The Prostate 71:445 – 452 (2011)

Receptors for Luteinizing Hormone-Releasing Hormone (LHRH) in Benign Prostatic Hyperplasia (BPH) as Potential Molecular Targets for Therapy With LHRH Antagonist Cetrorelix

Bernadett Rozsa,[1] Mehrdad Nadji,[2] Andrew V. Schally,[2,3,4] Balazs Dezso,[5] Tibor Flasko,[6] Gyorgy Toth,[6] Melinda Mile,[1] Norman L. Block,[2,3] and Gabor Halmos[1,2,4]*

[1]*Department of Biopharmacy, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary*
[2]*Department of Pathology, Miller School of Medicine, University of Miami, Miami, Florida*
[3]*Department of Medicine, Miller School of Medicine, University of Miami, Miami, Florida*
[4]*Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, Miami, FL*
[5]*Department of Pathology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary*
[6]*Department of Urology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary*

BACKGROUND. The majority of men will develop symptoms of benign prostatic hyperplasia (BPH) after 70 years of age. Various studies indicate that antagonists of LHRH, such as cetrorelix, exert direct inhibitory effects on BPH mediated by specific LHRH receptors. Our aim was to investigate the mRNA for LHRH and LHRH receptors and the expression of LHRH receptors in specimens of human BPH.
METHODS. The expression of mRNA for LHRH ($n = 35$) and LHRH receptors ($n = 55$) was investigated by RT-PCR in surgical specimens of BPH, using specific primers. The characteristics of binding sites for LHRH on 20 samples were determined by ligand competition assays. The LHRH receptor expression was also examined in 64 BPH specimens by immunohistochemistry.
RESULTS. PCR products for LHRH were found in 18 of 35 (51%) BPH tissues and mRNA for LHRH receptors was detected in 39 of 55 (71%) BPH specimens. Eighteen of 20 (90%) samples showed a single class of high affinity binding sites for [D-Trp^6]LHRH with a mean K_d of 4.04 nM and a mean B_{max} of 527.6 fmol/mg membrane protein. LHRH antagonist cetrorelix showed high affinity binding to LHRH receptors in BPH. Positive immunohistochemical reaction for LHRH receptors was present in 42 of 64 (67%) BPH specimens.
CONCLUSION. A high incidence of LHRH receptors in BPH supports the use of LHRH antagonists such as cetrorelix, for treatment of patients with lower urinary tract symptoms from BPH. *Prostate 71: 445–452, 2011.* © 2010 Wiley-Liss, Inc.

KEY WORDS: **human benign prostatic hyperplasia; luteinizing hormone-releasing hormone (LHRH) receptors; LHRH antagonist**

Disclosure Summary: Dr.A.V.Schally receives a grant from Aeterna-Zentaris through VA Foundation. All other authors have nothing to disclose.

Grant sponsor: Hungarian Ministry of Health Research Grants ETT 240/2006; Grant sponsor: Hungarian Scientific Research Fund (OTKA) K 81596; Grant sponsor: Medical Research Service of the Veterans Affairs Department; AETERNA ZENTARIS; Grant sponsor: South Florida VA Foundation for Research and Education; Grant sponsor: University of Miami; Grant sponsor: European Social Fund; Grant sponsor: European Regional Development Fund.

*Correspondence to: Gabor Halmos, Department of Biopharmacy, Medical and Health Science Center, University of Debrecen, Nagyerdei krt. 98, 4032 Debrecen, Hungary.
E-mail: halmos@king.pharmacol.dote.hu
Received 10 May 2010; Accepted 3 August 2010
DOI 10.1002/pros.21258
Published online 21 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

© 2010 Wiley-Liss, Inc.

INTRODUCTION

Benign prostatic hyperplasia (BPH), the most common age-related disease of the male, occurs clinically in a majority of all men over 70 years of age [1]. Improvement in the quality of life of patients suffering from BPH is an important issue in the medical management of this condition. Clinical BPH refers to lower urinary tract symptoms (LUTS), associated with benign prostatic enlargement and leading to bladder outlet obstruction. The pathophysiology, epidemiology, etiology, and natural history of BPH and the mechanism of progression from histologic to clinical BPH are incompletely understood [2,3]. Aging and chronic exposure to dihydrotestosterone (DHT) are required for the development of BPH [2,3]. However, the actions of androgen alone do not explain the hyperplastic process or the progression of the disease [4,5]. Other hormones and a variety of growth factors including epidermal growth factor (EGF), transforming growth factor-α (TGF-α) [6], members of the fibroblast growth factor (FGF) gene family [7], and other peptide growth factors have also been implicated in the pathogenesis and development of BPH [8]. Thus, the overall disease process that leads to the production of symptomatic BPH is very complex. Today, medical therapy is the preferred first-line approach to treating BPH because of the probability of clinical improvement and the patients' concern about surgery or other invasive treatments. Alpha1 adrenergic receptor blockers and 5-α reductase inhibitors are the two classes of drugs currently approved by the FDA for the treatment of symptomatic BPH, but these drugs do not offer long-term remission of LUTS after discontinuation and can be used only in a selected population of patients [9]. Other hormonal treatments, such as anti-androgens or LHRH agonists are either poor or associated with unacceptable side effects of medical castration such as hot flushes, decrease of potency and libido, and the risk of a deleterious effect on bone density [10]. In contrast, the use of LHRH antagonists can provide a dose-dependent submaximal blockade of testosterone and DHT avoiding the effects of medical castration [10–12]. Cetrorelix is one of the potent modern LHRH antagonists that induce an immediate inhibition of the pituitary–gonadal axis [13–15] and is the only LHRH antagonist that has been extensively studied in men with BPH [10–12,16,17]. Accumulating clinical experience with cetrorelix in men with BPH has shown that this drug is associated with a dose-dependent improvement of International Prostate Symptom Score (IPSS) and a reduction in prostate volume [10–12,16,17]. Recent studies show that the action of cetrorelix on BPH is likely mediated through LHRH receptors and involves inhibitory effects on growth factors as well as down-regulation of α1A adrenergic receptors and LHRH receptors [18]. Human prostate and prostate cancer specimens have been shown to express LHRH receptors [11,19]. These observations indicated the need for a more thorough investigation of LHRH receptors in surgical specimens of human BPH. To address this issue, in the present study we evaluated the expression of mRNA for LHRH receptors and the localization and characteristics of the LHRH receptor protein in 55 BPH specimens obtained from surgery or transurethral resection (TUR). We also examined 64 BPH tissues by immunohistochemistry. In addition, we investigated the expression of mRNA for LHRH as well as its incidence.

MATERIALS AND METHODS

Peptides and Chemicals

LHRH antagonist [D-Trp^6]LHRH (REF) was supplied by Debiopharm S.A. (Lausanne, Switzerland). LHRH antagonist, cetrorelix, first synthesized in our laboratory [13], was made by Aeterna Zentaris GmbH (Frankfurt am Main, Germany). Radioisotope ^{125}I-labeled sodium was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). All other chemicals were obtained from Sigma (St. Louis, MO), Bachem (Torrance, CA), R&D Systems (Minneapolis, MN), or California Peptide Research, Inc. (Napa, CA).

Tissue Samples From Patients

Human BPH specimens were obtained from patients 50–83 years of age at the time of initial open surgical treatment or TUR at Veterans Affairs Medical Center, New Orleans, LA; Department of Urology, University of Debrecen, Debrecen, Hungary and Department of Urology, University of Miami, Miami, FL. The local Institutional Review Boards approved the collection and use of these specimens for the current study. All analyses were first conducted to meet the primary clinical requirement for patient management, and only residual tissue was used for this study. After surgical removal, select portions of the prostate tissues were flash-frozen in liquid nitrogen and transported on dry ice. Histopathological examination of each specimen was undertaken to confirm the presence of BPH before the receptor studies. The intact specimens and their membrane fractions were stored at −80°C until analyses of LHRH binding sites and molecular biology studies were carried out.

The clinicopathological characteristics of the group of patients used for receptor binding studies are shown in Table I.

RNA Isolation

Tissue samples were homogenized and RNA was extracted with Nucleospin Total RNA and Protein

TABLE I. Clinicopathological Data, Expression of mRNA and Binding Characteristics of Receptors for LHRH (LHRH-R) in 20 Human Benign Prostatic Hyperplasia Specimens

Patient no.	Patient age (year)	PSA-value (ng/ml)	β-actin mRNA	LHRH-R mRNA	B_{max} (fmol/mg protein)	K_d (nM)
1	53	3.53	+	+	425.3	1.28
2	62	2.57	+	+	344.7	2.71
3	79	0.98	+	+	495.1	6.03
4	65	3.41	+	+	352.9	3.95
5	81	6.42	+	+	721.8	5.02
6	64	1.67	+	+	581.6	4.09
7	72	8.15	+	—	—	—
8	55	2.53	+	+	900.2	3.61
9	69	1.86	+	+	733.4	7.26
10	71	2.32	+	+	482.9	1.63
11	58	0.35	+	+	640.7	6.26
12	75	0.83	+	+	474.8	2.17
13	57	4.77	+	+	600.5	4.00
14	74	2.26	+	+	398.3	1.37
15	69	4.06	+	+	415.2	4.77
16	52	0.75	+	+	364.6	1.09
17	73	3.86	+	+	483.7	6.20
18	66	0.49	+	–	–	–
19	80	6.17	+	+	623.9	6.26
20	76	3.78	+	+	458.0	5.05

Isolation Kit (Macherey-Nagel, Germany). RNA concentration and purity were determined using the Nanodrop ND-1000 UV Spectrophotometer (Nanodrop Technologies, Wilmington, DE).

RT-PCR

cDNA was reverse transcribed from 150–250 ng total RNA using Oligo $(dT)_{15}$ primer (Promega Corporation, Madison,WI) with MMLV Reverse Transcriptase (Promega. Co.,) according to the manufacturer's instructions. For amplification from first-strand cDNAs, gene-specific primers for LHRH-R: sense 5′-GAC CTT GTC TGG AAA GAT CC-3′, antisense 5′-CAG GCT GAT CAC CAC CAT CA-3′, for LHRH: sence 5′-CTACTGACTTGGTGCGTGGA-3′ and antisence: 5′-CTGCCCAGTTTCCTCTTCAA-3′ and for β-actin housekeeping gene: sense 5′-GGC ATC CTC ACC CTG AAG TA-3′, antisense 5′-GGG GTG TTG AAG GTC TCA AA-3′ were used as described earlier [19]. After reverse transcription (RT), 2 µl of the reaction products were subjected to PCR amplification. The reaction mix contained 2 µl cDNA, 1.5 mM $MgCl_2$, 0.5 µM of each primer (Invitrogen), 1× PCR buffer, 200 µM of each dNTP, and 1 U Taq Polymerase (Invitrogen) in a final volume of 25 µl. After denaturation (3 min at 94°C) cDNA was amplified for 45 cycles (45 sec at 94°C; 30 sec at 60°C; and 1 min 30 sec at 72°C). β-actin was amplified with 30 cycles. A final elongation step at 72°C for 10 min was then applied. PCR products were separated electrophoretically on 1.5% agarose gel and stained with ethidium bromide. To avoid genomic deoxyribonucleic acid (DNA) contamination, all samples were subject to DNase treatment before RT-PCR.

Preparation of Membranes and Radioligand Binding Studies

Preparation of membranes for receptor studies was performed as described previously [19]. Briefly, the samples were thawed and cleaned, and then homogenized in 50 mM Tris–HCl buffer (pH 7.4), supplemented with protease inhibitors (0.25 mM phenylmethylsulfonyl fluoride, 0.4% (v/v) aprotinin, and 2 µg/ml pepstatin A) using an Ultra-Turrax tissue homogenizer (IKA Works, Wilmington, NC) on ice. The homogenate was centrifuged at 500*g* for 10 min at 4°C to remove nuclear debris and lipid layer. The supernatant containing the crude membrane fraction was ultracentrifuged (Beckman L8-80 M) twice at 70,000*g* for 50 min at 4°C after resuspending in fresh buffer. The final pellet was resuspended in homogenization buffer and stored at −80°C until assayed. Protein concentration was determined by the method of Bradford using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Radioiodinated derivatives of [D-Trp^6]LHRH were prepared by the chloramine-T method and purified by

reverse-phase HPLC in our laboratory [19]. LHRH receptor binding assays were carried out as reported [19] using in vitro ligand competition assays based on binding of $[^{125}I][D\text{-}Trp^6]$LHRH as radioligand to BPH membrane fractions. This radioligand has been well-characterized previously and shows high-affinity binding to human and rat pituitaries and human breast, prostate, and other cancers [15,19]. In brief, membrane homogenates containing 50–160 µg protein were incubated in duplicate or triplicate with 60–80,000 cpm $[^{125}I][D\text{-}Trp^6]$LHRH and increasing concentrations (10^{-12}–10^{-6} M) of non-radioactive peptides as competitors in a total volume of 150 µl of binding buffer. At the end of the incubation, 125 µl aliquots of suspension were transferred onto the top of 1 ml of ice-cold binding buffer containing 1.5% bovine serum albumin in siliconized polypropylene microcentrifuge tubes (Sigma). The tubes were centrifuged at 12,000*g* for 3 min at 4°C (Beckman J2-21M). Supernatants were aspirated and the bottoms of the tubes containing the pellet were cut-off and counted in a gamma counter (Micromedic System, Huntsville, AL). Preliminary experiments were performed with membrane protein concentrations ranging from 20–250 µg/tube in order to determine the minimal amount of protein required to assess specific binding at a satisfactory level. Our work showed that accurate results can be obtained over a range of 40–180 µg membrane protein in an incubation volume of 150 µl.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues from prostatectomy or TUR specimens were used for immunohistochemistry (n = 64). Three micron paraffin sections were stained by hematoxylin and eosin to confirm the presence of BPH. Adjacent serial sections were utilized for immunoperoxidase procedure following standard protocols. Briefly, the paraffin was melted at 37°C overnight and cleared in a bath of xylene for 10 min. The slides were then rehydrated in decreasing grades of ethanol (10 min each) and washed in phosphate-buffered saline solution. The antigen retrieval was performed in a pressure cooker with Dako Target Retrieval Solution (S1968; Dako, Carpinteria, CA) at 90°C for 15 min (pH 9.0). Antibodies to LHRH receptor (NCL-GnRHR A9E4; Novocastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom and GnRHR-N20, Santa Cruz Biotechnology, Santa Cruz, CA) were added to the slides and incubated for 30 min at room temperature. NCL-GnRHR is a monoclonal antibody raised in mouse and targets the human LHRH receptor extracellular region. The GnRHR N20 is an affinity-purified goat polyclonal antibody raised against a peptide, which maps near the N-terminus of human LHRHR.

Following the addition of the detection system, the reaction was visualized using diaminobenzidine (DAB) in the presence of hydrogen peroxide. The slides were counterstained with hematoxylin, mounted and examined using an Olympus DP50 microscope (Olympus, Hamburg, Germany). Human pituitary glands (anterior lobe) obtained from autopsy (n = 4) were used as positive controls.

Data Analysis

Specific ligand-binding capacities and affinities were calculated by the Ligand-PC computerized curve-fitting program of Munson and Rodbard [20]. To determine the type of receptor binding, equilibrium dissociation constants (K_d values), and the maximal binding capacity of receptors (B_{max}), LHRH binding data were also analyzed by the Scatchard method [21].

RESULTS

Molecular Biology Analysis

To investigate whether high-affinity binding sites for LHRH, present in the membranes of human BPH tissue, are the products of mRNA expression of LHRH receptors, we performed RT-PCR analysis on all samples collected for the study. Negative controls yielded no detectable signals, indicating that PCR products were generated from cDNA and not from genomic DNA. PCR amplification with β-actin-specific primers produced a single product from all samples, confirming that there was no degradation in the RNA preparation (Fig. 1A; Table II).

In 39 of 55 specimens examined (71%), RT of RNA followed by PCR amplification with specific primers produced a fragment of expected size (319 bp) for LHRH receptors (Fig. 1B; Table II).

To examine the mRNA expression of LHRH ligand, we also performed RT-PCR analyses on 35 samples of human BPH obtained from the University of Debrecen. In 18 of 35 specimens examined (51%), RT of RNA followed by PCR amplification with specific primers produced a fragment of expected size (230 bp) for LHRH (Fig. 1C; Table II).

Immunohistochemistry of LHRH Receptors in Benign Prostatic Hyperplasia

Altogether 64 human BPH specimens were obtained and examined by immunohistochemistry. A positive reaction in the form of brown granules was observed with both antibodies in the smooth muscle stroma and in the epithelial cells of BPH (Fig. 2). The immunoreaction with the NCL antibody, however, was more pronounced in the stromal smooth muscle cells (Fig. 2A), while the Santa Cruz antibody identified

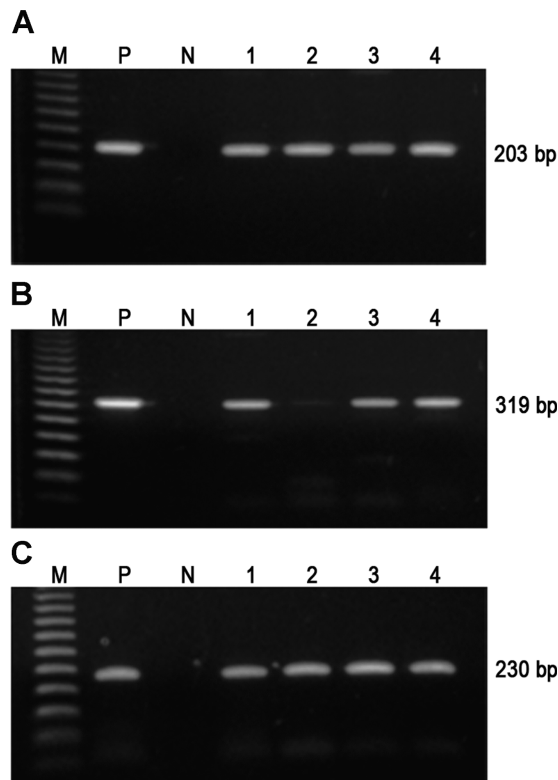

Fig. 1. Expression of mRNA for LHRH, receptors for LHRH and β-actin in human BPH specimens as revealed by RT-PCR. PCR products were separated electrophoretically on 1.5% agarose gels and stained with ethidium bromide. The integrity of RNA extracted was tested by PCR amplification of human β-actin cDNA from the same RT reaction. The PCR products were of the expected size of 203 bp for β-actin (panel A), 319 bp for LHRH receptors (panel B), and 230 bp for LHRH (panel C). Lane M, molecular marker (50-bp DNA ladder); Lane P, positive control (human pituitary tissue); Lane N, no template control; Lanes 1–4, representative human BPH specimens.

an epitope primarily in the epithelial cells (Fig. 2B). Among the immuno-positive BPH specimens, the majority stained strongly positive for LHRH receptors, whereas some specimens expressed the receptor at a moderate level. Overall, the positive reaction for LHRH receptor was present in 42 of 64 BPH specimens (67%) (Table II). The positive immuno-staining of LHRH receptors in BPH specimens was not related to the patients' age or other clinical and pathological findings.

Radioligand Binding Studies

The presence of specific LHRH binding sites and characteristics of binding of [^{125}I][D-Trp^6]LHRH to membrane receptors on human BPH tissue were determined using ligand competition assays. Of the 20 specimens examined, 18 showed LHRH receptor binding (90%; Tables I and II). Analyses of the typical displacement of radiolabeled [D-Trp^6]LHRH by the same unlabeled peptide revealed that the one-site model provided the best fit, indicating the presence of one class of high-affinity LHRH receptors in crude membranes derived from human BPH specimens. The computerized nonlinear curve-fitting and the Scatchard plot analyses of the binding data in 18 receptor-positive BPH specimens indicated that the single class of binding sites had a mean dissociation constant (K_d) of 4.04 nM (range, 1.09–7.26 nM) with a mean maximal binding capacity (B_{max}) of 527.6 fmol/mg of membrane protein (range, 344.7–900.2 fmol/mg protein). Biochemical parameters essential to establish the identity of specific binding sites were also determined. Thus, the binding of [^{125}I][D-Trp^6]LHRH was found to be reversible, time- and temperature-dependent, and linear with protein concentration in human BPH specimens examined (data not shown). The specificity of LHRH binding was demonstrated by competitive binding experiments using several peptides structurally related or unrelated to LHRH. The binding of radiolabeled [D-Trp^6]LHRH was completely displaced by increasing concentrations (10^{-12}–10^{-6} M) of LHRH agonist buserelin and LHRH antagonist cetrorelix (Fig. 3). Cetrorelix displaced radiolabeled [D-Trp^6]LHRH with an IC_{50} value of 0.94 ± 0.07 nM indicating its high-affinity binding to LHRH receptors expressed in BPH tissues (Fig. 3). None of the structurally and functionally unrelated peptides tested such as somatostatin-14, human growth hormone-releasing hormone, EGF, [Tyr^4]bombesin and insulin-like growth factor-I inhibited the binding of [^{125}I][D-Trp^6]LHRH at concentrations as high as 1 μM (Fig. 3).

TABLE II. Expression Pattern of LHRH and LHRH Receptors in Human BPH Specimens

	Positive/total no. of cases examined	% Positive	Age range of positive cases (year)
LHRH mRNA	18/35	51	54–79
LHRH receptor mRNA	39/55	71	51–82
β-actin	55/55	100	51–83
LHRH receptor binding	18/20	90	52–81
LHRH receptor immunostaining	42/64	67	50–83

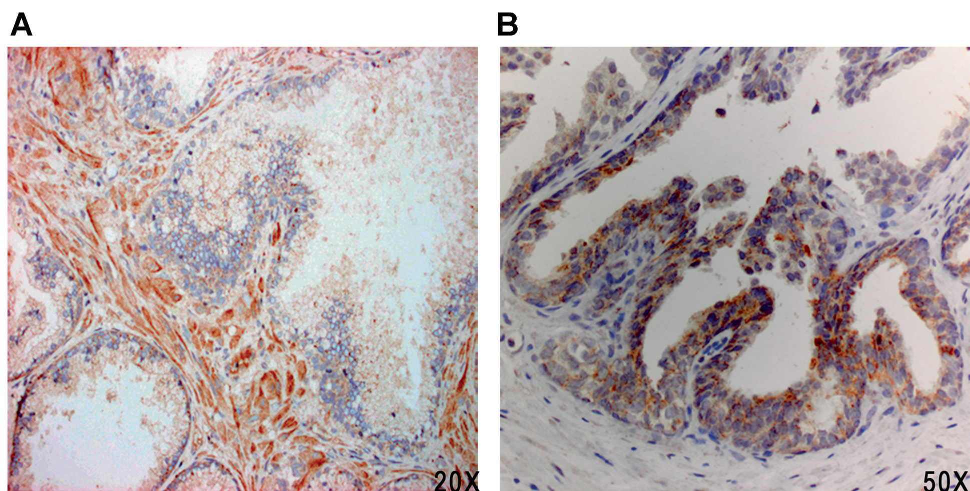

Fig. 2. Expression of receptors for LHRH in surgically removed human BPH specimens. The tissue was stained by immunohistochemistry with mouse LHRH receptor monoclonal antibody (clone A9E4; Novocastra) (panel A, magnification 20×) or with affinity-purified goat polyclonal antibody (N20; Santa Cruz; panel B, magnification 50×).

The expression of mRNA for LHRH receptors was accompanied by ligand binding in all samples examined (Table I). Two of 20 BPH specimens did not exhibit mRNA expression for LHRH receptors, or show ligand binding; conversely, all receptor-positive specimens expressed a detectable amount of the receptor gene (Table I). There was no correlation between receptor binding characteristics or mRNA expression and clinical and pathological findings.

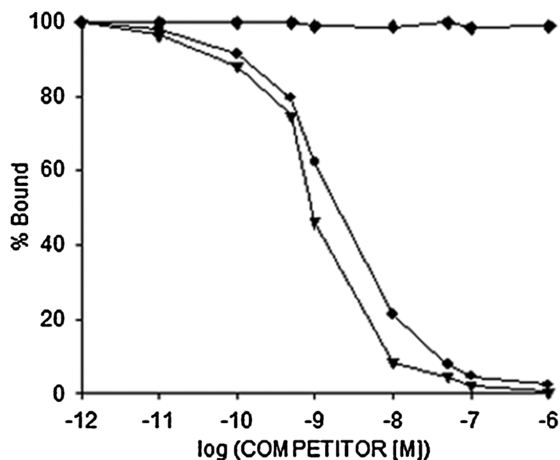

Fig. 3. Representative displacement of [^{125}I][D-Trp^6]LHRH binding to membrane fractions of human BPH specimens by increasing concentrations of Cetrorelix (▼) and Buserelin (●). Other unrelated peptides, like EGF (◆), somatostatin (◆), [Tyr^4]BN (◆), human growth hormone releasing hormone (◆), and IGF-I (◆) did not displace the radioligand. One hundred percent specific binding is defined as the difference between binding in the absence and in the presence of 10^{-5} M [D-Trp^6]LHRH. Each point represents mean of triplicate determinations.

DISCUSSION

BPH is a condition that will affect most men should they live long enough [1,4,5]. BPH is defined histologically by hyperproliferation of stromal and epithelial cells of the prostate, caused by complex cellular alterations including changes in proliferation, differentiation, and apoptosis [1,2]. However, the pathogenesis and the complex mechanism of evolution of BPH are still incompletely understood. Medical therapy is usually recommended first because of the probability of clinical improvement and the patients' concern about surgery or other invasive treatment. Inhibitors of 5α-reductase or α1-adrenergic receptor antagonists do not offer long-term remission of urinary symptoms after discontinuation and can be used only in a selected population of patients [9–11,14].

The clinical application of LHRH agonists in the treatment of human prostatic carcinoma is based on their ability to suppress testosterone secretion through the desensitization of the gonadotropes, down-regulation of LHRH receptors in the pituitary, and a reduction in the levels of their mRNA [11,15]. A repeated administration of LHRH agonists is required to continue the suppression of LH and FSH release and the reduction in the levels of sex steroids. This is clinically achieved by the periodic administration of depot preparations of LHRH agonists [11,15]. The initial testosterone ''flare'' may be prevented by pretreatment with estrogens or antiandrogens [9–11]. The side effects of LHRH agonists, including hot flashes, loss of libido, and erectile dysfunction, are acceptable when LHRH agonists are used for treatment of metastatic prostate cancer [11]. LHRH agonists were tested as a treatment for BPH, but the initial shrinkage

of the prostate, and improved urinary flow were reversed by 6 months after cessation of treatment [10,22,23]. Poor efficacy and unacceptable side effects also discouraged the use of LHRH agonists for treatment of BPH.

The advantage in the use of LHRH antagonists for the treatment of both prostate cancer and BPH is based on direct inhibition of LHRH receptors and their ability to thus lower LH, FSH, and sex steroid secretion immediately after a single injection, thereby reducing the time to the onset of therapeutic effect and avoiding a flare in disease [11,15]. A crucial therapeutic edge in the use of LHRH antagonists instead of agonists for the treatment of BPH is the capability to better control the level of testosterone suppression. In a clinical setting, low doses of cetrorelix or other LHRH antagonists, released from depot preparation induce only a partial and transient inhibition of the pituitary-gonadal axis without a marked down-regulation of pituitary LHRH receptors avoiding medical castration [24].

Cetrorelix, a highly active modern LHRH antagonist is the only LHRH antagonist that has been extensively evaluated in men with BPH. The principles of effective treatment of patients with BPH have been obtained from various uncontrolled and controlled clinical trials [10,16–17,25] using daily injections of cetrorelix acetate. Gonazales-Barecena et al. [16] reported an open-label study of 11 men with BPH who received 0.5 mg cetrorelix acetate s.c. twice daily for 28 days. A significant reduction in mean prostate volume, in LUTS and in mean peak urinary flow rate was observed [16]. These clinical outcomes were maintained well beyond the 1-month active treatment phase. Similar results were obtained in a second open-label study on BPH demonstrating, by subjective and objective parameters, including a lowering in IPSS, the long-term benefits of cetrorelix [17]. Lepor et al. [26] reported the first multicenter, randomized, placebo-controlled clinical trial evaluating the safety and efficacy of cetrorelix acetate in men with symptomatic BPH. Debruyne et al. [12] recently reported the results of another multicenter, randomized, double-blind, placebo-controlled study of cetrorelix gluconate in men with BPH. All active-treatment groups experienced significant improvements in the mean AUA symptom score and mean peak flow rate relative to placebo at 12 weeks. Debruyne also carried out a study with cetrorelix pamoate in BPH patients and obtained significant lowering of IPSS with cetrorelix, but not with placebo [27].

The mechanism for cetrorelix-mediated improvement in LUTS associated with benign prostatic enlargement is incompletely understood. This is due in part to the fact that the pathophysiology of LUTS in the aging male is poorly understood. Ongoing studies on the mechanism of action of cetrorelix in BPH indicate that its effects are mediated through the LHRH receptor and involve inhibition of growth factors and their receptors as well as α1A adrenergic receptors [18].

In the present study, we investigated the expression of mRNA for LHRH receptors in 55 BPH specimens. Our results show that 71% of human BPH specimens display mRNA for LHRH receptors. Furthermore, using ligand competition assay we examined the binding of [^{125}I][D-Trp^6]LHRH to membrane preparations of 20 BPH specimens. We found that 90% of human BPH samples investigated possessed specific LHRH receptors with a mean K_d of 4.04 nM and with a mean B_{max} of 527.6 fmol/mg membrane protein. Cetrorelix displaced radiolabeled [D-Trp^6]LHRH with an IC_{50} value of 0.94 ± 0.07 nM indicating its high affinity binding to LHRH receptors expressed in BPH tissues. It is also important to note that all receptor-positive specimens expressed a detectable amount of the receptor gene. The receptor protein encoded by mRNA for LHRH receptors was also demonstrated by immunohistochemistry in 42 of 64 BPH specimens. The difference between the immunoreactivity of the two LHRH receptor antibodies is most likely a reflection of the recognition of different epitopes in formalin-fixed, paraffin-embedded tissue. The lack of receptor protein in some specimens may be related to the heat damage caused by the mechanism of TUR from which source some of our samples were obtained.

In the current study, we were also able to demonstrate that 18 of the 35 BPH samples investigated (51%) expressed mRNA for LHRH ligand. The presence of both LHRH and its receptor in human BPH implies the existence of an autocrine mitogenic loop in accord with other findings [11,15].

CONCLUSIONS

The results of our study demonstrate, for the first time, that human BPH specimens obtained from surgery express LHRH and its receptors. Our findings that a high percentage of human BPH specimens express receptors for LHRH support the view that LHRH antagonists could be used for an effective treatment of BPH.

Since the LHRH receptors and mRNAs for receptor subtypes and transcript forms are variably expressed in BPH, a precise determination of LHRH receptors in samples of human BPH is necessary before therapy with LHRH analogs. Some BPH specimens may not have LHRH receptors and therefore would not respond to therapy with an antagonistic analog such as Cetrorelix. The response of individual patients with BPHs to LHRH analogs might be predicted by evaluating the LHRH receptors in their specimens. Thus, biopsy samples of BPH should be subjected to ligand competition assays for protein or RT-PCR

analyses for mRNA expression of receptors for LHRH. A rational therapy with LHRH antagonistic analog could be then implemented. The receptors could also be monitored during therapy to detect any changes in their levels and expression.

ACKNOWLEDGMENTS

We thank Maria Bessenyei and Aliz Juhasz for their experimental help and Dr. Andrea Treszl for her valuable advice in the preparation of the manuscript. This work was supported by Hungarian Ministry of Health Research Grants ETT 240/2006 (G.H.), Hungarian Scientific Research Fund (OTKA) K 81596 (G.H.), Medical Research Service of the Veterans Affairs Department; Aeterna Zentaris (Frankfurt on Main, Germany) through South Florida VA Foundation for Research and Education; University of Miami (all to A.V.S.); L.Austin Weeks Family Endowment for Urologic Research (N.L.B); The work is also supported by the TAMOP 4.2.1./B-09/1/KONV-2010-0007 project (G.H.). The project is implemented through the New Hungary Development Plan, co-financed by the European Social Fund and the European Regional Development Fund.

REFERENCES

1. Webber R. Benign prostatic hyperplasia. Clin Evid 2006;15: 1213–1226.
2. Lee KL, Peehl DM. Molecular and cellular pathogenesis of benign prostatic hyperplasia. J Urol 2004;172:1784–1791.
3. Lepor H. Pathophysiology, epidemiology, and natural history of benign prostatic hyperplasia. Rev Urol 2004;6:3–10.
4. Oesterling JE. Benign prostatic hyperplasia: A review of its histogenesis and natural history. Prostate Suppl 1996;6:67–73.
5. Isaacs JT. Importance of the natural history of benign prostatic hyperplasia in the evaluation of pharmacologic intervention. Prostate Suppl 1990;3:1–7.
6. De Bellis A, Ghiandi P, Comerci A, Fiorelli G, Grappone C, Milani S, Salerno R, Marra F, Serio M. Epidermal growth factor, epidermal growth factor receptor, and transforming growth factor-alpha in human hyperplastic prostate tissue: Expression and cellular localization. Journal Clin Endocrinol Metab 1996;81: 4148–4154.
7. Ropiquet F, Giri D, Lamb DJ, Ittmann M. FGF7 and FGF2 are increased in benign prostatic hyperplasia and are associated with increased proliferation. J Urol 1999;162:595–599.
8. 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.
9. Tammela T. Benign prostatic hyperplasia. Practical treatment guidelines. Drugs Aging 1997;10:349–366.
10. Lepor H. The role of gonadotropin-releasing hormone antagonists for the treatment of benign prostatic hyperplasia. Rev Urol 2006;8:183–189.
11. Engel JB, Schally AV. Drug insight: Clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. Nat Clin Pract Endocrinol Metab 2007;3:157–167.
12. 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;54:170–180.
13. Bajusz S, Csernus V, Janaky T, Bokser L, Fekete M, Schally AV. New antagonists of LH-RH: II. Inhibition and potentiation of LH-RH by closely related analogues. Int J Pept Protein Res 1988;32:425–435.
14. Reissmann TH, Engel J, Kutscher B, Bernd M, Hilgard P, Peukert M, Szelenyi I, Reichert S, Gonzalez-Barcena D, Nieschlag E. Cetrorelix. Drugs Future 1994;19:228–237.
15. Schally AV, Halmos G, Rekasi Z, Arencibia JM. GnRH analogs. In: Devroey P, editor. Infertility and Reproductive Medicine Clinics of North America. Philadelphia: Saunders; 2001; 120: 11–19.
16. 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.
17. 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.
18. Siejka A, Schally AV, Block NL, Barabutis N. Mechanism of action of inhibition of human benign prostatic hyperplasia in vitro by LHRH antagonist cetrorelix. Br J Urol Int 2010; Feb 11. [Epub ahead of print]
19. 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 cancers. J Urol 2000;163:623–629.
20. Munson PJ, Rodbard DA. A versatile computerized approach for characterization of ligand binding systems. Anal Biochem 1980;107:220–239.
21. Scatchard B. The attraction of proteins for small molecules and ions. Ann NY Acad Sci 1949;51:660–672.
22. Peters CA, Walsh PC. The effect of nafarelin acetate, a luteinizing hormone-releasing hormone agonist, on benign prostatic hyperplasia. N Engl J Med 1987;317:599–604.
23. Gabrilove JL, Levine AC, Kirschenbaum A, Droller M. Effect of long-acting gonadotropin-releasing hormone analog (leuprolide) therapy on prostatic size and symptoms in 15 men with benign prostatic hypertrophy. J Clin Endocrinol Metab 1989;69: 629–632.
24. Horvath J, Toller G, Schally AV, Bajo A-M, 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 fenale rats. Proc Natl Acad Sci U S A 2004;101:4996–5001.
25. De Reijke TM, Burk K, Kurth K. Clinical and endocrinological results of patients treated with the LHRH antagonist (cetrorelix). Eur Urol 1996;30:57 (Abstract no.245).
26. Lepor H, Dixon C, Crawford ED, Steidle C, Oesterling J. A randomized double blind placebo controlled phase II study of the safety and efficacy of cetrorelix in men with BPH. J Urol 1997;157:146 (abstract no.531).
27. Debruyne F, Tzvetkov M, Altarac S, Geavlete PA. Dose-ranging study of the LH-RH receptor antagonist cetrorelix pamoate in the treatment of patients with symptomatic benign prostatic hyperplasia. Urology 2010; (accepted, in print).