LHRH Antagonist Cetrorelix Reduces Prostate Size and Gene Expression of Proinflammatory Cytokines and Growth Factors in a Rat Model of Benign Prostatic Hyperplasia

Ferenc G. Rick,[1,2] Andrew V. Schally,[1,2]* Norman L. Block,[1,2] Gabor Halmos,[1,2,3] Roberto Perez,[1,2] Jesus B. Fernandez,[1,2] Irving Vidaurre,[1] and Luca Szalontay[1]

[1]*Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, Miami, Florida*

[2]*Departments of Pathology and Medicine, Division of Oncology/Hematology, Miller School of Medicine, University of Miami, Miami, Florida*

[3]*Department of Biopharmacy, School of Pharmacy, University of Debrecen, Debrecen, Hungary*

BACKGROUND. Recent findings suggest that BPH has an inflammatory component. Clinical trials have documented that therapy with LHRH antagonist Cetrorelix causes a marked and prolonged improvement in LUTS in men with symptomatic BPH. We investigated the mechanism of action and effect of Cetrorelix in a rat model of BPH.

METHODS. Adult male Wistar rats were used. BPH was induced in rats by subcutaneous injections of TE 2 mg/day for 4 weeks. Control animals received injections of corn oil. After induction of BPH, rats received depot Cetrorelix pamoate at the doses of 0.625, 1.25, and 12.5 mg/kg on days 1 and 22 and TE-control rats received vehicle injections. Whole prostates were weighed and processed for RNA and protein. Real-time RT-PCR assays for numerous inflammatory cytokines and growth factors were performed. Quantitative analyses of prostatic LHRH receptor, LHRH, androgen receptor (AR) and 5α-reductase 2 were done by real-time RT-PCR and immunoblotting; serum DHT, LH, PSA, and IGF-1 by immunoassays.

RESULTS. mRNA levels for inflammatory cytokines IFN-γ, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, IL-15, and IL-17 and for growth factors EGF, FGF-2, FGF-7, FGF-8, FGF-14, TGF-β1, and VEGF-A were significantly reduced by Cetrorelix 0.625 mg/kg ($P < 0.05$). Prostate weights were also significantly lowered by any dose of Cetrorelix.

CONCLUSIONS. This study suggests that Cetrorelix reduces various inflammatory cytokines and growth factors in rat prostate and, at doses which do not induce castration levels of testosterone, can lower prostate weights. Our findings shed light on the mechanism of action of LHRH antagonists in BPH. *Prostate 71: 736–747, 2011.* © 2010 Wiley-Liss, Inc.

KEY WORDS: **benign prostatic hyperplasia; LHRH; proinflammatory cytokines; growth factors; rats**

Dr. Ferenc G. Rick is on leave of absence from Department of Anatomy, University of Pecs Medical School, Pecs, Hungary.

Grant sponsor: Medical Research Service of the Veterans Affairs Department; Grant sponsor: Department of Pathology and Medicine, Division of Hematology/Oncology of the Miller Medical School, University of Miami and AEterna/Zentaris GmbH through South Florida Veterans Affairs Foundation for Research and Education; Grant sponsor: L. Austin Weeks Endowment for Urologic Research.

*Correspondence to: Andrew V. Schally, Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, 1201 NW 16th St., Research (151), Room 2A103C, Miami, FL 33125. E-mail: andrew.schally@va.gov

Received 12 May 2010; Accepted 7 September 2010
DOI 10.1002/pros.21289
Published online 14 October 2010 in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Benign prostatic hyperplasia is extremely common and is clinically characterized by prostate enlargement and lower urinary tract symptoms. Despite the enormous burden of BPH on public health, the pathogenesis of BPH is incompletely understood [1,2]. Age-related systemic/local hormonal and vascular changes appear to represent the predominant mechanism. However, an emerging body of evidence suggests that inflammation may play a key role in the development and progression of BPH [3,4]. Clinically, several cross-sectional studies have proposed that a relationship exists between the presence of inflammatory infiltrates and an increase in prostate volume. Di Silverio et al. [5] reported that presence of chronic inflammation increased as prostate volume augments, from 9% in prostates of 30–39 ml to more than 60% in prostates of 80–89 ml. A minor correlation was observed between presence of prostatic inflammation and lower urinary tract symptoms (LUTS) in participating men in the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial [6]. This minor correlation between prostatic inflammation and presence of LUTS may be expected given the relatively modest correlations that have been found between prostate volume and LUTS [7]. Additionally, in a Wistar rat model, administration of an immunostimulator resulted in epithelial proliferation [8].

Recent reviews of the pathogenesis of BPH highlighted the differential expression of cytokines and growth factors in BPH tissue suggesting a role for inflammation in the propagation of BPH [9,10]. An up-to-date outline of the current state of knowledge in regard to the impact of inflammation on the pathogenesis of BPH was reported by Kramer et al. [4]. Chronic inflammatory infiltrates, mostly composed of activated T cells and macrophages, are often associated with BPH nodules [11,12]. Infiltrating T cells and macrophages are responsible for the production of cytokines (IL-2 and IFNγ), which are believed to support fibromuscular growth through cascades of proinflammatory cytokines and growth factors [13–15], leading to LUTS [16]. Migration of T cells into the area is accomplished by increased production of proinflammatory cytokines such as IL-6, IL-8, IL-15, and IL-17 [4,10,17,18]. Subsequently, surrounding cells are killed by unknown mechanism and are replaced by fibromuscular nodules [19].Various growth factors such as FGF-2, FGF-7, IGF-I, IGF-II, TGFβ, and VEGF, are also involved in the pathogenesis of BPH [15,20].

Cetrorelix, a luteinizing hormone-releasing hormone antagonist, was shown to inhibit the growth of human prostatic and other cancers xenografted into nude mice with suppression of growth factors EGF and IGF-2 [21]. Therapy with Cetrorelix causes a marked and protracted improvement in LUTS, reduction in prostate volume and increase in urinary peak flow rate in men with BPH without impairment of gonadal function [22–24]. Improvement in LUTS could be due to direct inhibitory effects of Cetrorelix on the prostate exerted through prostatic LHRH receptors or to alterations in levels of proinflammatory cytokines and growth factors.

It is well known that enlargement of the prostate occurs in the presence of androgens [25] and that anabolic steroids increase prostatic volume and reduce urine flow, leading to increased urinary frequency [26]. Maggi et al. [27] described a model in male rats wherein BPH was produced by repeated injections of testosterone. The model described by Maggi et al. has been adapted for several studies [28–31]. Given that the mechanism of prostate growth is complex and heterogeneous in different species and the testosterone-induced models of BPH show an epithelial hyperplasia [31,32], the androgen-induced models of BPH have limitations. These include the fact that inflammation was not described as a main characteristic, but rather was incidental [31].

In this present study we used a testosterone-induced rat model of BPH based on the reports of Maggi et al. [27] and Scolnik et al. [31]. We estimated the therapeutic effect of LHRH antagonist Cetrorelix. We also investigated the mechanisms of action of Cetrorelix, including its in vivo effects on the expression of inflammatory cytokines and growth factors.

MATERIALS AND METHODS

Drugs and Chemicals

Testosterone enanthate (TE) (Watson Pharmaceuticals, Corona, CA) and corn oil vehicle (Sigma–Aldrich, St. Louis, MO) were used. Depot formulation of LHRH antagonist Cetrorelix (Ac-D-Nal (2)^{1},D-Phe(4Cl)^{2},D-Pal (3)^{3},D-Cit^{6},D-Ala-^{10})LHRH, was supplied by AEterna/Zentaris GmbH (Frankfurt, Germany) as Cetrorelix pamoate (D20762) and contained Cetrorelix peptide base, pamoic acid (in a molar ratio of 2:1) and mannitol suspended in distilled water [33]. This depot preparation can be injected every 21–30 days.

Animals

Adult male Wistar rats weighing 225–250 g (Charles River Laboratories, Wilmington, MA) were housed in a climate-controlled environment with a 12-h light/dark cycle and were fed standard laboratory diet, water ad libitum. The Institutional Animal Care and Use Committee approved the protocol. Body weights were determined weekly.

Study Design

After 7 days acclimatization, rats were randomly divided into four experimental groups and one negative control group of ten animals each (Fig. 1). BPH was induced in experimental groups by daily subcutaneous injection in the right flank of long acting testosterone enanthate (2 mg/day), dissolved in corn oil from Day −28 to Day 0 (induction phase). Negative control animals received subcutaneous injections of corn oil alone on the same schedule. The dosage and duration of testosterone treatment were based on the reports by Maggi et al. [27]. and Scolnik et al. [31]. Based on previous clinical [23,24] and experimental [34] reports, animals were administered subcutaneous injections of the LHRH antagonist Cetrorelix (0.625, 1.25, and 12.5 mg/kg body weight) in the left flank on days 1 and 22 after BPH induction (day −28 to day 0). Experimental groups consisted of: (1) TE only, (2) TE/Cetrorelix 0.625 mg/kg, (3) TE/Cetrorelix 1.25 mg/kg, and (4) TE/Cetrorelix 12.5 mg/kg body weight. TE only positive control animals were injected with mannitol instead of Cetrorelix on the same schedule. Venous blood samples were collected before the experiment and on the last day of the experiment (day 42). Serum was separated by centrifugation and stored at −80°C. Rats were weighed and sacrificed under anesthesia on the morning of day 42; whole prostates were immediately removed, weighed and snap frozen. Alternate prostrate lobes were immersed in phosphate-buffered 10% formalin (pH 7.4) and embedded in paraffin for histological analysis.

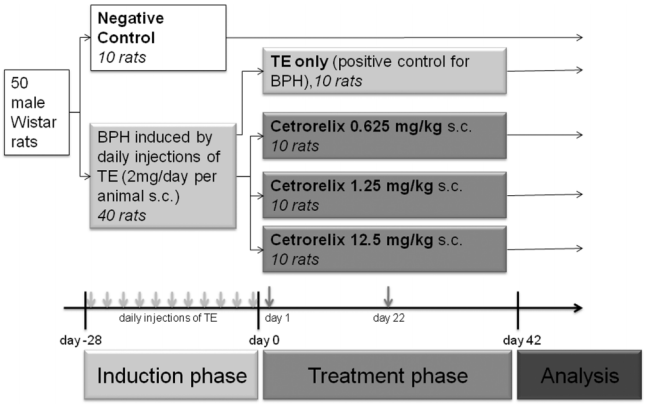

Fig. 1. Scheme of the study. Induction phase included daily injections of 2 mg TE s.c. per rat for 28 days. In the treatment phase, different doses of a depot preparation LHRH antagonist Cetrorelix pamoate (0.625, 1.25, or 12.5 mg/kg) were administered s.c. on days 1 and 22. Study groups were as follows: Control, TE, Cetrorelix 0.625 mg/kg, Cetrorelix 1.25 mg/kg and Cetrorelix 12.5 mg/kg. BPH, benign prostatic hyperplasia; LHRH, luteinizing hormone-releasing hormone; s.c., subcutaneous; TE, testosterone enanthate.

Histological Procedures and Morphological Analysis

Serial 5 μm-thick sections from each fixed tissue specimen were prepared, mounted on glass slides, and stained with hematoxyline-eosin for morphological analysis. The sections were analyzed with a Nikon Eclipse 90i microscope with a built-in digital camera. The digitalized images of ventral prostatic lobes, obtained by using NIS-Elements BR 3.00 for Windows image analyzer software, were used for stereological-morphometric analysis. The mean epithelial height was determined from a total of 500 random interactive measurements with 40× objective at 10 different points on 10 different fields from five different individual ventral prostate sections. Measurements were taken from the intermediate to distal regions of the prostate lobe ducts, which represent the major portions of the prostatic lobes [35].

Total RNA Isolation/Real-Time RT-PCR

Total RNA was isolated from ~30 mg of prostate tissue for each sample using NucleoSpin kit (Macherey-Nagel, Germany). Three prostate samples from each group were analyzed. Quality control of RNA samples, synthesis of cDNAs for various rat genes (Table I) and target gene amplification with real-time reverse transcriptase-polymerase chain reaction (RT-PCR) were as described [36]. Sequences for forward and reverse rat specific primers and thermal cycling conditions are shown in Table I. Pfaffl's method was used to evaluate the relative expression ratio for all genes normalized to β-actin [37].

RT^2 Profiler^{TM} PCR Arrays

Rat Inflammatory Cytokines and Receptors and Growth Factors RT^2 Profiler^{TM} PCR arrays (SABiosciences, Frederick, MD) were used to examine the mRNA levels of 168 genes related to inflammatory response and growth factors. Total RNA extraction was as described. Quality control of RNA samples, synthesis of cDNA and its amplification by real-time RT-PCR arrays were performed per manufacturer (SABiosciences). Fold-changes in gene expression were calculated using the ΔΔCt method. Normalization was performed using five housekeeping genes on the arrays.

Western Blot

Prostate tissue was homogenized with Mikro-Dismembrator (Goettingen, Germany). Protein was isolated with NucleoSpin Kit (Macherey-Nagel) and sonicated with Branson Sonifier (Danbury, CT). Protein lysates were adjusted to equal concentrations. Western

TABLE I. Oligonucleotide Primers Used for Quantitative Real-Time PCR

Gene	Accession no.	Forward (5′–3′)	Reverse (5′–3′)
IGF-1	NM_178866	GGCATTGTGGATGAGTGTTG	CGATAGGGGCTGGGACTT
IGF-2	NM_133519	CGCACCCACAGAGAAATAAAA	TCCGAGCACCTTCCTAACAC
TGF-α	NM_012671	GCCTTCTTGCTAACCCACAC	GATGTTTCCCCTTGTCCATTT
TGF-β1	NM_021578	CGCAATCTATGACAAAACCAAA	ACAGCCACTCAGGCGTATC
TGF-β2	NM_031131	CGCATCTCCTGCTAATGTTG	TTCGGGGTTTATGGTGTTGT
EGF	NM_012842	CCCGTGTTCTTCTGAGTTCC	TGTAACCGTGGCTTCCTTCT
FGF-2	NM_019305	CTGTCTCCCGCACCCTATC	CTTTCTCCCTTCCTGCCTTT
KGF/FGF-7	NM_022182	TCCACCTCGTCTGTCTTGTG	CCTTTCACTTTGCCTCGTTT
IL-1β	NM_031512	GTCACTCATTGTGGCTGTGG	GGGATTTTGTCGTTGCTTGT
IL-6	NM_012589	GCCAGAGTCATTCAGAGCAA	CATTGGAAGTTGGGGTAGGA
VEGF-A	NM_031836	GACACACCCACCCACATACA	ACATCCTCCTCCCAACTCAA
LHRH-R	NM_031038	GGGGCTGAGCATCTATAACACC	TGCTAACCTCTGGACAGGGATC
LHRH	NM_012767	GGCTTTCACATCCAAACAGAA	GCCTTCCAAACACACAGTCAA
AR	NM_012502	CAAAGGGTTGGAAGGTGAGA	GAGCGAGCGGAAAGTTGTAG
5α-reductase 2	NM_022711	GCAAAGTTTCTGTGGAGGA	AAGCAACTGGAATAACAAGAGA
β-Actin	NM_031144	GGGTTACGCGCTCCCTCAT	GTCACGCACGATTTCCCTCTC

Rat specific primers were designed according to the following criteria: (1) a product-size range of 70–180 bases, (2) a primer size range of 18–22 bases, (3) a T_m difference of 3°C, and (4) a GC content of 30–80%. The mRNA sequences used for the design of the primers were taken from the NCBI Reference Sequences. The primers were tested for sequence similarity to other genes with NCBI Basic Local Alignment Search Tool (BLAST). The thermal cycling conditions for each set of primers comprised an initial denaturation step at 95°C for 3 min and then 35–40 cycles of two-step PCR including 95°C for 30 sec and 60°C for 1 min. Data were collected during the 60°C annealing step and were further analyzed by the iCycler™ iQ Optical system software (Bio-Rad). Real-time PCR melting curve analyses revealed a single product for each primer set.

blot analyses were as described [38]. Primary antibodies for LHRH-R, LHRH, AR and 5α-reductase-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Radioimmunoassay (RIA) and ELISA

Serum DHT was determined by RIA using DSL-9600 kit (Diagnostic Systems Laboratories, Webster, TX) and serum LH by 29-AH-R002 RIA kit (Alpco Diagnostics, Salem, NH). For quantitative serum PSA and IGF-1 we used ELISA kits DSL-9700, DSL-10-2800. All immunoassays were done according to manufacturers' instructions.

Ligand Competition Assay

Receptors for LHRH on rat prostate tissues were characterized by ligand competition assay. Radioligand binding studies were performed as described [39].

Statistical Analysis

For statistical evaluation, SigmaStat 3.0 software (Sytat Software, San Jose, CA) was used. Results are expressed as mean ± SE. One-way analysis of variance (ANOVA) followed by Bonferroni *t*-test or a two-tailed Student's *t*-test were used where appropriate, and significance was accepted at $P < 0.05$.

RESULTS

Effect of LHRH Antagonist Cetrorelix on Rat Prostate Weight

All animals remained healthy throughout the experiment. Body weights at sacrifice were not affected by Cetrorelix + TE compared to TE-only treated rats (Table II). Corn oil-injected control prostates (negative control) weighed 264.8 ± 9.6 mg/100 g rat (Fig. 2); while in TE controls prostates were enlarged by 40.52% to 372.1 ± 25.3 mg/100 g rat ($P < 0.001$; Fig. 2). Cetrorelix pamoate 0.625 mg/kg significantly lowered prostate weights by 17.88% ($P = 0.02$). This decrease was similar to that obtained with 1.25 mg/kg Cetrorelix (18.65% reduction [$P = 0.01$]); further reduction occurred but smaller than with 12.5 mg/kg Cetrorelix (35.17% reduction [$P < 0.001$]).

Morphology and Morphometry

Macroscopically, no visible lesions were present in the prostates of Wistar rats in the different experimental groups, except for the obvious enlargement observed among TE-induced BPH rats.

In the ventral prostates of negative control rats, we noted normal histology [31] distinguished by monolayered low columnar epithelium with round-shaped nuclei adjacent to the intact basal membrane and

TABLE II. Effect of LHRH Antagonist Cetrorelix at Different Doses on Morphological Parameters

	Body weight (g)		Day 42	
	Day −28	Day 42	Prostate weight (g)	Epithelial height (μm)
Control	255 ± 3	481 ± 9	1.27 ± 0.05	11.65 ± 0.11
TE	257 ± 7	445 ± 20	1.64 ± 0.10‡	20.43 ± 0.39§
TE/Cetrorelix 0.625 mg/kg	258 ± 4	446 ± 15	1.36 ± 0.06*	13.15 ± 0.14***
TE/Cetrorelix 1.25 mg/kg	258 ± 3	449 ± 10†	1.35 ± 0.04*	14.62 ± 0.17***
TE/Cetrorelix 12.5 mg/kg	254 ± 2	445 ± 12†	1.06 ± 0.05***	12.57 ± 0.15***

Mean ± SE.
†$P < 0.05$, ‡$P < 0.01$ and §$P < 0.001$ as compared to control, *$P < 0.05$, ***$P < 0.01$ as compared to TE. Statistical analysis was performed by one-way ANOVA, followed by Bonferroni *t*-test.

regular acini surrounded by variable amounts of fibromuscular stroma. The acinar lumen contains only a small number of papillary folds (Fig. 3 and Table II).

Induction of BPH by testosterone resulted in hyperplastic acinar morphology in the ventral prostates characterized by high columnar epithelial cells with round nuclei placed near to the intact basal membrane, secretory vacuoles at the apical zone, numerous intraluminal papillary folds and variable amounts of stroma (Fig. 3). These changes in acinar morphology after treatment with testosterone correspond to the observations of Scolnik et al. [31]. We also found a 75% increase in the epithelial cell height compared to control (Table II).

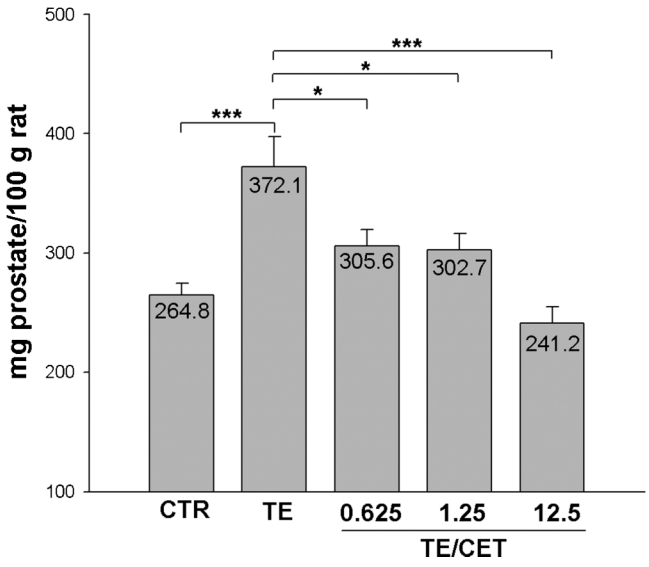

Fig. 2. Effect of TE and treatment with LHRH antagonist Cetrorelix at doses of 0.625, 1.25, and 12.5 mg/kg on relative prostate weight, evaluated 42 days after the start of treatment with Cetrorelix. Statistical analysis was performed by one-way ANOVA, followed by Bonferroni *t*-test. Significant differences are marked by asterisks (*$P < 0.05$ and ***$P < 0.001$). CTR, negative control; CET, Cetrorelix; ANOVA, analysis of variance.

After treatment with Cetrorelix 0.625 mg/kg, the ventral prostatic acinar morphology showed characteristics similar to that of normal rats (Fig. 3). The acini were lined with low columnar epithelium with round basal nuclei. The acinar lumina had only a small degree of infolding. Cetrorelix 0.625 mg/kg significantly decreased the epithelial height by 36% compared to TE-induced BPH controls (Table II). The amount of fibromuscular stroma was seemingly not affected by treatment with Cetrorelix at a low dose.

In addition to the primary pathological lesions of the prostatic acini, incidental inflammatory reactions including lymphocyte and mast cell infiltration, edema and blood congestion, were also observed in all experimental groups (Fig. 4). In some instances of TE-induced BPH, we found intraluminal infiltration as well (Fig. 4). These inflammatory infiltrates in the ventral prostates of Wistar rats are consistent with the observations of inflammatory exudates that have been previously reported for this model [31].

Effect of TE and Cetrorelix 0.625 mg/kg on Expression of Inflammatory Cytokine/growth Factor mRNA

Using real-time PCR arrays for rat inflammatory cytokines/receptors and growth factors on control, TE-induced BPH control and TE-induced BPH treated with Cetrorelix 0.625 mg/kg (=lowest dose), we identified important functional molecules affected by treatment with Cetrorelix and selected genes potentially related to prostate shrinkage. More than 30 genes were significantly altered after treatment with TE and Cetrorelix ($P < 0.05$; Table III). Cytokines including IL-3, IL-5, IL-6, IL-13, IL-15, IL-17β, and lymphotoxin-A (LTA) were upregulated by induction of BPH with TE, by 2.95-, 4.54-, 1.98-, 4.47-, 4.17-, 1.95-, and 4.62-fold, respectively. Treatment with Cetrorelix significantly downregulated cytokines IFN-γ, IL-1α, IL-3, IL-4, IL-5, IL-6, IL-13, IL-15, IL-17β and LTA by 2.35-, 3.96-, 4.06-, 4.14-, 2.03-, 2.73-, 4.09-, 2.68-, 5.35-, and 3.78-fold,

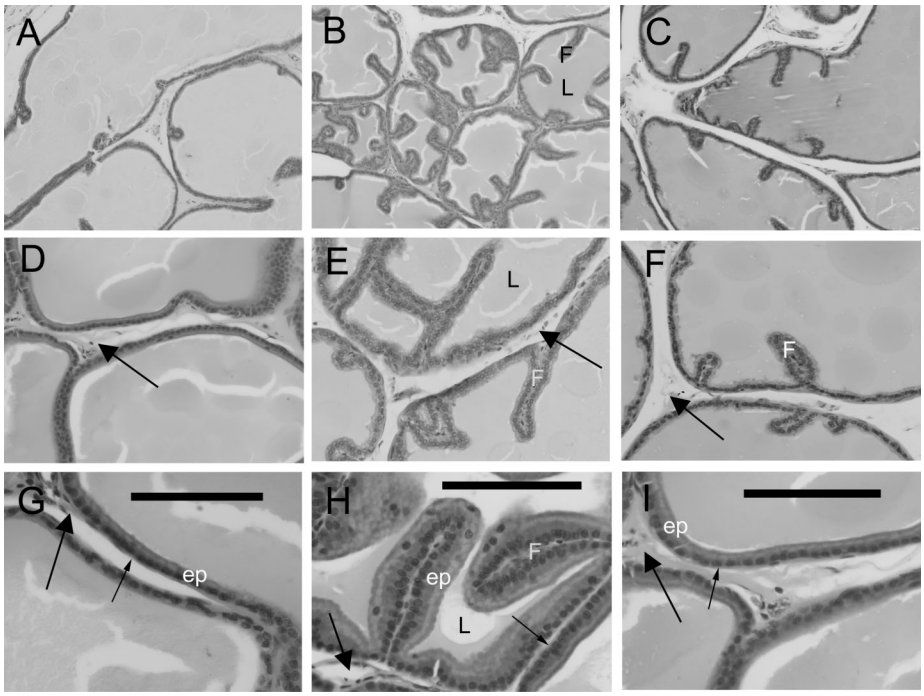

Fig. 3. Morphological evaluation of ventral prostate sections from control rats (**A**,**D**,**G**), rats with testosterone-induced BPH (**B**,**E**,**H**) and rats treated with Cetrorelix 0.625 mg/kg (**C**,**F**,**I**). Compared to control, induction of BPH by testosterone decreased luminal area, promoted epithelial folds (F) and increased the epithelial height (ep). Treatment with Cetrorelix 0.625 mg/kg increased the luminal area of the ducts (L), diminished the epithelial folds and reduced epithelial height, as compared to TE-induced BPH animals. Bars indicate 100 μm (G,H,I). Arrows indicate fibromuscular stroma, while fine arrows indicate intact basal membrane. Histomorphology is shown at 10× (A–C), 20× (D–F), and 40× (G,H,I) magnification (hematoxylin-eosin staining).

respectively. Among chemokines and chemokine receptors, expression of C5, CCL25, SPP1, CCR4, CCR9, and BLR-1/CXCR5 was significantly decreased by Cetrorelix by 3.07-, 4.06-, 2.33-, 3.76-, 12.30-, and 2.87-fold, respectively. Levels of FGF-2, FGF-7, FGF-8, and FGF-14 were significantly reduced by Cetrorelix by 2.02-, 2.07-, 4.72-, and 18.90-fold, respectively. Levels of mRNA for TGF-β superfamily members, TGF-β1 and BMP-7, showed 1.79- and 1.75-fold decrease after Cetrorelix. The level of VEGF-A was 1.83-fold lower after Cetrorelix.

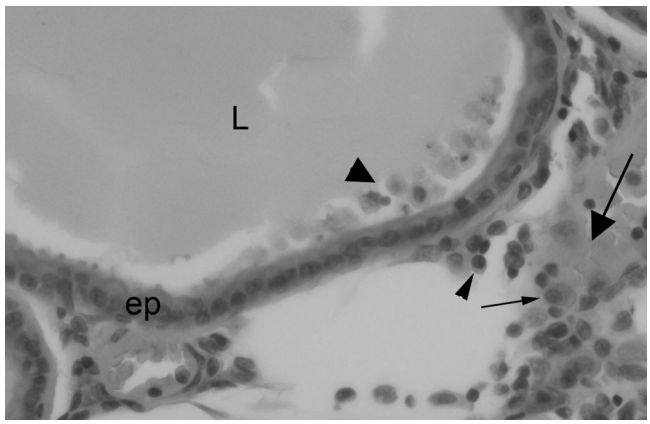

Fig. 4. Inflammatory infiltrate in ventral prostate of testosterone-induced BPH rats. In addition to the primary pathological lesions of the prostatic acini, we observed incidental inflammatory reactions in the stroma, including lymphocytes (fine arrowhead), mast cells (fine arrow), edema, and blood congestion (arrow). This inflammatory infiltrate seemed to be present in the adjacent acinar lumen (arrowhead) as well. We noted inflammatory infiltrates in all experimental groups. Hematoxylin-eosin staining, 40× magnification.

Evaluating our PCR array data, we used real-time RT-PCR to analyze selected proinflammatory and growth factor genes (Fig. 5). We found that levels of mRNA for EGF, FGF-2, FGF-7, and VEGF-A were significantly elevated in TE-induced BPH by 2.17-, 2.67-, 2.53-, and 2.20-fold, respectively ($P < 0.05$). Treatment with Cetrorelix 0.625 mg/kg significantly decreased expression of EGF (4.37-fold [$P < 0.01$]), TGF-β1 (1.89-fold [$P < 0.01$]), TGF-β2 (1.46-fold [$P < 0.05$]), FGF-2 (2.11-fold [$P < 0.05$]), FGF-7 (2.75-fold [$P < 0.05$]), VEGF-A (2.39-fold [$P < 0.001$]), IL-1β (1.64-fold [$P < 0.001$]), and IL-6 (1.76-fold [$P < 0.05$]).

Effect of Cetrorelix 0.625 mg/kg on Expression of LHRH-R, LHRH, AR, and 5α-reductase 2 in Rat Prostate

mRNA for LHRH-R and LHRH-R protein and its LHRH ligand were detected in rat prostate (Fig. 6.1,2).

TABLE III. Gene Expression of Rat Inflammatory Cytokines and Receptors and Growth Factors in Prostates Harvested From TE Treated and Cetrorelix 0.625 mg/kg Treated Rats 42 Days After Start of Treatment With Cetrorelix

	Fold change		
Gene	TE vs. control	TE/Cetrorelix 0.625 mg/kg vs. TE	Description
Cytokine genes			
IFN-γ	1.29	**−2.35**	Interferon gamma, induction of proliferation of BPH stromal cells [13,19]
IL-1α	1.41	**−3.63**	Interleukin 1 alpha, stimulates the production of epithelial growth promoting FGF-7 in fibroblastic stromal cells [45]
IL-1β	1.56	−1.47	Interleukin 1 beta, important mediator of the inflammatory response [18,19]
IL-2	1.14	−1.27	Interleukin 2, stimulation of growth of stromal cell clones [13]
IL-3	**2.95**	**−4.06**	Interleukin 3, secreted by activated T cells to support growth and differentiation of T cells in an immune response [46]
IL-4	1.12	**−4.14**	Interleukin 4, inhibition of proliferation of slowly growing stromal cell clones, stimulation of growth of fibroblasts [13,19]
IL-5	**4.54**	**−2.03**	Interleukin 5, produced by T helper-2 cells and mast cells, stimulates B cell growth and increase immunoglobulin secretion
IL-6	**1.98**	**−2.73**	Interleukin 6, stimulates epithelial cell growth [13]
IL-f6	**4.49**	**−4.02**	Interleukin 1 family, member6, function unknown
IL-13	**4.47**	**−4.09**	Interleukin 13, a central mediator of the physiologic changes induced by allergic inflammation in many tissues
IL-15	**4.17**	**−2.68**	Interleukin 15, a growth factor for BPH memory T cells [18,19]
IL-17β	**1.95**	**−5.35**	Interleukin 17B, a potent inductor of IL-6 and IL-8 production by prostate epithelial and stromal cells [17]
LTA	**4.62**	**−3.78**	Lymphotoxin A, produced by lymphocytes, mediates a large variety of inflammatory, immunostimulatory, and antiviral responses
Cytokine receptors			
IL-5Rα	**4.51**	**−4.15**	Interleukin 5 receptor alpha, receptor for IL-5
Chemokines			
C5	1.04	**−3.07**	Complement component 5, a protein involved in the complement system
CCL25	**4.42**	**−4.06**	Chemokine (C−C motif) ligand 25, chemotactic for macrophages
CXCL2	**3.89**	−1.43	Chemokine (C−X−C motif) ligand 2, chemotactic for polymorphonuclear leukocytes [42,43]
SPP1	**2.75**	**−2.33**	Secreted phosphoprotein 1 or osteopontin (OPN), immunmodulator and adhesion protein, involed in chemotaxis and wound healing
Chemokine receptors			
CCR4	1.04	**−3.76**	Chemokine (C−C motif) receptor 4, a receptor for certain CC chemokines
CCR9	1.20	**−12.30**	Chemokine (C−C motif) receptor 9, the specific ligand of this receptor is CCL25
BLR-1/ CXCR5	**2.08**	**−2.87**	Chemokine (C−X−C motif) receptor 5, plays an essential role in B cell migration [47]
Growth factors			
EGF	**3.93**	**−2.47**	Epidermal growth factor, potent mitogenic growth factor for prostatic epithelial cells [20]
IGF-1	1.52	−1.45	Insulin-like growth factor 1, a mitogen for prostatic epithelial cells [15]
IGF-2	−1.15	1.12	Insulin-like growth factor 2, the predominant mitogenic IGF produced chiefly by prostatic stromal cells [15,20]
FGF-2	**2.64**	**−2.02**	Fibroblast growth factor 2, a potent growth factor for prostatic epithelial and stromal cells [15]
FGF-7	1.41	**−2.07**	Fibroblast growth factor 7, a potent mitogen for prostatic epithelial cells [15]
FGF-8	**8.07**	**−4.72**	Fibroblast growth factor 8, a FGF family member possessing broad mitogenic and cell survival activities
FGF-9	1.32	−1.25	Fibroblast growth factor 9, a potent mitogen for both prostatic epithelial and stromal cells
FGF-14	**24.25**	**−18.90**	Fibroblast growth factor 14, involved in cell growth, morphogenesis, tissue repair, tumor growth and invasion

TABLE III. (*Continued*)

Gene	Fold change: TE vs. control	Fold change: TE/Cetrorelix 0.625 mg/kg vs. TE	Description
TGF-β superfamily			
BMP5	**4.29**	−1.36	Bone morphogenetic protein 5, function unknown in the prostate
BMP7	**4.00**	**−1.75**	Bone morphogenetic protein 7, function unknown in the prostate
TGF-β1	1.23	**−1.79**	Transforming growth factor beta 1, inhibition of stromal cell growth by induction of differentiation and angiogenesis [13,15,19]
TGF-β2	1.16	−1.41	Transforming growth factor beta 2, inhibition of stromal cell growth by induction of differentiation and angiogenesis [13,15,19]
Angiogenic factors			
VEGF-A	**1.75**	**−1.83**	Vascular endothelial growth factor A, angiogenesis
VEGF-B	**−2.64**	−1.27	Vascular endothelial growth factor B, angiogenesis
VEGF-C	−1.07	−1.03	Vascular endothelial growth factor C, angiogenesis

Multiple genes related to inflammatory response and growth factors were evaluated for expression using real-time PCR via RT^2 Profiler^{TM} PCR Array system. The table lists the genes of interest evaluated and their fold increase or decrease in prostates obtained from TE treated and TE/Cetrorelix 0.625 mg/kg treated rats 42 days after the start of treatment with Cetrorelix. Data represent fold differences of individual gene expression between study groups TE and control prostate or TE/Cetrorelix 0.625 mg/kg and TE. Positive values mark upregulation of individual genes, while negative values mark downregulation. Three experiments were run for each study group. The data were evaluated by two-tailed Student's *t*-test. Boldface depicts significant changes ($P < 0.05$).

There were no significant changes in the mRNA and the protein levels of LHRH-R in TE-induced BPH and also after treatment with Cetrorelix (Fig. 6.1A,B). Expression of LHRH was elevated after Cetrorelix; this is significant compared to control (Fig. 6.2B). There were no significant changes in the expression of prostatic androgen receptor (AR) in TE-induced BPH, while Cetrorelix significantly downregulated mRNA for AR and AR protein levels (Fig. 6.3A,B [$P < 0.001$ and $P < 0.05$, respectively]). Prostatic 5α-reductase 2 protein increased after TE treatment compared to control ($P < 0.01$); Cetrorelix significantly lowered 5α-reductase 2 mRNA and protein levels (Fig. 6.4A,B [$P < 0.01$ and $P < 0.05$, respectively]).

Binding Assay for LHRH Receptors in Rat Prostate

Receptor analyses revealed a single class of high affinity binding sites for LHRH in rat prostate

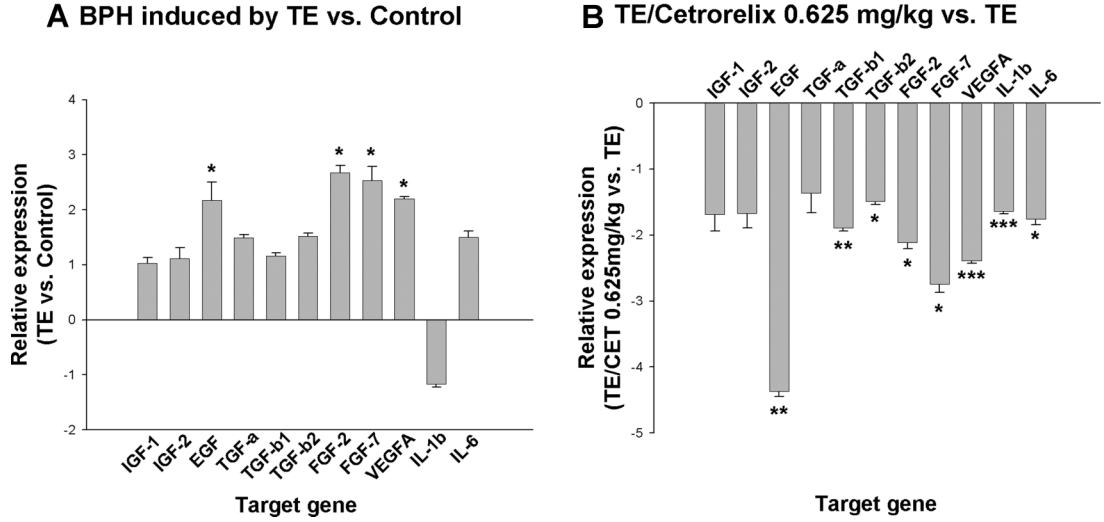

Fig. 5. Real-time PCR quantification of various growth factors involved in prostatic growth and function. Bars represent fold differences of individual gene expression between prostate samples from (**A**) TE and CTR (negative control) groups or (**B**) Cetrorelix 0.625 mg/kg and TE groups. Positive values (>1.00) mark upregulation of individual genes, while negative values (<1.00) mark downregulation. Data are shown as mean ± SE. Asterisks indicate a significant difference (*$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ Student's *t*-test).

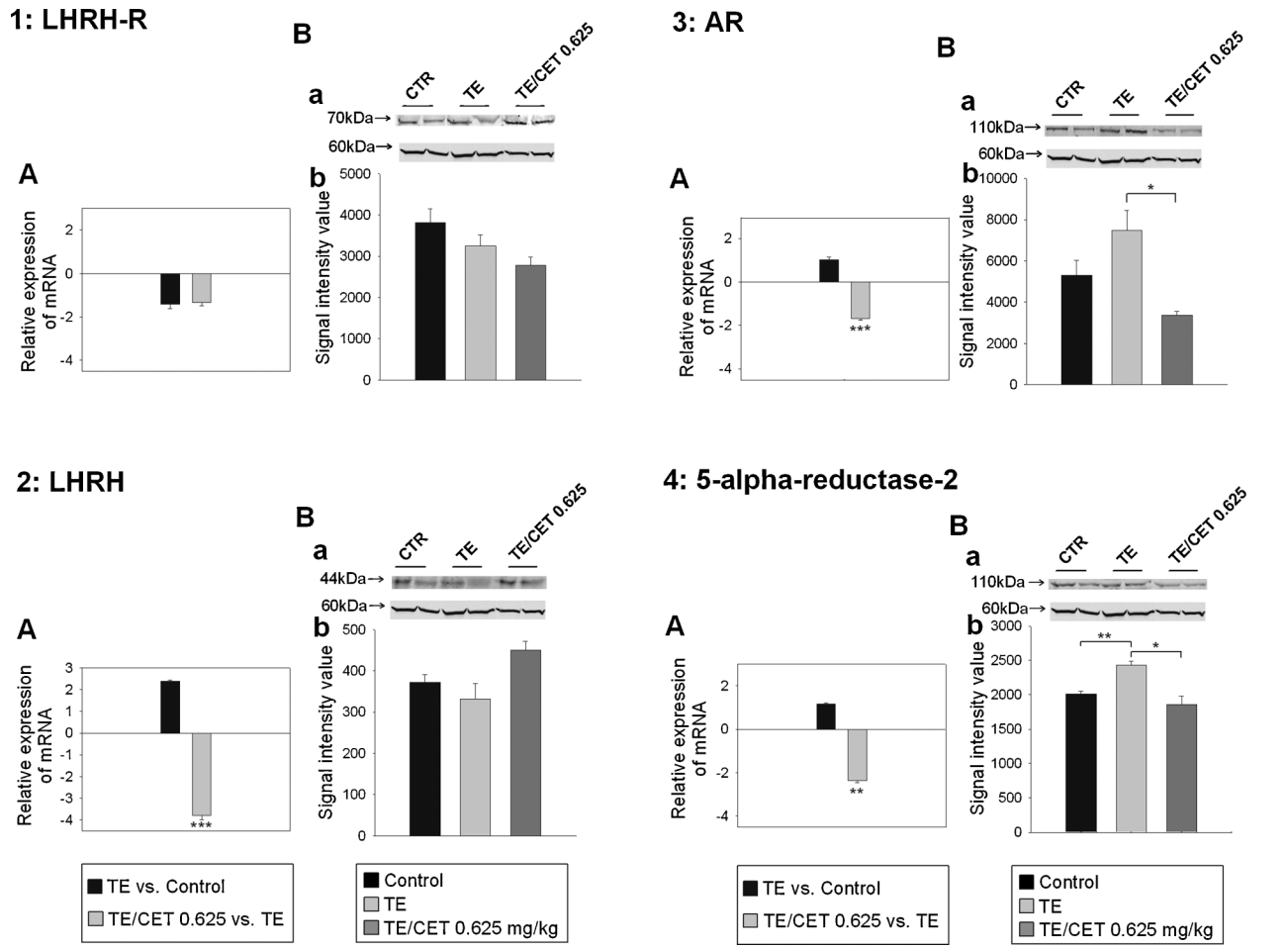

Fig. 6. **A**: Real-time PCR and **B**: Western blot analysis of LHRH-R (**1**), LHRH (**2**), AR (**3**) and 5-alpha-reductase 2 (**4**) in rat prostate after induction of BPH with TE and treatment with Cetrorelix at low doses of 0.625 mg/kg. **A**: Real-time PCR analysis of individual genes. Bars represent fold differences of individual gene expression between prostate samples from TE and control groups or Cetrorelix 0.625 mg/kg and TE groups. Positive values (>1.00) mark upregulation of individual genes, while negative values (<1.00) mark downregulation. Data are shown as mean ± SE. Asterisks indicate a significant difference (*$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ by Student's *t*-test). **B**: Western blot analysis of individual proteins showing immunoblot images (**a**) and corresponding signal intensity values (**b**). Representative blots are presented and include 60 kDa internal standard α-tubulin. Molecular masses are shown. Data are presented as mean of scaled signal intensity values ± SE. Asterisks indicate a significant difference (*$P < 0.05$ and **$P < 0.01$ by Student's *t*-test).

with dissociation constant (K_d of 0.82 ± 0.12 nM) and a mean B_{max} (maximal receptor binding capacity) value of 143.0 ± 3.81 fmol/mg membrane protein. Non-significant changes were found in K_d or B_{max} values in TE-induced BPH. B_{max} for LHRH was significantly ($P < 0.01$) reduced to 68.9 ± 8.28 fmol/mg membrane protein after Cetrorelix (12.5 mg/kg), compared with control; but K_d remained unchanged.

Effect of Cetrorelix on Serum DHT, LH, IGF-I, and PSA

In TE-induced BPH there was a 10-fold increase in the serum DHT as compared to control at day 42 (328.52 ± 44.51 pg/ml [$P < 0.001$]), while serum DHT was lowered after all doses of Cetrorelix. A significant reduction in DHT was observed with 1.25 and 12.5 mg/kg doses of Cetrorelix only (146.97 ± 28.24 and 134.06 ± 21.73 pg/ml, respectively [$P < 0.05$]), but not with 0.625 mg/kg. TE markedly decreased serum LH as compared to control (0.22 ± 0.02 ng/ml [$P < 0.001$]). Cetrorelix caused an additional decrease in serum LH, however, significant changes were only seen with the highest dose (0.15 ± 0.01 ng/ml [$P < 0.05$]). Serum PSA and IGF-1 changes were not significant (Table IV).

DISCUSSION

Clinical data have demonstrated that therapy with LHRH antagonist Cetrorelix resulted in long-lasting improvement in LUTS [22,23]. This improvement,

TABLE IV. Effect of Treatment With Cetrorelix on Serum Levels of DHT, LH, PSA, and IGF-1

	DHT (pg/ml)		LH (ng/ml)		PSA (ng/ml)		IGF-1 (ng/ml)	
	Day −28	Day 42	Day −28	Day 42	Day −28	Day 42	Day −28	Day 42
Control	39.75 ± 5.61	36.11 ± 7.84	0.36 ± 0.02	0.35 ± 0.02	0.62 ± 0.21	0.55 ± 0.35	3.35 ± 0.53	4.40 ± 0.22
TE	39.89 ± 2.03	328.52 ± 44.51†	0.35 ± 0.01	0.22 ± 0.02†	0.53 ± 0.30	0.50 ± 0.23	4.21 ± 0.25	4.84 ± 0.77
TE/Cetrorelix 0.625 mg/kg	35.74 ± 6.40	227.37 ± 41.63	0.34 ± 0.03	0.18 ± 0.01	0.48 ± 0.18	0.34 ± 0.06	3.67 ± 0.27	5.05 ± 0.49
TE/Cetrorelix 1.25 mg/kg	40.44 ± 7.52	146.97 ± 28.24*	0.36 ± 0.02	0.16 ± 0.01	0.46 ± 0.25	0.30 ± 0.14	5.58 ± 0.25	3.75 ± 0.33
TE/Cetrorelix 12.5 mg/kg	38.62 ± 4.72	134.06 ± 21.73*	0.32 ± 0.05	0.15 ± 0.01*	0.51 ± 0.11	0.29 ± 0.05	4.93 ± 1.13	4.06 ± 0.50

Mean ± SE.
†$P < 0.001$ as compared to control; *$P < 0.05$ as compared to TE. The data were evaluated by two-tailed Student's *t*-test.

including reduction in prostate volume and increase in urinary peak flow rate, appears to be superior to that produced by α-blockers or 5α-reductase inhibitors [24]. Low doses of Cetrorelix used in recent clinical trials cause only a partial suppression of pituitary-gonadal axis and testosterone levels.

In the present study, we have shown that Cetrorelix significantly reduced prostate weights by 18% in non-castrating doses of 0.625 mg/kg [34]. Histological observations in our rat model of BPH revealed marked hyperplastic morphological changes in the prostates of testosterone-treated BPH animals, while treatment with a low dose of Cetrorelix (0.625 mg/kg) caused an involution of these hyperplastic changes resulting in a morphology similar to that of normal animals. Although inflammation is not a main characteristic of the BPH model used in this study, we also observed incidental inflammatory infiltrates in all experimental groups.

The presence of LHRH and LHRH-R in rat prostate was demonstrated by real-time PCR and Western blot. Furthermore, ligand competition assay detected specific high affinity receptors for LHRH in rat prostate. Cetrorelix 0.625 mg/kg significantly lowered prostatic AR and 5α-reductase 2 levels, however, serum DHT and LH were only slightly decreased. Changes in serum PSA were not significant after treatment with Cetrorelix at 0.625 mg/kg. Moreover, the expression of LHRH and LHRH-R and direct antiproliferative effects of LHRH and its analogs have been demonstrated in many malignant human tumors [21,40]. Recently we showed that Cetrorelix inhibits the proliferation of human prostate epithelial BPH-1 cell line in vitro [41]. These findings suggest that low doses of Cetrorelix did not impair gonadal function in rats as was also shown by experimental [34] and clinical findings [22–24]. Prostate shrinkage is a result of direct inhibitory effects of Cetrorelix exerted through prostatic LHRH receptors, implies the presence of an LHRH-based autocrine regulatory system.

Herein, we used real-time PCR arrays to investigate the beneficial molecular mechanisms of Cetrorelix in a BPH-model. The analyses showed several proinflammatory pathways and growth factors were upregulated in control animals with induced BPH and markedly downregulated in Cetrorelix-treated animals. Verifying PCR array results and more precisely determining changes in gene expression, we studied the expression of selected proinflammatory and growth factor encoding genes by real-time RT-PCR. Of the 11 genes analyzed, 9 (IGF-1, EGF, TGF-β1 and-β2, FGF-2, FGF-7, VEGF-A, IL-1β, and IL-6) showed the same change pattern as in the PCR arrays, that is, upregulation after TE treatment and downregulation after Cetrorelix. These insulin-like, transforming and fibroblast growth factors and downstream effector molecules as well as a variety of interleukins, can lead to abnormal stromal and epithelial prostate cell growth [13,15,17].

Our observation of the transcriptional activation of inflammatory chemokine ligands (e.g., C5, CCL25, and CXCL2) and interleukins (e.g., IL-3, IL-5, IL-6, IL-13, IL-15, and IL-17) in the prostate of rats with induced BPH is consistent with clinical findings [42] and with experimental findings in rats [43]. Chemokines promote neutrophil recruitment and T-lymphocyte infiltration and the subsequent progression of the inflammation associated with BPH. We found that Cetrorelix significantly lowered transcriptional expression of several chemokines including C5 and CCL25.

Low doses of Cetrorelix caused a marked reduction in cytokine mRNA levels for IFN-γ, IL-1α, IL-3, IL-4, IL-5, IL-6, IL-13, IL-15, and IL-17. These cytokines are part of an inflammatory network in BPH including several growth factors [4,14,44]. IFN-γ, produced by infiltrating T-cells, is a natural antagonist of growth inhibiting TGF-β; FGF-2 stimulates growth [13]. IFN-γ also

stimulates IL-15, thereby augmenting increased influx of T-lymphocytes [18]. These T-cells further produce lymphokines such as IL-4 and IL-13, facilitating formation of active androgens and estrogens by inducing 3β-hydroxydehydrogenase/isomerase. T-cell derived IL-17 fine tunes immune response, stimulating IL-6, IL-8, and IL-1α and β [14,17–19].

In summary, our wide-range analysis of gene expression in the prostate of rats with testosterone-induced BPH revealed the transcriptional activation of several genes including those for proinflammatory interleukins, chemokines and prostatic growth factors. The expression of these genes was suppressed in Cetrorelix-treated animals. These findings suggest that Cetrorelix exerts its beneficial effects on BPH by suppressing proinflammatory cytokines and growth factors at the transcriptional level.

CONCLUSIONS

The results of this study indicate that the reduction in prostate volume could be due to direct inhibitory effects of Cetrorelix exerted through prostatic LHRH receptors as well as transcriptional suppression of proinflammatory cytokines and growth factors. These findings shed light on the mechanism of action of LHRH antagonists in BPH and also suggest a role for LHRH as a locally acting growth factor in BPH. It is possible that LHRH antagonists could be clinically used for therapy of BPH in combination with other agents.

ACKNOWLEDGMENTS

This study was supported by the Medical Research Service of the Veterans Affairs Department, Departments of Pathology, and Medicine, Division of Hematology/Oncology of the Miller Medical School, University of Miami and AEterna/Zentaris GmbH through South Florida Veterans Affairs Foundation for Research and Education (all to A.V.S.) and the L. Austin Weeks Endowment for Urologic Research (N.L.B.). We thank Professor J. Engel (AEterna/Zentaris GmbH) for his useful suggestions and encouragement.

REFERENCES

1. Roehrborn CG, Siami P, Barkin J, Damiao R, Becher E, Minana B, Mirone V, Castro R, Wilson T, Montorsi F, CombAT Study Group. The influence of baseline parameters on changes ininternational prostatesymptomscore with dutasteride, tamsulosin, and combination therapy among men with symptomatic benign prostatic hyperplasia and an enlarged prostate: 2-year data from the CombAT study. Eur Urol 2009; 55:461–471.
2. Parsons JK, Kashefi C. Physical activity, benign prostatic hyperplasia, and lower urinary tract symptoms. Eur Urol 2008; 53:1228–1235.
3. Briganti A, Capitanio U, Suardi N, Gallina A, Salonia A, Bianchi M, Tutolo M, Di Girolamo V, Guazzoni G, Rigatti P, Montorsi F. Benign prostatic hyperplasia and its aetiologies. Eur Urol Suppl 2009;8:865–871.
4. Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? Eur Urol 2007;51:1202–1216.
5. Di Silverio F, Gentile V, De Matteis A, Mariotti G, Giuseppe V, Luigi PA, Sciarra A. Distribution of inflammation, pre-malignant lesions, incidental carcinoma in histologically confirmed benign prostatic hyperplasia: A retrospective analysis. Eur Urol 2003;43: 164–175.
6. Nickel JC, Roehrborn CG, O'leary MP, Bostwick DG, Somerville MC, Rittmaster RS. Examination of the relationship between symptoms of prostatitis and histological inflammation: Baseline data from the REDUCE chemoprevention trial. J Urol 2007; 178:896–900, discussion 900–901.
7. St Sauver JL, Jacobson DJ, Girman CJ, Lieber MM, McGree ME, Jacobsen SJ. Tracking of longitudinal changes in measures of benign prostatic hyperplasia in a population based cohort. J Urol 2006;175(3 Pt 1):1018–1022, discussion 1022.
8. Kessler OJ, Keisari Y, Servadio C, Abramovici A. Role of chronic inflammation in the promotion of prostatic hyperplasia in rats. J Urol 1998;159:1049–1053.
9. Lee KL, Peehl Donna M. Molecular and cellular pathogenesis of benign prostatic hyperplasia. J Urol 2004;172:1784–1791.
10. Untergasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: Age-related tissue-remodeling. Exp Gerontol 2005; 40:121–128.
11. Kohnen PW, Drach GW. Patterns of inflammation in prostatic hyperplasia: A histologic and bacteriologic study. J Urol 1979; 121(6):755–760.
12. Theyer G, Kramer G, Assmann I, Sherwood E, Preinfalk W, Marberger M, Zechner O, Steiner GE. Phenotypic characterization of infiltrating leukocytes in benign prostatic hyperplasia. Lab Invest 1992;66:96–107.
13. Kramer G, Steiner GE, Handisurya A, Stix U, Haitel A, Knerer B, Gessl A, Lee C, Marberger M. Increased expression of lymphocyte-derived cytokines in benign hyperplastic prostate tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation. Prostate 2002;52:43–58.
14. Djavan B, Eckersberger E, Espinosa G, Kramer G, Handisurya A, Lee C, Marberger M, Lepor H, Steiner GE. Complex mechanisms in prostatic inflammatory response. Eur Urol Suppl 2009;8:872–878.
15. Lucia MS, Lambert JR. Growth factors in benign prostatic hyperplasia: Basic science implications. Curr Urol Rep 2008;9: 272–278.
16. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC, Rittmaster RS. The relationship between prostate inflammation and lower urinary tract symptoms: Examination of baseline data from the REDUCE trial. Eur Urol 2008;54:1379–1384.
17. Steiner GE, Newman ME, Paikl D, Stix U, Memaran-Dagda N, Lee C, Marberger MJ. Expression and function of pro-inflammatory interleukin IL-17 and IL-17 receptor in normal, benign hyperplastic, and malignant prostate. Prostate 2003;56:171–182.

18. Handisurya A, Steiner GE, Stix U, Ecker RC, Pfaffeneder-Mantai S, Langer D, Kramer G, Memaran-Dadgar N, Marberger M. Differential expression of interleukin-15, a pro-inflammatory cytokine and T-cell growth factor, and its receptor in human prostate. Prostate 2001;49:251–262.
19. Steiner GE, Stix U, Handisurya A, Willheim M, Haitel A, Reithmayr F, Paikl D, Ecker RC, Hrachowitz K, Kramer G, Lee C, Marberger M. Cytokine expression pattern in benign prostatic hyperplasia infiltrating T cells and impact of lymphocytic infiltration on cytokine mRNA profile in prostatic tissue. Lab Invest 2003;83:1131–1146.
20. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Hittmair A, Zhang J, Thurnher M, Bartsch G, Klocker H. Regulation of prostatic growth and function by peptide growth factors. Prostate 1996;28:392–405.
21. Schally AV. New approaches to the therapy of various tumors based on peptide analogues. Horm Metab Res 2008;40:315–322.
22. Gonzalez-Barcena D, Vadillo-Buenfil M, Gomez-Orta F, Fuentes Garcia M, Cardenas-Cornejo I, Graef-Sanchez A, Comaru-Schally AM, Schally AV. Responses to the antagonistic analog of LH-RH(SB-75, Cetrorelix) in patients with benign prostatic hyperplasia and prostatic cancer. Prostate 1994;24:84–92.
23. Comaru-Schally AM, Brannan W, Schally AV, Colcolough M, Monga M. Efficacy and safety of luteinizing hormone-releasing hormone antagonist cetrorelix in the treatment of symptomatic benign prostatic hyperplasia. J Clin Endocrinol Metab 1998;83: 3826–3831.
24. Debruyne F, Gres AA, Arustamov DL. Placebo-controlled dose-ranging phase 2 study of subcutaneously administered LHRH antagonist cetrorelix in patients with symptomatic benign prostatic hyperplasia. Eur Urol 2008;54:170–177.
25. McConnell JD. Benign prostatic hyperplasia. Hormonal treatment. Urol Clin North Am 1995;22:387–400.
26. Wemyss-Holden SA, Hamdy FC, Hastie KJ. Streoid abuse in athletes, prostatic enlargement and bladder outflow obstruction-Is there a relationship? Br J Urol 1994;74:476–478.
27. Maggi CA, Manzini S, Giuliani S, Meli A. Infravesical outflow obstruction in rats: A comparison of two models. Gen Pharmacol 1989;20(3):345–349.
28. Constantinou CE. Influence of hormone treatment on prostate growth and micturition characteristics of the rat. Prostate 1996; 29:30–35.
29. Liu HP, Chen GL, Liu P, Xu XP. Amlodipine alone or combined with terazosin improves lower urinary tract disorder in rat models of benign prostatic hyperplasia or detrusor instability: Focus on detrusor overactivity. BJU Int 2009;104:1752–1757.
30. Pandita RK, Persson K, Hedlund P, Andersson KE. Testosterone-induced prostatic growth in the rat causes bladder overactivity unrelated to detrusor hypertrophy. Prostate 1998;35:102–108.
31. Scolnik MD, Servadio C, Abramovici A. Comparative study of experimentally induced benign and atypical hyperplasia in the ventral prostate of different rat strains. J Androl 1994;15:287–297.
32. Kojima Y, Sasaki S, Oda N, Koshimizu TA, Hayashi Y, Kiniwa M, Tsujimoto G, Kohri K. Prostate growth inhibition by subtype-selective alpha(1)-adrenoceptor antagonist naftopidil in benign prostatic hyperplasia. Prostate 2009;69:1521–1528.
33. Bajusz S, Csernus VJ, Janaky T, Bokser L, Fekete M, Schally AV. New antagonists of LHRH. II. Inhibition and potentiation of LHRH by closely related analogues. Int J Pept Protein Res 1988;32:425–435.
34. Horvath JE, Toller GL, Schally AV, Bajo AM, Groot K. Effect of long-term treatment with low doses of the LHRH antagonist Cetrorelix on pituitary receptors for LHRH and gonadal axis in male and female rats. Proc Natl Acad Sci USA 2004;101:4996–5001.
35. Nemeth JA, Lee C. Prostatic ductal system in rats: Regional variation in stromal organization. Prostate 1996;28:124–128.
36. Heinrich E, Schally AV, Buchholz S, Rick FG, Halmos G, Mile M, Groot K, Hohla F, Zarandi M, Varga JL. Dose-dependent growth inhibition in vivo of PC-3 prostate cancer with a reduction in tumoral growth factors after therapy with GHRH antagonist MZ-J-7-138. Prostate 2008;68:1763–1772.
37. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45.
38. Bellyei S, Schally AV, Zarandi M, Varga JL, Vidaurre I, Pozsgai E. GHRH antagonists reduce the invasive and metastatic potential of human cancer cell lines in vitro. Cancer Lett 2010;293:31–40.
39. Halmos G, Arencibia JM, Schally AV, Davis R, Bostwick DG. High incidence of receptors for luteinizing hormone-releasing hormone (LHRH) and LHRH receptor gene expression in human prostate cancer. J Urol 2000;163:623–629.
40. Emons G, Gründker C, Günthert AR, Westphalen S, Kavanagh J, Verschraegen C. GnRH antagonists in the treatment of gynecological and breast cancers. Endocr Relat Cancer 2003;10:291–299.
41. Siejka A, Schally AV, Block NL, Barabutis N. Mechanisms of inhibition of human benign prostatic hyperplasia in vitro by LHRH antagonist Cetrorelix. BJU Int 2010; DOI:10.1111/j.1464-410X.2010.09215.x.
42. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. BJU Int 1999;84:976–981.
43. Tagaya M, Oka M, Ueda M, Takagaki K, Tanaka M, Ohgi T, Yano J. Eviprostat suppresses proinflammatory gene expression in the prostate of rats with partial bladder-outlet obstruction: A genome-wide DNA microarray analysis. Cytokine 2009;47: 185–193.
44. Konig JE, Senge T, Allhoff EP, Konig W. Analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. Prostate 2004;58:121–129.
45. Giri D, Ittmann M. Interleukin-1alpha is a paracrine inducer of FGF7, a key epithelial growth factor in benign prostatic hyperplasia. Am J Pathol 2000;157:249–255.
46. Martinez-Moczygemba M, Huston DP. Biology of common beta receptor-signaling cytokines: IL-3, IL-5, and GM-CSF. J Allergy Clin Immunol 2003;112:653–665.
47. Förster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell 1996;87:1037–1047.