Insulin-Like Growth Factor (IGF)-I Controls Prostate Fibromuscular Development: IGF-I Inhibition Prevents Both Fibromuscular and Glandular Development in Eugonadal Mice

David L. Kleinberg, Weifeng Ruan, Douglas Yee, Kalman T. Kovacs, and Sergio Vidal†

The Bunnie Joan Sachs Laboratory (D.L.K., W.R.), Neuroendocrine Unit, Department of Medicine, New York University School of Medicine, New York, New York 10016; Department of Medicine (D.Y.), University of Minnesota, Minneapolis, Minnesota 55455; Department of Pathology (K.T.K.), St. Michaels Hospital, University of Toronto, Toronto, Ontario, Canada M5B 1W8; and University of Santiago de Compostela (S.V.), Campus Universitario de Lugo, Department of Anatomy, Lugo 2700-2, Spain

Although antiandrogen therapy has been shown effective in treating prostatic tumors, it is relatively ineffective in treating benign prostatic hyperplasia (BPH). In an attempt to understand better the role of androgens in the development of the normal prostate and BPH, we studied the relative effects of testosterone and IGF-I on the development of the two compartments of the prostate in castrated IGF-I(−/−) male mice. Here we report that IGF-I stimulated the development of the fibromuscular compartment, but testosterone inhibited it (stromal epithelial ratio 2.17 vs. 0.83, respectively; P < 0.001). Testosterone also impaired IGF-I induced insulin receptor substrate-1 phosphorylation and cell division, and increased apoptosis in fibromuscular tissue. In sharp contrast IGF-I and testosterone both stimulated the development of the glandular compartment individually and together. The combined effects were either additive or synergistic on compartment size, cell division, insulin receptor substrate-1 phosphorylation, and probasin production. Together they also had a greater inhibitory effect on apoptosis in gland tissue. To determine whether IGF-I inhibition would inhibit both fibromuscular and glandular compartments, we tested the effect of IGF binding protein-1 on prostate development in two different models: castrated Ames dwarf mice and eugonadal normal male mice. IGF binding protein-1 blocked bovine GH-induced fibromuscular and glandular development in both. It also inhibited epithelial cell division and increased apoptosis in both prostate compartments in the eugonadal mice. The observed discordance between IGF-I and testosterone control of prostate compartment development might explain the relative failure of 5α-reductase inhibition in BPH and why testosterone inhibition might theoretically reduce gland volume but increase fibromuscular tissue. The work also provides a rationale for considering IGF-I inhibition as therapy for BPH to reduce the size of both prostate compartments. (Endocrinology 148: 1080–1088, 2007)

THE PROSTATE IS composed of two compartments, glandular and fibromuscular. Testosterone is a central factor in prostate development (1, 2). We previously showed that, like testosterone, IGF-I was also necessary for the development of the glandular component of the prostate (3). We also showed that IGF-I was necessary for the development of the fibromuscular compartment, but testosterone inhibited it (stromal epithelial ratio 2.17 vs. 0.83, respectively; P < 0.001). Testosterone also impaired IGF-I induced insulin receptor substrate-1 phosphorylation and cell division, and increased apoptosis in fibromuscular tissue. In sharp contrast IGF-I and testosterone both stimulated the development of the glandular compartment individually and together. The combined effects were either additive or synergistic on compartment size, cell division, insulin receptor substrate-1 phosphorylation, and probasin production. Together they also had a greater inhibitory effect on apoptosis in gland tissue. To determine whether IGF-I inhibition would inhibit both fibromuscular and glandular compartments, we tested the effect of IGF binding protein-1 on prostate development in two different models: castrated Ames dwarf mice and eugonadal normal male mice. IGF binding protein-1 blocked bovine GH-induced fibromuscular and glandular development in both. It also inhibited epithelial cell division and increased apoptosis in both prostate compartments in the eugonadal mice. The observed discordance between IGF-I and testosterone control of prostate compartment development might explain the relative failure of 5α-reductase inhibition in BPH and why testosterone inhibition might theoretically reduce gland volume but increase fibromuscular tissue. The work also provides a rationale for considering IGF-I inhibition as therapy for BPH to reduce the size of both prostate compartments. (Endocrinology 148: 1080–1088, 2007)

Materials and Methods

Experimental animals

Three animal models were used. The first was the IGF-I(−/−) male mouse. They were raised in our Association for Assessment and Accreditation of Laboratory Animal Care-approved facility at the Harbor VA Medical Center (New York, NY) by crossing male and female mice heterozygous for the absence of an IGF-I gene, originally supplied by Lynn Powell-Braxton of Genentech, Inc. (South San Francisco, CA) (3, 14). PCR on DNA extracted from mouse-tails confirmed the absence of an IGF-I gene. At 56-d-old they were castrated under anesthesia. Two weeks later, hormone therapy was begun and continued for 7 d. The second animal model was the Ames dwarf male mouse. These animals are deficient in GH, prolactin, and TSH. They were bred in our Laboratory using breeding pairs obtained from Andrzej Bartke of the School of Medicine, Southern Illinois University (Carbondale, IL). They were...
castrated at 8 wk of age, and experiments were started at 10 wk. They received I-131 0.25 mg/pellet imbedded sc (Innovative Research of America, Sarasota, FL). The third model was a 28-d-old intact (cesarean derived) mouse (Charles River Laboratories, Inc., Wilmington, MA). The institutional committee on animal care approved these studies, and animals were maintained in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

Hormones and hormone inhibitors

Hormone therapy was begun and continued for 7 d. Testosterone was administered in SILASTIC capsules (inside diameter 0.16 cm, outside diameter 0.32 cm, length 1 cm; Dow Corning, Corp., Midland, MI) imbedded sc on the backs of animals as previously described (3). Des (1–3) IGF-I [40 μg per Alzet pump (No. 1007D; Alzet Corp., Palo Alto, CA)] was a gift from Genentech Inc. In this paper it is referred to as IGF-I. The pumps were also implaned sc. Animals were treated with IGF-I plus testosterone, IGF-I alone, testosterone alone or saline in controls. The Alzet pump was used to administer bovine GH (bGH) (100 μg/pump) or saline for controls. The pumps were also implanted sc. Animals were maintained in accordance with the Health guidelines for the Care and Use of Laboratory Animals.

Implantation of SILASTIC capsules and Alzet miniosmotic pumps. SILASTIC capsules (inside diameter 0.16 cm, outside diameter 0.32 cm, length 1 cm) containing testosterone (Calbiochem, La Jolla, CA) and Alzet pumps containing IGF-I or bGH, or IGF-BP-1 alone or in combination, or saline were implanted sc on the backs of animals under anesthesia as previously described (3).

Histology

At the end of each experiment, the entire urogenital complex was excised. The seminal vesicles, accessory glands, prostate, urinary bladder, and most of the ureter were removed, and the remaining prostate gland with attached ureter was examined. Whole prostate glands were fixed in 10% phosphate formalin solution. After 24 h the prostate tissues were transversely embedded in paraffin along the same orientation so that the sections were perpendicular to the urethra. The paraffin block was cut into 4-μm serial sections. Three adjacent sections from three different levels of each gland were taken to make a single observation. In the case of probasin, only one section from each animal was assessed because the results were so obvious. Anatomical analysis was performed on sections stained with hematoxylin and eosin or smooth muscle α-actin. Adjacent sections from each level were stained by immunohistochemistry for phosphorylated insulin receptor substrate 1 (pY-IRS1), Ki67, and terminal deoxynucleotidyl transferase-mediated DUTP-biotin end labeling of fragmented DNA (TUNEL). The histology sections, which included both ventral and dorsolateral prostate lobes, were examined using a Nikon E400 microscope (Nikon Corp., Tokyo, Japan). Photographs were taken at ×200 and ×400 by an attached Nikom digital camera using DXM1200 software Nikon ACT-1 on a personal computer system. All prostate tissue on each slide was assessed for the area occupied by each of the components of the prostate. Fibromuscular development was assessed by outlining the periglandular sheath of fibromuscular tissue and then calculating the area. The area between the inner aspects of the fibromuscular layer and the outer limit of the lumen was measured to assess size of the glandular compartment. The lumen was measured separately. A semiautomatic computer system (Image-Pro plus, version 4.5; Media Cybernetics, Baltimore, MD) assisted morphological analysis. The method of Doehring et al. (10) was used to calculate the stromal epithelial ratio.

Cloning, expression, and purification of IGFBP-1

The IGF-BP-1 gene was isolated by PCR from pcBP25 (16) and ligated into pPICZαA. This plasmid was then transformed into Pichia pastoris strain X-33 per manufacturer’s instructions (Invitrogen Easy Select Pichia Expression Kit; Invitrogen, Corp., Carlsbad, CA). Transformed yeast cells were initially grown overnight in sterile buffered glycerol-complex medium plus 1 mg/ml Zeocin (1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.0; 1.34% YNB; 440 ng/liter biotin; and 1% glycerol) at 28 C. Cells were aseptically diluted 10-fold in sterile buffered methanol-complex medium (1% yeast extract, 2% peptone, 100 mM potassium phosphate pH 6.0, 1.34% YNB, 440 ng/liter biotin, and 0.5% methanol) and incubated for an additional 24 h at 28 C to induce expression. Cell suspensions were centrifuged (5000 × g; 10 min); the protein in the culture supernatant was then precipitated in 60% ammonium sulfate for 1 h. Precipitated protein was harvested by centrifugation (7500 × g; 30 min) and resuspended in Tris buffer (0.5 mM NaCl; 20 mM Tris-HCl, pH 7.9; 20 μg/ml leupeptin; 20 μg/ml aprotinin; 1 mM phenylmethylsulfonyl fluoride). Protein was further purified by fast protein liquid chromatography using Ni-NTA Superflow Agarose (QIA-GEN, Inc., Valencia, CA). The fractions containing protein determined by the Bradford assay (Pierce Biotechnology, Rockford, IL) were dialyzed (Spectrum, Spectra/Por MWCO 6–8000) in 100 mM HEPES pH 6.0 (100 mM HEPES salt, 50 mM NaH2PO4/H2O) for at least 1 h. The Bradford assay determined the final concentration of the protein. Western blotting with antihuman IGFBP-1 (Upstate Biotechnology, Waltham, MA) confirmed identification of IGFBP-1.

Surgical procedures

Orchietomy was performed under anesthesia with tribromoethane (1.5% isoflurane in 100% O2; mask). The testes, epididymides, deferent ducts, seminal vesicles, ampullary gland, urinary bladder, and most of the ureter were removed, and the remaining prostate gland with attached ureter was examined. Whole prostate glands were fixed in 10% phosphate formalin solution. After 24 h the prostate tissues were transversely embedded in paraffin.
International, Inc., Temecula, CA), was used to detect apoptotic cells. The results were expressed as the percentage of labeled cells in both epithelial and stromal compartments. At least 2000 cells per three sections were counted.

Western blot
Specificity of the antibody to pY-IRS1 was determined by measuring pY-IRS1 by Western blotting. MCF-7 cells were treated with IGF-I. Protein samples from control and IGF-I treated cells were analyzed by SDS-PAGE on a 10–15% gradient gel. We found that there was an increase in signal of phosphorylated IRS1 in protein extracted for IGF-I treated cells.

Statistical analysis
Statistics were performed using groups of three animals each for each hormone or hormone combination in each of the three animal models. The two-tailed unpaired Student’s t test was used between groups.

Results
Relative effects of IGF-I and testosterone on anatomical parameters of development of fibromuscular and glandular components of the prostate
We tested the individual and combined effects of testosterone and amino-terminally shortened des (1–3) IGF-I on glandular and fibromuscular development in castrated IGFI−/− male mice, in which prostate gland development is markedly impaired (Fig. 1A and Table 1) (3).

Fibromuscular development. In the fibromuscular compartment, IGF-I stimulated hyperplasia (P < 0.001 vs. control), while testosterone antagonized the IGF-I stimulated development (Fig. 1 and Table 1). Endpoints were assessed on slides stained with hematoxylin and eosin, and others immunostained with smooth muscle α-actin antibody. Although testosterone alone had a slight, but significant, stimulatory effect over control on fibromuscular development, this effect was significantly less than the effect of IGF-I alone.

Glandular development. In contrast, in the glandular compartment both IGF-I alone and testosterone alone stimulated epithelial mass and lumen size, and when administered together, there was an additive effect on the epithelial cell area and a synergistic one on lumen size.

When the relative effects of these hormones were expressed as a ratio between stromal and epithelial elements (SER), testosterone alone and testosterone together with IGF-I caused a small but significant reduction in the SER compared with control and a much greater reduction compared with the effect of IGF-I alone (Fig. 1C). The stimulatory effect of IGF-I alone was greater on stromal development than any other treatment. The SER is calculated from the area of stromal tissue to epithelial tissue and does not consider the lumen area (10).

Fig. 1. Relative effects of IGF-I and testosterone on the development of fibromuscular vs. glandular compartments of the prostate. A, Effects of IGF-I, testosterone (T), or IGF-I + testosterone or no treatment on representative histological sections of prostate from castrated IGF-I−/− mice at two magnifications: ×200 (top row) and ×400 (bottom row). IGF-I alone caused fibromuscular hyperplasia around each glandular unit (second column), while testosterone alone had a more pronounced effect on glandular and lumen size (third column). IGF-I + testosterone mainly affected the glands by causing hyperplastic changes (fourth column). There was an increase in cellularity, and some variation in nuclear size and shape. The papillary structures reflect areas of glandular hyperplasia caused by IGF-I + testosterone (arrowheads). B, Relative amounts of hyperplasia in fibromuscular and epithelial tissues are expressed as the ratio of fibromuscular tissue over epithelial. This is called SER or stromal epithelial ratio. C, A photomicrograph of four representative sections through prostate glands stained for smooth muscle α-actin (arrow). Magnification, ×400. Again the greatest increase was obtained when IGF-I alone was given. D, Bar graph of the area stained by antismooth muscle α-actin in three sections from each prostate and three prostates per treatment group. Data are expressed as the mean ± SEM. P < 0.05 was considered significant.
TABLE 1. Effect of IGF-I and testosterone on prostate development in castrated IGF-I−/− male mice

<table>
<thead>
<tr>
<th></th>
<th>Fibromuscular, μm² (×10^3)</th>
<th>Epithelium, μm² (×10^3)</th>
<th>Lumen, μm² (×10^3)</th>
<th>Total, μm² (×10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.0 ± 3.7</td>
<td>46.7 ± 2.4</td>
<td>80.0 ± 4.4</td>
<td>195.7 ± 10.7</td>
</tr>
<tr>
<td>Testosterone alone</td>
<td>98.1 ± 5.8b</td>
<td>112.1 ± 4.1b</td>
<td>256.3 ± 10.1c</td>
<td>466.5 ± 12.5c</td>
</tr>
<tr>
<td>IGF-I alone</td>
<td>207.9 ± 9.4d</td>
<td>95.5 ± 3.9e</td>
<td>115.1 ± 9.2f</td>
<td>418.5 ± 10.2g</td>
</tr>
<tr>
<td>IGF-I + testosterone</td>
<td>123.0 ± 1.8s</td>
<td>148.9 ± 8.9i</td>
<td>427.6 ± 16.6j</td>
<td>699.6 ± 25.1l</td>
</tr>
</tbody>
</table>

\( ^a P < 0.04 \) compared with control.
\( ^b P < 0.02 \) compared with IGF-I + testosterone, \( P < 0.002 \) compared with control.
\( ^c P < 0.001 \) compared with control and IGF-I + testosterone.
\( ^d P < 0.03 \) compared with testosterone and IGF-I + testosterone, \( P < 0.001 \) compared with control.
\( ^e P < 0.05 \) compared with testosterone, \( P < 0.01 \) compared with control and IGF-I + testosterone.
\( ^f P < 0.001 \) compared with testosterone and IGF-I + testosterone, \( P < 0.03 \) compared with control.
\( ^g P < 0.05 \) compared with testosterone.
\( ^h P < 0.05 \) compared with testosterone, \( P < 0.001 \) compared with control.
\( ^i P < 0.01 \) compared with control.
\( ^j P < 0.001 \) compared with control.

Relative effects of IGF-I and testosterone on cell division.

Apoptosis and phosphorylation of IRS1

Mechanisms by which the various hormone combinations acted on the fibromuscular compartment were explored. Endpoints were cell division, apoptosis, and phosphorylation of IRS1 (Fig. 2). IGF-I alone increased cell division (\( P < 0.001 \); Fig. 2, A and C) and IRS1 phosphorylation (\( P < 0.001 \); Fig. 2, A and B), and inhibited apoptosis (\( P < 0.05 \); Fig. 2, A and D) in the fibromuscular compartment. Addition of testosterone to IGF-I significantly reversed each of these effects. Testosterone alone had a slight stimulatory effect on phosphorylation of IRS1, but when given together with IGF-I, it inhibited IRS1 phosphorylation.

In the glandular compartment, testosterone alone increased cell division and phosphorylation of IRS1 and inhibited apoptosis. IGF-I together with testosterone had an additive effect on IRS1 phosphorylation and a synergistic one on cell division. There was also a stimulatory effect of IGF-I alone on cell division and IRS1 phosphorylation, and an inhibitory one on apoptosis, but the independent effect of testosterone and combined effects of testosterone and IGF-I were both greater than those of IGF-I alone.

Effects of IGF-I and testosterone on probasin

Probasin, an androgen sensitive protein (Fig. 3), was not found in the glandular epithelium in control prostates. Testosterone alone led to an increase in the percent of epithelial cells that were positive for probasin. IGF-I alone very slightly increased the number of epithelial cell that produced probasin, but when testosterone and IGF-I were given together, the increase in the percent of cells positive for probasin was synergistic. There were no obvious qualitative differences in probasin staining in ventral and dorsolateral prostate lobes.

Effects of IGFBP-1 on prostate development induced by bGH in castrated Ames dwarf mice and on normal prostate development in 4-wk-old intact wild-type mice

To determine whether IGF-I inhibition could impair prostate development induced by GH-stimulated IGF-I and normal development in eugonadal animals, we tested the effect of IGFBP-1 in two models.

Ames dwarf mice. Ames dwarf mice have impaired prostate development because they are deficient in GH. They are also deficient in prolactin, TSH, and gonadotrophins (18). To determine if IGFBP-1 could prevent bGH-induced IGF-I action, we treated 10-wk-old castrated Ames animals with bGH with or without infusion of IGFBP-1 for 7 d. IGFBP-1 inhibited GH-induced fibromuscular and glandular development (Fig. 4 and Table 2).

Eugonadal intact mice. The effect of treatment with IGFBP-1 on prostate development in 4-wk-old intact wild-type male mice was also studied. When compared with saline-treated control animals, IGFBP-1 inhibited all fibromuscular development, epithelial development, lumen size, prostate area, and prostate weight (Fig. 5, A–E). IGFBP-1 inhibited IGF-I induced fibromuscular and glandular development (Fig. 4 and Table 2).

Discussion

Complex stromal-epithelial interactions mediate the effect of testosterone in prostate development. Testosterone acts via androgen receptor in stromal cells, which in turn stimulates ductal morphogenesis and epithelial differentiation (5, 11, 19, 20). Castration causes atrophy of the glands (2, 21). Other hormones, including estrogen (5) and prolactin, play important roles in prostate development as well (6, 7).

IGF-I induced prostate development begins with GH. Animals with low GH have impaired prostate development. Thus, mice overexpressing a GH antagonist that prevents the action of GH (3, 22) and Ames dwarf mice that have low GH also have impaired prostate development (unpublished results). GH has previously increased transcripts coding for IGFBP-1 in two models.
digestion so that the effects of the hormones on the glands free of fibromuscular elements could be analyzed. We found that IGF-I was necessary for full glandular development. IGF-I was found to substitute for the IGF-I deficiency in IGF-I(-/-) mice. That bGH was incapable of doing the same suggested that the entire effect of GH in prostate glandular development is mediated via IGF-I. In that study we did not assess effects on the fibromuscular compartment. We were also not able to assess the relative effects of testosterone vs. IGF-I because the animals were not castrated. In the present study the mice were castrated to remove endogenous testosterone, and the prostates were studied in serially sectioned whole glands so that both compartments could be studied simultaneously.

Our results revealed novel discordance between the hormonal control of the development of fibromuscular and glandular compartments. In keeping with previous observations that IGF-I can cause DNA synthesis in smooth muscle cells and fibroblasts (25), that prostatic stromal cells express IGF-I receptor (IGF-IR), that IGFs can increase stromal cell proliferation (26), and that des (1–3) IGF-I stimulates cell division and inhibits apoptosis in BPH stromal cells (27), we found that the development and hyperplasia of the fibromuscular compartment were under the control of IGF-I. When added
to IGF-I, testosterone had an inhibitory effect on this IGF-I induced process, raising the possibility that testosterone might prevent fibromuscular hyperplasia. Interestingly, when IGF-I was absent, testosterone had a slight but significant effect on fibromuscular development. However, testosterone alone also significantly inhibited SER in comparison to controls. Because androgen has been shown to stimulate IGF-I production in the stromal compartment of the prostate (28), it is possible that testosterone alone might have had a greater effect on fibromuscular development had the animal model been capable of expressing IGF-I.

Why testosterone would inhibit the action of IGF-I in the fibromuscular compartment is unclear. This unanticipated finding suggests a far more complicated interaction between the two hormones in which the mechanisms of development are different. One unsupported suggestion is that testosterone might induce an intermediary substance that could inhibit IGF-I action in the fibromuscular compartment, as it is known to stimulate substances that stimulate gland development (2).

The events in the glandular compartment are in sharp contrast to those observed in the fibromuscular compartment. For glandular development both IGF-I and testosterone are required (3). Both had significant independent effects on the development of epithelial mass and lumen size, although the effect of testosterone alone was greater than that of IGF-I alone. The effect of testosterone on phosphorylation of IRS1 and cell division was also greater than that of IGF-I alone in epithelial cells. Testosterone was also more effective than IGF-I in inhibiting apoptosis. When administered in combination, IGF-I and testosterone had greater effects on each of these parameters of IGF-I action than either hormone alone. The high rate of apoptosis in the castrated IGF-I(-/-) animals compared with that in wild-type mice could be caused by complete deficiency of IGF-I, or castration or both (29).

The combination of IGF-I and testosterone also had a synergistic effect on probasin production in the epithelial cells of the prostate. Staining was more intense in response to the two hormones than with either alone. That IGF-I and testosterone acted in synergy compared with testosteron alone suggests that IGF-I potentiates the effect of testosterone on probasin production. Although probasin production is known to be more or less sensitive to testosterone depending upon the specific prostate lobes (30–32), we did not note qualitative differences between the ventral and dorsolateral lobes of the prostate.

Another way of understanding the relative effects of the hormones on the two compartments of the prostate is by expressing the effects on prostate development as the ratio of stromal to epithelial development (10). The stimulatory effect of IGF-I on fibromuscular development is underscored by a marked increase in SER when given alone and by the reduction in SER by testosterone alone or testosterone combined with IGF-I. One caveat is that the doses of IGF-I used may have been
FIG. 5. Effect of IGFBP-1 treatment on prostate structures in intact 28-d-old male mice. After 7 d of treatment with IGFBP-1 or vehicle, prostate development in both fibromuscular and glandular compartments was analyzed. Effects on fibromuscular development (A), epithelial development (B), lumen size (C), total prostate area (D), and prostate weight (E) are shown. Effects of IGFBP-1 (compared with control) on cell division and programmed cell death are shown in representative photomicrographs (F) showing the effect of IGFBP-1 (lower row) on cell division (left) and apoptosis (right) compared with control animals (top row). Arrows point to Ki67 or TUNEL staining in stroma, and arrows point to those effects in glands. Magnification, ×400. Effects of Ki67 staining (G) for cell division (left) and effects on programmed cell death by TUNEL (right). WT, Wild type.

TABLE 2. Effect of IGFBP-1 on inhibition of GH-induced prostate development in castrated Ames dwarf mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fibromuscular, ( \mu m^2 ) ( (\times 10^3) )</th>
<th>Epithelium, ( \mu m^2 ) ( (\times 10^3) )</th>
<th>Lumen, ( \mu m^2 ) ( (\times 10^3) )</th>
<th>Total, ( \mu m^2 ) ( (\times 10^3) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>bGH</td>
<td>253.4 ± 18.6(^{a,b})</td>
<td>160.5 ± 4.0(^{a,d})</td>
<td>274.7 ± 11.9(^{b,c})</td>
<td>688.6 ± 32.4(^{a,b})</td>
</tr>
<tr>
<td>bGH + BP-1</td>
<td>156.9 ± 8.7(^{e})</td>
<td>145.0 ± 2.9</td>
<td>239.1 ± 4.8(^{d})</td>
<td>540.3 ± 8.9(^{e})</td>
</tr>
<tr>
<td>Control</td>
<td>122.6 ± 6.7</td>
<td>144.7 ± 3.2</td>
<td>188.2 ± 10.4</td>
<td>455.5 ± 18.1</td>
</tr>
</tbody>
</table>

\(^{a}\) \( P < 0.02 \) compared with bGH + BP-1.
\(^{b}\) \( P < 0.01 \) compared with control.
\(^{c}\) \( P < 0.05 \) compared with bGH + BP-1.
\(^{d}\) \( P < 0.04 \) compared with control.
\(^{e}\) \( P < 0.04 \) compared with control.
supraphysiological, and, therefore, we may be seeing an exaggerated response regarding fibromuscular hyperplasia.

Our results demonstrate that IGFBP-1 can prevent GH-induced fibromuscular and glandular development of the prostate in Ames dwarf male animals, and also inhibit multiple aspects of prostate development in both the fibromuscular and glandular compartments of intact male mice through inhibiting cell division and stimulating apoptosis (33). It is likely that IGFBP-1 acted by competitively inhibiting IGF-I action (34), but other mechanisms have also been proposed (35). Presumably, other inhibitors of IGF-I action would behave similarly.

That IGFBP-1 inhibited the normal development of the fibromuscular compartment in eugonadal intact mice supports the likelihood that IGF-I inhibition would be effective in reducing the size of the stromal compartment, even in the absence of supraphysiological concentrations of IGF-I and in the presence of circulating testosterone.

BPH is a disease of unknown etiology that affects millions of men (5, 36), and causes obstructive and irritative symptoms. It is a disease of hyperplasia of both fibromuscular and glandular tissue, with fibromuscular tissue being the larger component (9, 10). The SER is higher in men with BPH than in controls, and younger men with familial forms of BPH have a much higher SER than older men with BPH (10). Monti et al. (37) examined IGF-IR in periurethral, intermediate and subcapsular regions of prostates from men with BPH. They found that IGF-IR mRNA was highest in the periurethral portion of the tissue, the problem area in BPH. There is also crosstalk between IGF-I and IGFBPs in the prostate (33, 38, 39). Additionally, high-serum IGF-I is associated with an increased risk for BPH (40, 41). A relative reduction in apoptosis compared with cell proliferation has been noted in stromal tissue (42, 43). It should be pointed out that mouse prostate development is not a model for BPH because periurethral fibromuscular hyperplasia does not occur in mice as it does in dogs or human beings (44, 45).

Because testosterone, through dihydrotestosterone, is centrally involved in prostate development, and castration causes shrinkage of the prostate (46), most attempts to treat BPH hormonally have centered on inhibition of dihydrotestosterone formation by 5α-reductase inhibitors (11, 12). Although these medications may reduce symptoms of BPH and the need for surgical intervention in select individuals, the overall effect has been disappointing (47). Assuming that the hormones that control prostate development in human beings are similar to those in mice, we would expect that testosterone inhibition would reduce the size of the glandular compartment (13) and increase the size of the fibromuscular compartment because the inhibitory effect of testosterone on IGF-I stimulated fibromuscular development would be removed. This could explain the relative failure of 5α-reductase inhibitors in BPH. Although IGF-I inhibitors would not necessarily impair the action of testosterone on the glandular compartment, the relative reduction of both compartments might be sufficient to relieve the obstructive aspects of BPH or prevent further BPH, or both. This suggestion is only theoretical in that the possible use of IGF-I inhibition in BPH would have to be proven in other models, including the human being. If it is proven, IGF-I inhibition might prevent or inhibit prostate hyperplasia, and treat associated signs and symptoms without the need for reducing or specifically inhibiting circulating testosterone.

Although this work implies a potential therapeutic benefit of IGF-I inhibition for the treatment of BPH, risks posed by systemic IGF-I inhibition must be considered. Local administration of IGF-I inhibitors deserves consideration, as do drugs that might inhibit IGF-I action locally in the prostate. Although we are not aware of such candidate drugs for prostate, an example of such a drug that inhibits IGF-I activity in the mammary gland via somatostatin receptors is the somatostatin analog SO230 (15).

Conclusions

This work provides evidence that the development of the two compartments of the prostate differs in regard to the actions of testosterone and IGF-I. In the glandular compartment these hormones stimulate development both individually and together. Testosterone has a greater effect on epithelial and luminal development than IGF-I. When given together IGF-I and testosterone increase epithelial (additive) and luminal (synergistic) development. IGF-I also potentiates the effect of testosterone on probasin production in the epithelial cells. In sharp contrast, IGF-I stimulated fibromuscular development, and testosterone inhibited this action of IGF-I. IGFBP-1, an inhibitor of IGF-I action, was found to inhibit both glandular and fibromuscular development. Therefore, these results suggest that if prostate development in human beings is similar to the mouse, IGF-I inhibition could be more effective than 5α-reductase inhibitors in the treatment of BPH.

Acknowledgments

We thank Inn Ling Eng for expert animal handling and sectioning of tissue blocks.

Received September 15, 2006. Accepted November 17, 2006.

Address all correspondence and requests for reprints to: David L. Kleinberg, The Bunnie Joan Sachs Laboratory, Neuroendocrine Unit, Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, New York 10016. E-mail: david.kleinberg@med.nyu.edu.

This work was supported by NIH R01 CA064709, The Foundation for Growth and Endocrinology, Variety the Children’s Charity, and the Bunnie Joan Sachs Family Foundation.

Disclosure Statement: Regarding D.L.K. and W.R., NYU School of Medicine is applying for a patent based on this work. K.T.K., and D.Y. have nothing to disclose.

References


34. EFSA 2004 A critical analysis of the scientific literature on the potential role of diet and nutrition in the prevention of chronic diseases. EFSA J 2:365–1444


Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.