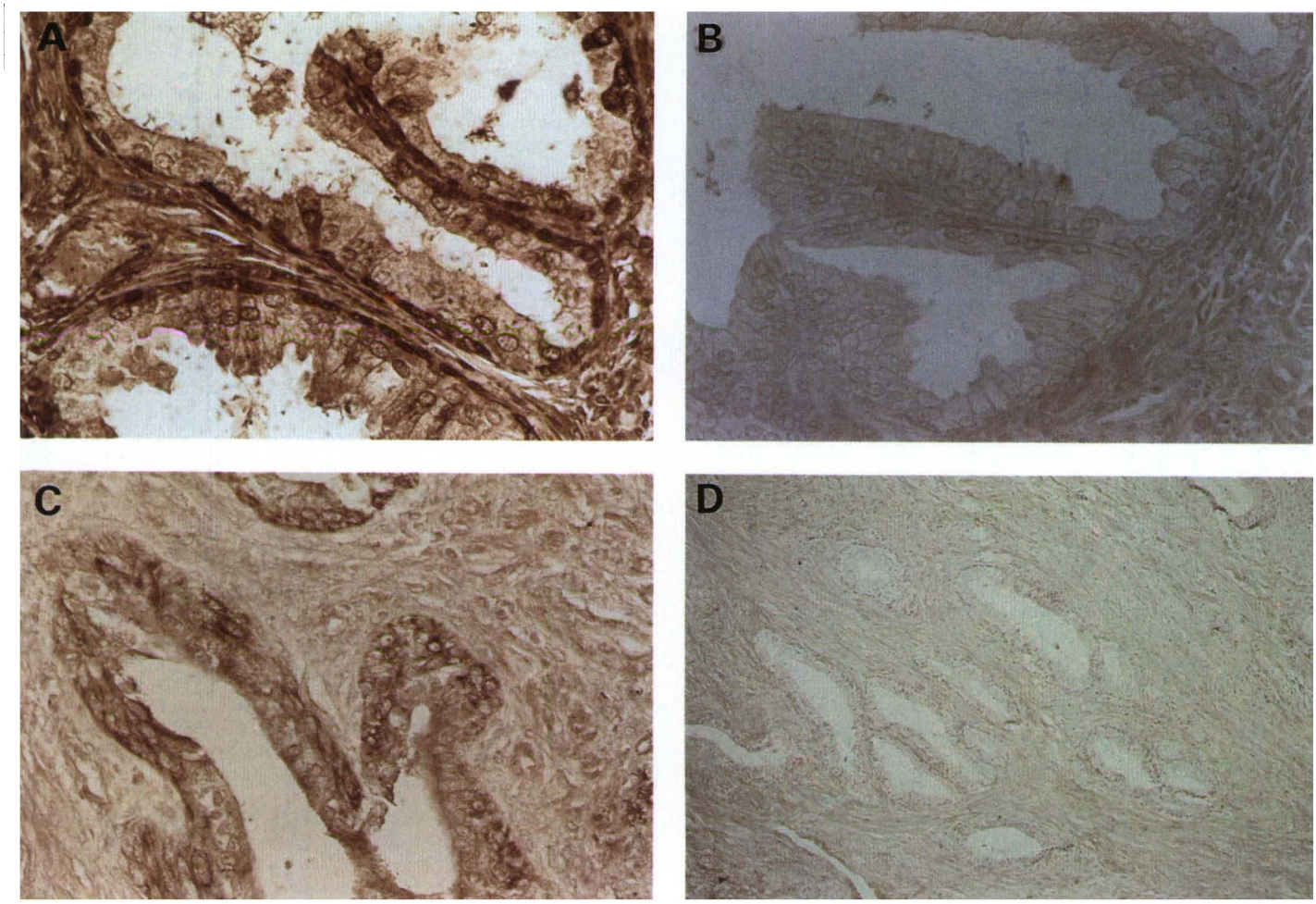

FIG. 7. Immunohistochemical localization of IGF-I receptors in BPH prostatic tissue. Monoclonal antibody raised against IGF-I receptor (α IR-3, 11 μg/mL) was used in paraffin sections of untreated (A, B) and treated (C, D) BPH prostatic tissue. Immunoperoxidase staining was present in basal epithelial cells in section A (magnification, ×375), and extended also to the glandular epithelium in section C (magnification, ×250). Preincubation of sections with 1 μmol/L IGF-I abolished the staining (B, magnification, ×375; C, magnification, ×125).

or by pharmacological testicular suppression (present study, 3), and in poorly differentiated prostatic carcinoma (4) suggest that *in vivo* androgens may down-regulate IGF-I and EGF binding capacities in prostatic tissue. Thus, breakdown of androgenic action could unleash the action of these growth-promoting factors. Morphologic analysis of BPH prostatic tissues from the patients here investigated showed that testicular suppression induced the regression of prostatic glandular epithelium, in agreement with a previous report (24). This phenomenon may be explained by the fact that prostatic epithelium seems to be the major target of androgens in BPH prostate tissue (25), and testicular suppression strongly reduces nuclear androgen receptor content in this tissue (26). Moreover, immunostaining of IGF-I receptors shows that, while immunoreactivity is confined to the basal epithelial cells in BPH tissue from untreated subjects, under androgen deprivation positive staining of IGF-I receptors is extended also to the glandular epithelium.

In conclusion, the epithelial localization of IGF-I receptors and EGF receptors (2), the increase of their binding capacities and the lack of glandular differentiation as a consequence of androgen deprivation, may be coincidental events that suggest the involvement of these growth-promoting factors as prostatic tissular response to the regression of glandular epithelium.

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