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Regional distribution of epidermal growth factor, testosterone and dihydrotestosterone in benign prostatic hyperplasia tissue

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Abstract In benign prostatic hyperplasia (BPH), basic fibroblast growth factor (bFGF) is found to have a regional distribution, with concentrations in the periurethral zone (where the primitive fibrostromal nodule originates) higher than those of the peripheral subcapsular zone. The aim of the present investigation was to verify whether androgens and epidermal growth factor (EGF) are uniformly distributed from the periurethral to the peripheral zone or whether they show regional differences. Tissue samples, removed by transvesical resection from nine untreated BPH patients, sectioned in periurethral, subcapsular, and intermediate zones, were examined. In the periurethral zone, dihydrotestosterone (DHT), testosterone, and EGF, determined by radioimmunoassay (RIA) techniques after purification on Celite microcolumns and Sep-pak C18 cartridge, showed values significantly higher (mean ± SD: 1121 ± 482 pg, 250 ± 129 pg, and 6.89 ± 3.28 ng/mg DNA, respectively; $P < 0.01$) than those of the subcapsular zone (489 ± 190 pg, 114 ± 70 pg, and 3.40 ± 1.90 ng/mg DNA, respectively). A positive linear correlation between EGF, testosterone, and DHT was also observed. The regional distribution of EGF, testosterone, and DHT was similar to that found for bFGF: the highest levels of these factors in the periurethral region allow us to hypothesize on their possible involvement in the rewakening of mesenchymal tissue, leading to the formation of the primitive fibrostromal nodule and then to BPH development.

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Introduction

Morphological investigations have shown that benign prostatic hyperplasia (BPH) is primarily a stromal disease and that the first lesion is represented by the fibrostromal nodule in the periurethral region [1, 2, 5, 12, 19]. In this area of BPH, Lawson [11] has found concentrations of basic fibroblast growth factor (bFGF) higher than those found in the peripheral subcapsular region and in the normal adolescent and adult gland. It has been hypothesized, therefore, that in BPH bFGF is responsible for the development of the primitive fibrostromal nodule.

According to Cunha et al. [3, 4], the mesenchyme-epithelium balance plays an important role in the regulation of prostatic growth, which is a function of the amount of stroma available for epithelium proliferation: in fact experiments with tissue recombinants prepared with embryonic urogenital sinus mesenchyme and adult prostatic acini have shown that epithelium proliferation is regulated by stromal cells, probably through androgen-dependent paracrine mediators, represented by peptide growth factors such as bFGF and epidermal growth factor (EGF). It has been postulated that in BPH the different glandular regions receive the same androgenic support and that the different regional production of growth regulatory factors depends on the different capacity of the cells – stroma, smooth muscle, and epithelium – to respond to the androgen stimulation [13, 20].

The aim of the present investigation was to verify whether, in BPH, androgens are uniformly distributed from the periurethral to the peripheral zone and whether EGF levels show regional differences comparable with those observed for bFGF.

Materials and methods

Tissue samples removed by transvesical resection from nine BPH patients were sectioned in periurethral, subcapsular, and intermediate zones. Each section parallel to the longitudinal axis of the urethra was about 0.5 cm thick and 1 g in weight. The patients, aged 56–68 years, were in good general health, had never been treated for BPH, but had experienced urinary obstructive symptoms for 1–3 years.

Tissue specimens were pulverized in liquid nitrogen and homogenized in 5 vol. of TEGM buffer, as already described [15]. Three thousand counts per minute of [^{3}H]testosterone and [^{3}H]dihydrotestosterone were added to the homogenates for recovery calculation.

Intraprostatic testosterone and dihydrotestosterone (DHT) were determined in duplicate by radioimmunoassay (RIA) in tissue homogenate after acetone and ether extraction: purification was performed on Celite microcolumns, eluted with isoctane/benzene 60:40 (v/v) for testosterone and 65:35 (v/v) for DHT. Specific antibody for testosterone was purchased from Cea-Ire-Sorin and specific antibody for DHT from Biodata Serono, Milan, Italy. The sensitivity calculated was 70 pmol/l for testosterone and DHT. The interassay and intraassay variabilities were 8.2% and 2.5% for T, and 9.8% and 3.4% for DHT [18, 23].

Immunoreactive EGF was evaluated in duplicate by RIA in tissue homogenate using a homologous kit (Diagnostic System Laboratories, Webster, Tex., USA). After extraction of EGF with ice-cold acetone and, from the acetone powder, by homogenization in 10 vol. (w/vol) of ice-cold solution: 1% trifluoroacetic acid, 1% sodium chloride, 5% formic acid in 1 N hydrochloric acid, the homogenate was centrifuged and purified on Sep-pak C18 cartridges (from Waters Associates, Milford, Mass., USA), then eluted with 80% acetonitrile-20% H_2O containing 0.1% trifluoroacetic acid [15]. To each homogenate, 3000 cpm of 125I-EGF was added, for the recovery calculation. The sensitivity of the homologous RIA for EGF was 25 pg/tube. The inter- and intraassay coefficients of variation were 5.1% and 4.7%, respectively.

No cross-reactivity of the EGF antiserum, measured against several polypeptides such as transforming growth factor (TGFα), FGF, insulin-like growth factor (IGF), and pituitary hormones, expressed as the ratio of the EGF concentration to the concentration of the reacting peptide at 50% binding of the 0 ng/ml standard, was found.

A stereological analysis was also performed on 4-μm tissue sections stained with hematoxylin and eosin, according to Bartsch et al. [1]. Volume densities of the stroma and epithelium in the three zones were determined.

The statistical analysis was made utilizing the PC Statistician software for the IBM PC (Human System Dynamics, Northridge, Calif., USA) and the values were reported as mean ± SD. Correlation of the degree of association for any two parameters was determined using the Pearson *r* correlation test, validated by the Spearman's rank and Kendal tau correlation tests.

Results

Stereological analysis

Volume density of stromal tissue was higher in the periurethral zone (87 ± 5.7% SD) than in the subcapsular zone (78 ± 9.4%; $P < 0.01$). No relationship between EGF, testosterone, and DHT concentrations and the relative proportion of the epithelial and stromal component were found.

EGF concentrations in BPH

Mean (± SD) EGF concentrations were 5.10 ± 2.86 ng/mg DNA. In the three zones of BPH tissue the highest EGF levels (6.89 ± 3.28 ng/mg DNA) were found in the periurethral section and the lowest (3.40 ± 1.90 ng/mg DNA) in the subcapsular zone, differences being statistically significant ($P < 0.01$; Table 1).

Androgen concentrations in BPH

The results, expressed in picograms per milligram DNA, show that DHT displays the highest concentrations (731 ± 428) when compared with testosterone (174 ± 110). Mean androgen levels in the three zones are reported in Table 1. The highest values of DHT and testosterone (1121 ± 482 pg/mg DNA and 250 ± 129 pg/mg DNA, respectively) are observed in the periurethral zone and the lowest values (489 ± 190 pg/mg DNA and 114 ± 70 pg/mg DNA, respectively), in the subcapsular region, differences being statistically significant ($P < 0.01$).

A positive linear correlation between testosterone and DHT ($r = 0.575$, $P < 0.05$), EGF and testosterone ($r = 0.562$, $P < 0.02$), and EGF and DHT ($r = 0.652$, $P < 0.001$) was observed and is reported in Fig. 1.

Discussion

The results of the present investigation performed on BPH tissue samples, removed from nine untreated

Table 1 Percentage of stromal tissue and testosterone (*T*), dihydrotestosterone (*DHT*), and epidermal growth factor (*EGF*) content in the three zones of benign prostatic hyperplasia tissue

Prostatic zones	Stroma (%)	T (pg/mg DNA)	DHT (pg/mg DNA)	EGF (ng/mg DNA)
Periurethral				
Mean ± SD	87 ± 5.7	250 ± 129	1121 ± 482	6.89 ± 3.28
Range		469 – 109	1875 – 640	12 – 3.9
Intermediate				
Mean ± SD	81 ± 7.1	158 ± 94	585 ± 282	4.96 ± 2.48
Range		317 – 82	1115 – 370	8.30 – 2.1
Subcapsular				
Mean ± SD	78 ± 9.4	114 ± 70	489 ± 190	3.40 ± 1.90
Range		244 – 39	750 – 227	6.5 – 1

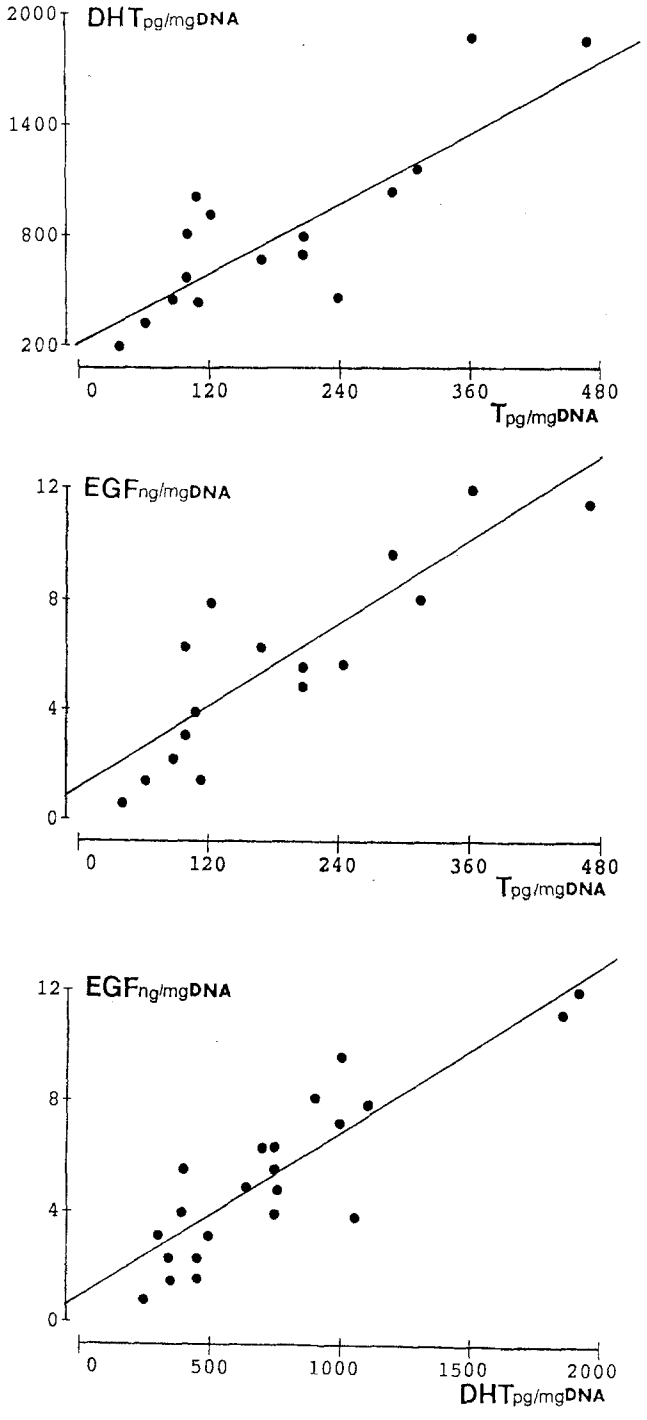

Fig. 1 Correlation between testosterone (*T*) and dihydrotestosterone (*DHT*), epidermal growth factor (*EGF*) and T, and EGF and DHT values obtained in the periurethral, subcapsular, and intermediate zones of each benign prostatic hyperplasia sample

patients in good general health, show a regional distribution of EGF and androgens, the highest values being in the periurethral zone of the prostate. In fact, periurethral values of EGF, testosterone, and DHT are about two-fold those of the subcapsular zone, with differences statistically significant. The regional variations of these factors are independent of stromal to epithelial ratio.

Attempts to correlate the steroid levels with the EGF concentrations found in the periurethral, intermediate, and subcapsular regions, employing the linear regression curve, yield a positive correlation between testosterone and EGF, and DHT and EGF. These findings are consistent with the notion of the androgen dependence of EGF release by prostatic tissue, as suggested by many authors [6, 8–10, 14, 15, 22, 24, 25].

Levine et al. [13] demonstrated that fetal and adult human prostatic fibroblasts contain nuclear androgen receptors and proliferate in response to DHT addition to the culture medium; but this effect is in part indirect and mediated by growth factors.

Since BPH is a stromal disease, the main growth factor involved in stroma cell proliferation may be the bFGF, possible inducer of the "mesenchymal rewakening," as hypothesized by McNeal [17], responsible for the origin of the primitive fibrostromal nodule. In fact, in vitro experiments [21] on isolated prostatic stromal and epithelial cells reveal that bFGF stimulates only the proliferation of the stroma, which contains specific receptors, whilst EGF influences mainly the epithelial growth and secondarily that of the stroma [4, 16, 21].

At present, the meaning of the highest EGF concentrations in the periurethral zone of the prostate, which are perfectly in agreement with the highest levels of bFGF found by Lawson in the same area , is difficult to explain. The question is whether the periurethral EGF levels, which correspond to the highest values of testosterone and DHT and of stromal content, reflect a local hyperproduction and express the biological effects of this peptide. A hypothesis might be that EGF promotes stromal cell growth in synergy with bFGF or the proliferation of adjacent epithelial cells with paracrine effect, the primitive fibrostromal nodule acting as primitive mesenchyme [11].

A response to these questions could in part be given by the simultaneous measurement of the androgen and EGF receptor content and of the respective messanger RNA by northern-blot analysis in the three BPH regions.

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