J. Steroid Biochem. Molec. Biol. Vol. 40, No. 1–3, pp. 199–205, 1991
Printed in Great Britain

0960-0760/91 $3.00 + 0.00
Pergamon Press plc

GROWTH FACTORS IN THE HUMAN PROSTATE

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Summary—Recent studies have focused on the potential role of local polypeptide growth-regulating factors in the etiology of benign prostatic hyperplasia (BPH) and prostatic carcinoma. In our studies we confirmed the presence of specific receptors for epidermal growth factor (EGF) in prostatic tissues from patients affected by BPH. In addition, we demonstrated that specific receptors for insulin-like growth factor type I (IGF-I) are present in BPH tissues. In order to identify a possible interaction between androgens and these growth-regulating factors, we investigated the effect of testicular suppression-induced androgen withdrawal on both EGF and IGF-I receptor concentrations in prostatic tissue from patients affected by BPH treated with a long-acting luteinizing hormone-releasing hormone analog. Both EGF and IGF-I binding capacities were significantly increased after treatment. This finding suggests that *in vivo* IGF-I and EGF receptor levels may be under negative androgenic regulation, indicating a potential role for these growth-regulating factors in the mechanism of response to the castration-induced regression of androgen-dependent prostatic tissue. Moreover, preliminary studies indicate that in human BPH prostatic tissue multiple IGF-binding proteins (IGF-BP) are present. This finding suggests a possible role of IGF-BP in modulating IGFs biological activities at the prostate level.

INTRODUCTION

Gonadal androgens are essential for the development, differentiation, and maintenance of the human prostate. However, it remains a challenge to establish the nature of the relationship between prostatic androgens and the growth-response of prostatic tissue to androgens in either benign prostatic hyperplasia (BPH) and prostatic carcinoma, the most common neoplasiae in aging men. It is undefined whether testicular androgens play a permissive or active part in the induction of these tumors, and whether they continue to exert an important growth-promoting role in BPH and prostatic carcinoma, after their induction.

Recent studies have established that growth-promoting polypeptides related to epidermal growth factor (EGF) [1, 2] and fibroblast growth factor (FGF) [3, 4], or apparently novel peptides [5] are present in extracts of normal, hyperplastic and carcinomatous prostatic tissues. In addition, EGF-specific receptors have been characterized in tissue specimens of hyperplastic [6–9], and carcinomatous prostate tissue [8, 10], and it has been reported that their binding capacity is inversely related to androgen receptor content [8, 9].

In vitro studies have provided evidence that a variety of growth factors affect prostatic epithelial growth, with or without interceding androgenic regulation [11–14]. This subject will be discussed extensively elsewhere in this publication. Although at this time no definite link between growth factors and prostatic growth has been established, circumstantial evidence has raised attractive hypotheses regarding the role of growth factors in BPH and prostatic carcinoma. Among them, it has been postulated that androgen–growth factor interactions may be an important feature of prostatic growth aberration.

In this report we present the work from our laboratory, undertaken in an attempt to add some information about the possible relationship between androgens and EGF receptors in BPH tissue. In addition, we report our investigation addressed to study whether insulin-like growth factor type I (IGF-I) exerts a possible role in prostatic tissue, and whether it may be considered as a potential mediator of androgen action in BPH. Likewise, it has been postulated

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.

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that IGF-I may function as a mediator of estrogen action in hormone-dependent tissues such as uterine and breast tissues [15, 16]. To explore this hypothesis we have investigated in human BPH tissue (a) the affinity of IGF-I receptor, (b) the receptor structure and (c) receptor binding capacity under androgen deprivation. Moreover, since there is increasing evidence that IGF-binding proteins are produced by many normal and transformed cells and may modulate IGFs action with a paracrine and/or autocrine mechanism [17–20], and they are present in human seminal fluid [21], we have investigated the possible presence of IGF-binding proteins in BPH tissue by affinity cross-linking.

EXPERIMENTAL

Patients

We studied 35 subjects (age range 60–81 yr) undergoing suprapubic adenomectomy for benign prostatic hyperplasia (BPH). The patients gave written consent, and the study was approved by the local institutional review. Two groups of 5 and 6 men were treated for three and two months, respectively, with monthly subcutaneous injections of the GnRH agonist analog Goserelin (Zoladex, ICI Pharma, Milan, Italy; a 3.6 mg/cylindrical rod depot formulation), before surgical intervention. Plasma samples for testosterone (T) measurement were obtained from patients before treatment and on the day of surgery. Tissues from treated and untreated patients were immediately frozen and kept in liquid nitrogen until processed for membrane preparations.

Preparation of prostatic membranes

BPH prostatic membranes for EGF receptor assay were prepared as previously reported [7]. BPH prostatic membranes for IGF-I receptor assay and affinity cross-linking were prepared as for EGF receptor assay, with some modifications [22]. Briefly, about 2–3 g of frozen tissue was homogenized in Tris–HCl buffer pH 7.4 (1:4, w/v) containing 0.25 M sucrose and 1 mM phenylmethylsulfonylfluoride (PMSF) at 4°C. All subsequent procedures were carried out at 4°C. The homogenate was filtered through a nylon gauze and sedimented at 1000 *g*. The supernatant was then centrifuged for 40 min at 105,000 *g*. The pellet was resuspended in Tris–HCl buffer (pH 7.4), and an aliquot was used to measure protein concentration by the method of Bradford [23]. 0.5% BSA was added to the membrane suspension and the sample was kept in liquid nitrogen until radioreceptor assay.

EGF receptor assay

The [^{125}I]EGF (900 Ci/mmol, Amersham) binding assay was carried out as previously described [7]. Duplicate aliquots of membrane suspension were incubated with increasing concentrations of labeled EGF (0.025–0.2 nM) in tubes without unlabeled EGF and with a fixed concentration of labeled peptide (0.2 nM) in tubes with increasing concentrations of unlabeled EGF (Amersham, 1–50 nM) at 35°C for 30 min in a final volume of 200 μl binding buffer (10 mM Tris–HCl, 150 mM NaCl, 0.1% BSA, pH 7.4). The incubations were stopped by the addition of 1 ml cold binding buffer and the samples were filtered under vacuum on Whatman GF/B filters. The filters were extensively washed and counted in a counter.

Scatchard analysis of binding data was performed using the computer program LIGAND [24].

IGF-I receptor assay

The [^{125}I]IGF-I (2000 Ci/mmol, Amersham) binding was carried out as previously described [22]. Briefly, duplicate aliquots of membrane preparation were incubated with increasing concentrations of labeled peptide (5–30 pM) and with a fixed concentration of [^{125}I]IGF-I (30 pM) in tubes with increasing concentrations of unlabeled IGF-I (Amersham, 50–300 pM), at 4°C for 24 h in a final volume of 200 μl binding buffer (50 mM Tris–HCl, 0.5% BSA, pH 7.4). The incubation was stopped by the addition of 0.8 ml cold binding buffer containing 1.0% BSA and centrifugation at 6000 *g* for 30 min at 4°C. The pellet was counted in a counter. Scatchard analysis of binding data was performed using the computer program LIGAND.

Affinity cross-linking of IGF-I to its receptor

After binding of [^{125}I]IGF-I to BPH membrane preparation in the absence and presence of unlabeled peptides, the samples were pelleted and resuspended in Tris–HCl buffer containing the cross-linking reagent disuccinimidyl suberate (DSS) 0.5 mM. After 30 min at 22°C the reaction was stopped by the addition of cold 50 mM Tris–HCl, pH 8.8. The crude membranes were solubilized by 1% Triton X-100

at 22°C for 30 min. Samples were then pelleted and electrophoresis sample buffer [24] with or without 5% 2-mercaptoethanol (2-ME) was added to the solubilized fraction. Samples were boiled and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) on linear gradient gel from 5 to 15% (w/v) acrylamide. The gels were stained, destained, dried and autoradiographed with Hiperfilm-MP (Amersham). The molecular weight standards (Bio-Rad) ranged from 14 to 200 kDa.

Affinity cross-linking of IGF-I to its binding proteins (IGF-BP)

The cytosolic fraction, obtained by BPH tissue homogenization, was treated with acetic acid (1:8, v/v) and with 95% ethanol (1:4, v/v). After 30 min, the sample was centrifugated at 10,000 *g* for 30 min. The precipitated proteins obtained from 1 g tissue were neutralized by the addition of Tris–HCl 1.5 M, pH 8.8, and then diluted with binding buffer to 0.5 ml, final volume. After [^{125}I]IGF-I binding to IGF-BP for 24 h at 4°C, in the absence and presence of unlabeled peptides, 0.5 mM DSS was added. The samples were then incubated at room temperature for 30 min and concentrated, using the filter unit Ultrafree-MC (Millipore, cut-off 10,000). Electrophoresis sample buffer with 5% 2-ME was then added. The samples were boiled and subjected to SDS–PAGE, linear gradient gel from 10 to 15% acrylamide. The gels were processed as described for SDS–PAGE of IGF-I–receptor complex. The molecular weight standards ranged from 14 to 97 kDa.

RESULTS

Patients

The treatment with Zoladex for three (5 patients) and two months (6 patients) gave similar results in terms of serum T levels with castrate values ranging from 1.3 to 0.1 nM.

EGF radioreceptor assay

Scatchard analysis of the binding data obtained from 17 different samples of BPH prostatic tissues (12 untreated and 5 treated patients) gave similar results. Figure 1 depicts a typical Scatchard plot, showing two different classes of binding sites for [^{125}I]EGF, one with high affinity and low capacity (Site 1), and one with lower affinity and higher capacity (Site 2).

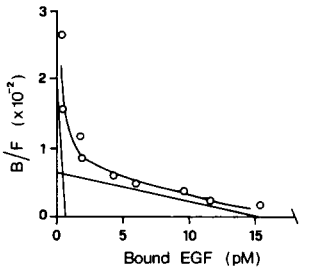

Fig. 1. Scatchard plot of [^{125}I]EGF binding to BPH prostatic membranes.

The mean (±SE) K_d values and the mean binding capacities of the two sites obtained in 12 untreated and 5 treated BPH tissues are reported in Table 1. No significant change in the binding affinity of Sites 1 and 2 was observed in BPH tissues from treated patients compared to that of untreated patients. On the contrary, the mean binding capacities of both Sites 1 and 2 in treated tissues were significantly ($P < 0.001$) higher than those of the 12 untreated patients.

IGF-I radioreceptor assay

[^{125}I]IGF-I binding to BPH prostatic membrane preparations was specific and time- and temperature-dependent [22]. Scatchard analysis of the binding data obtained from 18 different samples of BPH prostatic tissues (12 untreated and 6 treated patients) gave similar results. A typical Scatchard plot of IGF-I binding to BPH prostatic membranes is reported in Fig. 2, showing the presence of two classes of binding sites, one with high affinity and low capacity (Site 1) and one with lower affinity and higher capacity (Site 2). The mean (±SE) K_d values and the mean binding capacities of the two binding sites obtained in prostatic samples from untreated and treated patients are reported in Table 2. No significant change in the binding affinity of Sites 1 and 2 was observed in BPH tissues from treated patients compared to that of untreated patients. On the contrary, the mean binding capacities of both Sites 1 and 2 were significantly ($P < 0.001$) higher in treated tissues than those of untreated patients.

Table 1. Mean (±SE) K_d values and binding capacities (B_{max}, fmol/mg protein) of EGF binding sites in BPH prostatic tissue from untreated and treated patients

	Site 1		Site 2	
	K_d (pM)	B_{max}	K_d (nM)	B_{max}
Controls ($n = 12$)	25.0 ± 0.5	2.5 ± 0.2	2.2 ± 0.3	68.0 ± 7.8
Treated ($n = 5$)	40.0 ± 3.0	7.5 ± 1.0*	4.3 ± 0.6	198.0 ± 27.0*

*$P < 0.001$ vs controls.

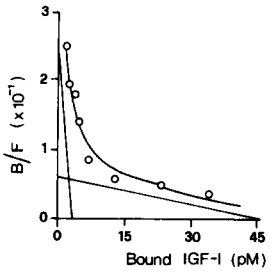

Fig. 2. Scatchard plot of [^{125}I]IGF-I binding to BPH prostatic membranes.

Table 2. Mean (±SE) K_d values and binding capacities (B_{max}, fmol/mg protein) of IGF-I binding sites in BPH prostatic tissue from untreated and treated patients

	Site 1		Site 2	
	K_d (pM)	B_{max}	K_d (nM)	B_{max}
Controls ($n = 12$)	14.0 ± 1.0	9.2 ± 0.8	0.7 ± 0.1	117.5 ± 10.3
Treated ($n = 6$)	34.0 ± 5.0	42.0 ± 2.9*	1.5 ± 0.5	369.9 ± 25.9*

*$P < 0.001$ vs controls.

Affinity cross-linking of IGF-I to its receptor

A typical autoradiography of cross-linking experiment of [^{125}I]IGF-I to BPH solubilized membranes in absence and presence of 300-fold molar excess of unlabeled peptides, is shown in Fig. 3. Linear gradient (5–15% acrylamide) SDS–PAGE under non-reducing conditions gave one protein band (lane 1) of approximately molecular weight (M_r) 300 kDa, reduced by molar excess of unlabeled IGF-II and abolished by molar excess of unlabeled IGF-I. The autoradiographic pattern observed under reducing conditions gave two labeled bands of approximately 270 and 130 kDa, respectively. The intensity of these bands was reduced by excess unlabeled IGF-II and abolished by excess unlabeled IGF-I. The radioactivity migrating in the 270 kDa region more probably represents cross-linking to type Iα subunit dimers.

Affinity cross-linking of IGF-I to IGF-binding proteins

Figure 4 illustrates the autoradiographic result of IGF-binding proteins (IGF-BP) present in the cytosolic fraction of BPH tissue, cross-linked to [^{125}I]IGF-I, and subjected to linear gradient (10–15% acrylamide) SDS–PAGE under reducing conditions. BPH tissue showed five major bands of approximately 48, 42, 38, 36 and 24 kDa (line 1). Incubation with 1000-fold molar excess of unlabeled IGF-II (line 2) did not change the intensity of bands in the region between 48 and 36 kDa, and slightly reduced the band at 24 kDa. Incubation with 1000-fold molar excess of unlabeled IGF-I substantially reduced all labeled bands.

DISCUSSION

The present report was focused on recent work from our laboratory on the characterization of specific receptors for EGF and IGF type I in prostatic tissues obtained from patients affected by benign prostatic hyperplasia (BPH). Additionally, we demonstrated that androgen deprivation resulted in a significant increase of both EGF and IGF-I receptor levels in BPH prostate tissues.

The binding characteristics of EGF prostatic receptors revealed the presence of two classes of binding sites of higher and lower affinity, according to those reported by other authors [6, 8, 9]. The differences in absolute values are probably due to the methodological conditions employed in the radioreceptor

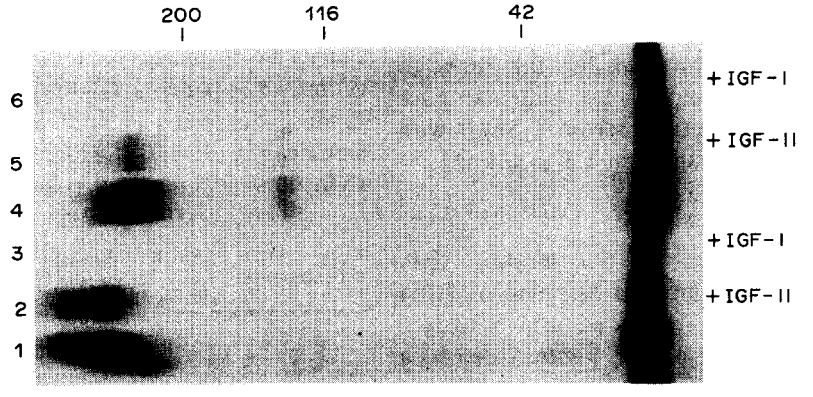

Fig. 3. Autoradiograph of [^{125}I]IGF-I cross-linked to prostatic membranes, as analyzed by 5–15% gradient SDS–PAGE under non-reducing (lanes 1–3) and reducing (lanes 4–6) conditions, in the absence (lanes 1 and 4) and in the presence of molar excess of unlabeled IGF-II and IGF-I. The migration (mol. wt, $\times 10^{-3}$) of standard proteins is indicated.

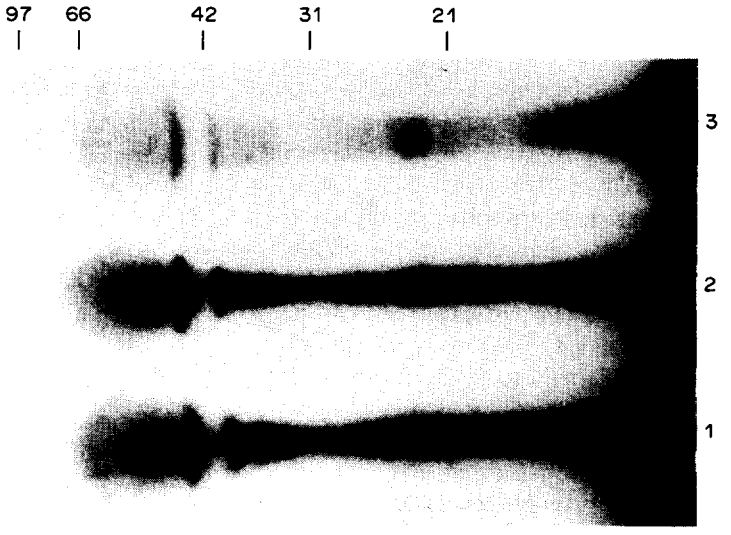

Fig. 4. Autoradiograph of [^{125}I]IGF-I cross-linked to soluble cytosolic BPH proteins, as analyzed by 10–15% gradient under reducing conditions, in the absence (lane 1) and in the presence of molar excess of unlabeled IGF-II (lane 2) and IGF-I (lane 3). The migration (mol. wt, $\times 10^{-3}$) is indicated.

assay and to the heterogeneity of BPH tissue specimens.

The evaluation of specific IGF-I receptors in BPH tissue was undertaken using multiple approaches. The specificity of IGF-I binding to prostatic membranes was tested by competitive binding studies and by the use of receptor monoclonal antibody αIR-3 as binding inhibitor [22]. The binding characteristics of IGF-I to prostatic membranes revealed the presence of two classes of binding sites, one with high affinity and low capacity and one with lower affinity and higher capacity. Affinity cross-linking studies revealed that in BPH tissue IGF type I receptors are predominant.

An understanding of the possible relationship between androgens and growth factors is essential for the study of prostatic pathological growth and may have a therapeutic and clinical interest. In the attempt to add some information to this end, we studied the effect of pharmacological androgen deprivation on BPH prostatic levels of EGF and IGF-I receptors. Three and two months treatment with a GnRH analog resulted in a significant increase of receptor levels. This increase might be explained by a decrease in EGF and IGF-I prostatic concentrations, leading to an increase in unoccupied receptors. Although this possibility remains to be clarified by experimental data, binding assays were performed under conditions of maximal exchange between endogenous and exogenous ligands. Moreover, the hormonal treatment did not result in changing the binding affinity of receptors for both EGF and IGF-I. Thus, it is reasonable to consider that under androgen deprivation there is a real increase of these receptors. In addition, our observations are in agreement with recent *in vivo* findings on the effect of castration on the rat prostate. In fact, castration of mature rats resulted in a substantial increase of EGF prostatic receptor levels, which were restored to pre-castration values by androgen administration [26]. Castration and subsequent androgen administration produced the same effect on the rat prostatic binding capacity for transforming growth factor β (TGFβ) and prostatic TGFβ expression [27, 28]. These findings and our observations suggest that some growth factors are under negative androgenic regulation at the prostate level. Thus, androgen deprivation could unleash the action of these growth factors.

Testicular suppression induced by GnRH agonist analogs in patients affected by BPH results in a preferential regression of androgen-dependent epithelial cells [29]. Moreover, immunostaining of EGF and IGF-I receptors shows that the majority of positivity is localized in the basal cells of the glandular epithelium [6, 22]. Under androgen deprivation positive staining of IGF-I receptors is extended also to the damaged luminar cells [22]. Thus, tissular localization of EGF and IGF-I receptors and their increased binding capacities under androgen deprivation, indicate that these growth-regulating factors may play a potential role in the mechanism of response to the castration-induced regression of androgen-dependent prostatic tissue.

The physiopathological meaning of our findings remains to be clarified, but may contribute to explain clinical observations. Medical castration, induced by GnRH agonists, results in a large variability of prostatic volume reduction in patients affected by BPH [29], while peripheral and tissular concentrations of androgens are homogeneously reduced by the treatment [30]. The disagreement between these observations could be in part explained by the different individual response of local peptide growth-regulating factors to the treatment.

Our preliminary results obtained with affinity cross-linking of radiolabeled IGF-I to the cytosolic fraction of BPH tissue indicate that five predominant labeled complexes of approx. molecular weight 25, 36, 38, 42 and 48 kDa are present. Formation of the complexes was substantially inhibited by IGF-I, while IGF-II produced only a faint inhibition of the complex in the 25 kDa region. The experimental conditions employed in the present study, i.e. the use of only radiolabeled IGF-I cross-linked to crude cytosol fraction, and the lack of a further structural characterization, do not allow a comparison of IGF-BP here reported with those described in different tissues and cell types [17–20], in human serum [31], and in seminal plasma [21]. Presumably they reflect some of them. Studies are in progress to characterize prostatic IGF-BP and possibly to elucidate their potential role at the prostate level.

Acknowledgements—The present work was supported in part by a Grant from the Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.), by a Grant from C.N.R. (91.00356.40) and by a Grant from the University of Florence.

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