Effects of hyperprolactinemia on rat prostate growth: evidence of androgeno-dependence

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Van Coppenolle, Fabien, Christian Slomianny, Francoise Carpentier, Xuefen Le Bourhis, Ahmed Ahidouche, Dominique Croix, Guillaume Legrand, Etienne Dewailly, Sarah Fournier, Henri Coussé, Dominique Authie, Jean-Pierre Raynaud, Jean-Claude Beauvilain, Jean-Paul Dupouy, and Natalia Prevarskaya. Effects of hyperprolactinemia on rat prostate growth: evidence of androgeno-dependence. Am J Physiol Endocrinol Metab 280: E120–E129, 2001.—The effects of the polypeptide hormone prolactin (PRL) in the development and regulation of benign prostate hyperplasia (BPH) and also in prostate cancer are not very well characterized. This study examines the action of PRL, either alone or in association with androgens [testosterone (T) or dihydrotestosterone (DHT)], in the rat prostate gland. The effects of PRL and androgens were investigated after 30 and 60 days in control, castrated, castrated with a substitutive implant of T or DHT, and sham-operated Wistar rats. To enhance PRL release, we induced hyperprolactinemia by administering chronic injections of sulpiride (40 mg·kg−1·day−1). Chronic hyperprolactinemia induces enlargement and inflammation of the lateral rat prostate without any histological changes on ventral and dorsal lobes. We also demonstrate that hyperprolactinemia induces Bcl-2 overexpression in the lateral rat prostate and that this could inhibit the level of apoptosis. The in vivo model established here is a useful in vivo approach for studying the hormonal regulation of normal and pathological prostate development.

prolactin; testosterone; dihydrotestosterone

THE CONSTANT INCREASE IN LIFE EXPECTANCY has enhanced the incidence of benign prostate hyperplasia (BPH) and prostate cancer. In particular, prostate cancer is the second cause of male cancer-related death in the Western world (67).

It has now been clearly established that the growth, differentiation (16, 20, 64), and programmed cell death (22) of prostate cells are regulated by androgens. For this reason, the main treatment for prostate tumors consists of inhibiting cell growth by suppressing the action or production of endogenous androgens (6). However, despite this treatment, almost all tumors, and especially malignant tumors, continue to progress. The background of this clinical phenomenon is poorly understood. It has become obvious that other nonandrogenic factors, such as peptide hormones (1, 12, 56) and growth factors (17), are involved in prostate cell growth regulation.

Prolactin (PRL) is one of the nonsteroidal factors assumed to be involved in the proliferation of prostate cells (9) and in the development and regulation of BPH and prostate cancer (24, 25, 37, 39).

PRL levels increase with age (17, 62) whereas testosterone levels decrease (10, 36), indicating that the action or production of endogenous androgens (6). However, despite this treatment, almost all tumors, and especially malignant tumors, continue to progress. The background of this clinical phenomenon is poorly understood. It has become obvious that other nonandrogenic factors, such as peptide hormones (1, 12, 56) and growth factors (17), are involved in prostate cell growth regulation.

PRL levels increase with age (17, 62) whereas testosterone levels decrease (10, 36), indicating that the role of PRL in the development of prostate hyperplasia becomes increasingly important with age. Using organ cultures, Nevalainen et al. (39) showed that PRL induces differentiation and proliferation in rat and human prostate. These PRL actions are mediated through the signal transduction pathways triggered by both the short and long forms of PRL receptors. Furthermore, these authors showed that rat prostatic epithelial cells express prolactin, and they demonstrated an overall distribution of prolactin mRNA in the dorsal and lateral prostate (37). Other groups have suggested that PRL promotes the growth and proliferation of

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prostate cells in synergism with androgens (49). It has also been proposed that PRL could increase free steroid concentrations in the blood, as well as the uptake of testosterone (T) in prostate cells (14). On the other hand, it has also been suggested that PRL has an independent action on prostatic growth and metabolism (48, 52, 58). Some in vitro models of the prostate have been developed to investigate these problems, but an analysis of the independent and combined in vivo actions of these hormones is lacking. The importance of PRL was also shown by a study of PRL-transgenic mice (66), demonstrating a dramatic prostate enlargement.

In a recent study, we demonstrated that hyperprolactinemia induced a lateral rat prostate hyperplasia (60). We induced hyperprolactinemia by daily injections of 40 mg/kg of sulpiride, an antagonist of the type 2 dopamine receptor (D2), a mediator of dopamine-inhibited secretion of PRL (11, 35). In the present study, using the same in vivo model, we investigated the effects of hyperprolactinemia alone or in association with androgens (T or dihydrotestosterone (DHT)) on the different lobes of rat prostate gland. The lateral lobe was more sensitive to an increase in plasma PRL levels than the ventral and dorsal lobes. The hyperprolactinemia induced enlargement and inflammation of the lateral rat prostate without any histological changes on ventral and dorsal lobes. We also demonstrated that PRL may inhibit lateral lobe epithelial cell apoptosis by overexpressing Bcl-2.

**MATERIALS AND METHODS**

*Animals.* One hundred and ten male Wistar rats (200–220 g) from Iffa Credo, France, were used. These animals were conditioned for 1 wk before experimentation. Rats were randomized and housed five per cage on a 12:12-h light-dark cycle. They were provided ad libitum with water and a standard laboratory chow.

During this work, all animal studies were conducted in accordance with the European Community’s Council ruling of 24 November 1986 (86/609/EEC).

*Surgical procedures.* All surgeries were performed on day 1 under ether anesthesia and strict sanitized conditions. The operated animals were treated with antibiotics (penicillin) to prevent infections.

Castrations were performed on day 1 via scrotal route by removing epididymal fat pads with the testes. The sham-castrated rats were opened, and their testes were dissected but not removed. Operated animals were then sutured, and the injured areas were disinfected with betadine solution and sprayed with aluspray (Vetoquinol).

To add the desired quantity of exogenous androgens for comparison with control animals, we implanted Silastic medical-grade silicone tubing (1 cm length, 0.078 ID × 0.125 OD; Dow Corning, Midland, MI) filled with either T (Sigma) or DHT (Sigma) subcutaneously over the scapula. One end of the tubing was sealed with adhesive (Silastic Medical Adhesive; Dow Corning). After loading with the hormone, the unsealed end was sealed with adhesive. After the adhesive had hardened, the implants were put overnight in distilled water. The implants were inserted on day 8 in pockets formed over the dorsal area of the scapula. The incised area was disinfected and then sutured.

**Hyperprolactinemia induction.** Hyperprolactinemia was induced by daily intraperitoneal injections of a 40 mg/kg aqueous sulpiride solution (± sulpiride, Sigma). Control animals were intraperitoneally injected daily with the carrier alone (NaCl 0.9%).

Another experimental approach was used to induce hyperprolactinemia: Alzet 2ML4 osmotic pumps delivering 2.5 μl/h of PRL (0.173 mg/ml) were implanted for 1 mo by use of the same surgical procedure as described above.

**Sampling.** Table 1 gives the surgical event (castrations, sham castrations, and implants) and treatment (daily ip injections of sulpiride or NaCl 0.9%) schedule for the various experimental groups.

On the day animals were killed, blood samples were collected in EDTA-coated capillaries and stored immediately on ice. Blood was centrifuged at 3,000 rpm for 10 min. Plasma samples were frozen at −80°C until the hormonal assay was performed. The prostate lobes were dissected, weighed, and treated for light microscopy by fixing in 10% neutral buffered formalin, dehydrating, and embedding in paraffin.

**Hormonal assays.** Plasma levels of PRL and luteinizing hormone (LH) were measured by RIA with materials supplied by the National Institute of Diabetes and Digestive and Kidney Diseases rat pituitary hormone distribution program (Torrance, CA), with rat RP3-PRL and rat RP3-LH as reference preparations. T and DHT levels were measured by RIA with a TRK 600 kit (Amersham) according to a protocol from the manufacturer.

**Histology.** Tissue pieces were fixed in 10% neutral buffered formalin and embedded in paraffin. Histological analysis was performed on serial sections obtained from prostatic samples stained by hematoxylin-erythrosin-safron.

**Bcl-2 labeling.** Immediately after dissection of the lateral prostate, pieces of ~1 mm × 1 mm were fixed by immersion in paraformaldehyde (1.5% in PBS) for 1 h at 4°C. After several washes (the final wash lasted all night), the blocks were infused for the next 24 h in a mixture of sucrose (2.5 M) and polyvinyl pyrrolidone (20%) and then frozen in liquid nitrogen. Sections (0.2 μm) were cut and positioned on glass slides. The sections were blocked with 1.2% gelatin in PBS (PBSG) for 30 min to avoid nonspecific binding and were subsequently incubated overnight at 4°C in 100% humidity with the primary antibodies for Bcl-2 (polyclonal rabbit IgG and AB-2 from Oncogene Research Products, Cambridge, MA).

**Table 1. Surgical events and treatments for experimental groups**

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Surgery on Day 1</th>
<th>Surgery on Day 8</th>
<th>Treatments from Day 8 to the Day Animals Were Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, XII</td>
<td></td>
<td></td>
<td>sulpiride</td>
</tr>
<tr>
<td>III, XIV</td>
<td>castration</td>
<td></td>
<td>sulpiride</td>
</tr>
<tr>
<td>IV, XV</td>
<td>castration</td>
<td>DHT implant</td>
<td>sulpiride</td>
</tr>
<tr>
<td>V, XVI</td>
<td>castration</td>
<td>DHT implant</td>
<td>sulpiride</td>
</tr>
<tr>
<td>VI, XVII</td>
<td>castration</td>
<td>T implant</td>
<td>sulpiride</td>
</tr>
<tr>
<td>VII, XVIII</td>
<td>castration</td>
<td>T implant</td>
<td>sulpiride</td>
</tr>
<tr>
<td>VIII, XIX</td>
<td>sham castration</td>
<td></td>
<td>sulpiride</td>
</tr>
<tr>
<td>IX, XX</td>
<td></td>
<td></td>
<td>solvent</td>
</tr>
<tr>
<td>X, XI</td>
<td></td>
<td></td>
<td>solvent</td>
</tr>
<tr>
<td>XI, XXII</td>
<td></td>
<td></td>
<td>solvent</td>
</tr>
</tbody>
</table>

At the end of the study, animals were killed (on day 39 for groups I to XI and on day 69 for groups XII to XXII). Ventral, lateral, and dorsal prostate lobes were excised and weighed. Trunk blood was collected for hormone level measurements, and one hemilobe of each prostate lobe was histologically analyzed.
MA). After several washes in PBSG, the slides were incubated for 1 h at 37°C with secondary antibodies (donkey anti-rabbit IgG labeled with FITC), washed in PBS, and mounted in Mowiol. The sections were observed under a Zeiss Axioshot microscope equipped with epifluorescence (excitation: 450–490 nm, emission: 520 nm). Negative controls consisted of omission of the primary antibody.

**Western analysis.** The cells or the tissues were disrupted in a Kontes glass tissue grinder fitted with a tight pestle. After centrifugation (10,000 g), the pellets were fractionated in a standard SDS-PAGE gel (15%) (29). After electrophoresis was complete, the proteins were transferred onto a nitrocellulose membrane by use of a semi-dry electroblotter (BioRad). After the transfer was complete, the membrane was cut into thin strips that were further processed for Western blot. The strips were blocked for 1 h at room temperature in TNT (15 mM Tris buffer, pH 8, 140 mM NaCl, 0.05% Tween 20, and 5% nonfat dry milk), washed in TNT (3 times), and then soaked in primary antibodies (1 µM/ml in TNT; rabbit polyclonal antibody for the rat tissues (AB-2 from Oncogene Research Products), mouse monoclonal antibody for the cells (Sc-509 from Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit polyclonal anti-actin (Sigma), for 1 h at room temperature. After thorough washes in TNT, the strips were transferred to the corresponding horseradish peroxidase-linked secondary antibodies (Zymed Laboratories, San Francisco, CA) diluted in TNT (1/7,500) for 1 h. After several washes in TNT without milk, the strips were processed for chemiluminescent detection using Supersignal West Pico chemiluminescent substrate (Pierce Chemical, Rockford, IL) according to the manufacturer’s instructions. The blots were then exposed to X-Omat AR films (Eastman Kodak, Rochester, NY).

**Statistical analysis.** We expressed prostate weight relative to body weight (54). Variations in both prostate weight and plasma hormone levels were studied. Tukey's test was used to establish significant differences. Significance was established at levels of P < 0.05, P < 0.01, and P < 0.001.

**RESULTS**

**Hormonal assays: induction of chronic hyperprolactinemia in rats treated by sulpiride.** We measured PRL, T, DHT, and LH levels from blood plasma of rats.

Sulpiride induced a rise in basal plasma PRL levels in all groups of animals treated (Table 2). It enhanced the basal PRL level by a factor of 6.2 under control conditions and 3.6 in castrated animals. In castrated DHT-implanted rats, sulpiride injections increased the basal PRL level by a factor of 12. Because previous reports (13, 15, 23) indicated an increase of PRL during stress, sham-castrated and solvent-injected groups were also evaluated. It had previously been shown that empty tubing implants had no effect on rat prostate growth (42, 53, 54). PRL levels in solvent-injected animals were not significantly different from those in controls (data not shown).

In noncastrated animals, hyperprolactinemia induced a decrease of 55.68% in LH levels and 30.83% in T levels. As anticipated, hyperprolactinemia did not modify T and DHT levels in castrated and castrated T- or DHT-implanted groups.

Orchidectomy induced a dramatic decrease in T and DHT levels compared with control. T (1 cm) and DHT (1 cm) implants restored T levels to 45.78% and DHT to 336.60% of control, respectively. It has been found that a 2.5-cm implant mimics physiological T level (53). The chosen 1-cm implants produced a subnormal T release.

We also studied castrated-adrenalectomized, castrated-adrenalectomized and sulpiride-injected, sham-castrated and sham-adrenalectomized, and sham-castrated and sham-adrenalectomized solvent-injected groups to assess the role of adrenals in prostate growth. We did not notice any significant differences between T or DHT levels in castrated and castrated-adrenalectomized animals (data not shown).

**Effects of hyperprolactinemia on the wet weight of prostate lobes.** The wet weight of the prostate was examined when the animals were killed after 30 and 60 days of treatment with sulpiride, respectively. Because rat prostate is divided into three parts: the ventral lobe (VP), the lateral lobe (LP), and the dorsal lobe (DP), we show the different results obtained for each lobe.

Figure 1 illustrates the wet weight of the LP after 30 days of sulpiride treatment under various experimental conditions. In castrated and implanted animals, T and DHT induced an increase in the wet weight of the lateral lobe by factors of 3.8 and 5.1, respectively, compared with castrated rats.

Sulpiride injections enhanced the weight of lateral lobes in noncastrated animals 4.1 times compared with control. In castrated, T-implanted animals, sulpiride induced a 2.4-fold increase in the LP weight compared with noninjected rats. In castrated, DHT-implanted animals, hyperprolactinemia in those treated with sulpiride enhanced the growth of the LP by a factor of 1.6 compared with noninjected rats. There were no

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Prolactin, ng/ml</th>
<th>T, pg/ml</th>
<th>DHT, pg/ml</th>
<th>LH, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.16 ± 2.57</td>
<td>2,087 ± 272</td>
<td>183 ± 32</td>
<td>0.728 ± 0.096*</td>
</tr>
<tr>
<td>Control, sulpiride</td>
<td>62.54 ± 26.77</td>
<td>1,444 ± 184</td>
<td>181 ± 12</td>
<td>0.323 ± 0.019*</td>
</tr>
<tr>
<td>Castrated</td>
<td>25.40 ± 72.01</td>
<td>ND</td>
<td>ND</td>
<td>15.572 ± 1.348</td>
</tr>
<tr>
<td>Castrated, sulpiride</td>
<td>91.50 ± 18.98</td>
<td>ND</td>
<td>ND</td>
<td>15.25 ± 2.927</td>
</tr>
<tr>
<td>Castrated, DHT</td>
<td>5.84 ± 1.678</td>
<td>202 ± 62</td>
<td>617 ± 95</td>
<td>0.170 ± 0.027</td>
</tr>
<tr>
<td>Castrated, DHT, sulpiride</td>
<td>70.60 ± 44.20</td>
<td>219 ± 25</td>
<td>439 ± 27</td>
<td>0.372 ± 0.187</td>
</tr>
<tr>
<td>Castrated, T</td>
<td>25.87 ± 6.036</td>
<td>955 ± 75</td>
<td>203 ± 47</td>
<td>4.603 ± 0.834</td>
</tr>
<tr>
<td>Castrated, T, sulpiride</td>
<td>68.60 ± 15.01</td>
<td>971 ± 232</td>
<td>154 ± 18</td>
<td>3.044 ± 1.124</td>
</tr>
</tbody>
</table>

T, testosterone; DHT, dihydrotestosterone; LH, luteinizing hormone. Trunk blood was collected from Wistar rats after long-term treatment (60 days) and analyzed by RIA. Data are expressed as group means ± SE in pg/ml; n = 5 group. ND, nondetected values. P < 0.01: *b, P < 0.05: c,d.
apparent differences between the LPs of castrated and castrated sulpiride-injected groups. No enlargement of the LP was observed after sham surgery and/or solvent injections.

Similarly, after 60 days, T and DHT enhanced the weight of the LP in castrated rats implanted with T or DHT by factors of 5.3 and by 3.6, respectively, compared with castrated animals (Fig. 1). Sulpiride injections induced a twofold increase in the LP wet weight in noncastrated animals compared with control, and a threefold increase in those of castrated, T-implanted, and sulpiride-injected animals compared with noninjected rats. In the castrated, DHT-implanted, and sulpiride-injected group, PRL enhanced the wet weight of the LP by a factor of 3.2 compared with noninjected animals.

No significant differences were observed between the LPs of castrated and castrated sulpiride-injected groups after 30 and 60 days. No enlargement of the LP occurred after sham surgery and solvent injections.

We also measured the relative weights of the VP and the DP after all the types of treatment described in Table 1. The analysis of these data showed that hyperprolactinemia has little or no effect on the growth of the VP and DP in castrated, adrenalectomized, sulpiride-injected animals were similar to those of castrated-adrenalectomized and castrated rats (data not shown).

Induction of glandular hyperplasia of LP in sulpiride-treated rats. Similar histological aspects were observed after 30 and 60 days of experimentation. Sulpiride had no effect on VP and DP morphology.

The LP of normal (Fig. 4A), sham-castrated, and solvent-injected rats (data not shown) was most fre-

Fig. 1. Histogram showing total lateral prostate (LP) weight (mg) divided by total body weight (g). Animals received treatment described in Table 1. Values are means ± SE; n = 5. Solid columns, 30 days of treatment; open columns, 60 days of treatment. Ctl, control animals; Su, sulpiride treated; Cast, castrated; DHT, dihydrotestosterone (DHT) implanted; T, testosterone (T) implanted; Sh Cast, sham castrated; Solv, solvent injected. Values of treatments shown by the following superscript letters are significantly different: $P < 0.001$: a, b, f; $P < 0.01$: c, g, h, i; $P < 0.05$: d, e, j.

Fig. 2. Histogram showing total ventral prostate (VP) weight (mg) divided by total body weight (g). Animals received treatment described in Table 1. Values are means ± SE; n = 5. Solid columns, 30 days of treatments; open columns, 60 days of treatment. Values of the treatments shown by the following superscript letters are significantly different: $P < 0.001$: a, b, d, e; $P < 0.05$: c, f.

Fig. 3. Histogram showing total dorsal prostate (DP) weight (mg) divided by total body weight (g). Animals received treatment described in Table 1. Values are means ± SE; n = 5. Solid columns, 30 days of treatments; open columns, 60 days of treatment. Values of treatments shown by the following superscript letters are significantly different: $P < 0.01$: a, c, d; $P < 0.05$: b, e.
quently composed of the same proportion of small and large glands, both limited by columnar epithelial cells. The LPs of castrated rats without any substitutive treatment presented atrophy with a large majority of small glands (Fig. 4B). The LP atrophy of castrated animals receiving a substitutive treatment with T (Fig. 4C) or with DHT (data not shown) was particularly attenuated compared with LP of sham-castrated rats (Fig. 4B).

LPs from castrated, T-implanted rats (Fig. 4D) and normal rats (data not shown) receiving additional sulpiride treatment showed an increased proportion of large glands, some containing numerous neutrophils (Fig. 4E). Similar histological aspects were observed in the LP of castrated, DHT-implanted animals receiving additional sulpiride treatment (data not shown). The connective tissue surrounding these inflamed glands was focally infiltrated by lymphocytes and a few neutrophils and macrophages and was thickened by a discrete fibroblastic and collagenic fibrosis, sometimes associated with a slight muscular hyperplasia (Fig. 4F).

Thus, in the presence of androgenic activity, sulpiride treatment seems to induce LP glandular hyperplasia and, to a lesser extent, a fibromuscular hyperplasia focally associated with nonspecific, acute, chronic inflammation.

**Overexpression of Bcl-2 in the LP of control and sulpiride-treated rats.** The control LP sections showed a spotted cytoplasmic labeling (organelles, except the nucleus) on the whole of epithelial cells (Fig. 5A). In control and sulpiride-treated LP, the labeling was intensified in the epithelial cells bordering the acini, giving target-like images that become brighter at the periphery of the acini (Fig. 5B). The infiltrated cells were only faintly labeled. In the VP, the labeling observed under control and control sulpiride-treated conditions was similar to that shown in the control LP sections (data not shown).

To confirm the overexpression of Bcl-2 in the LP in this series of experiments, we performed a Western blot analysis (Fig. 6). As an additional control of this study, we also analyzed rats implanted with osmotic pumps filled with PRL. Such an approach is commonly used to increase hormonal level (41). As negative control, we used androgen-insensitive human cancer prostate LNCaP (lymph node carcinoma prostate) cells. We also studied native LNCaP cells and MCF7 human breast cancer cells known to express Bcl-2 (33). The level of Bcl-2 in control VP, LP, and DP is slight (Fig. 6, lanes 1, 2, and 3). On the contrary, the expression of Bcl-2 is higher in the LP of sulpiride-treated rats (lane 5) or in the osmotic pump-implanted rats (lane 7) than in the control LP (lane 1) or in the VP (lane 4) or the DP (lane 6) of sulpiride-injected rats. LNCaP cells (lane 8) express Bcl-2 in a low level. As expected, we did not notice any labeling in DU 145 (lane 9). Bcl-2-transfected LNCaP (lane 10) and MCF7 (lane 11) express a high level of Bcl-2. As control, the similar expression of a housekeeping protein (actin) in each lane shows equal amounts of total proteins in each well.

**DISCUSSION**

In this study we demonstrate the effects of chronic hyperprolactinemia on rat prostate growth. A particularly significant alteration in the rats with chronic hyperprolactinemia was a dramatic enlargement of the LP. This LP hyperplasia was focally associated with acute, chronic inflammation.

To induce a chronic hyperprolactinemia, we used a model of long-term (30 and 60 days) androgen treatment associated with daily sulpiride injections. Sulpiride, a specific D2 inhibitor, is known to stimulate PRL secretion from the pituitary (11, 35). It also has two actions on PRL plasma levels: sulpiride initially induces a peak of prolactinemia in ~30 min (26 times the initial value), followed by a gradual reduction in PRL levels. After 2 h of treatment, PRL levels remain higher (6 times the basal level) than the initial value before injection. Thus, a chronic (60-day) treatment with sulpiride causes a significant increase in plasma PRL level (62.54 ± 26.77 ng/ml) compared with control (10.16 ± 2.57 ng/ml) or solvent-injected (18.9 ± 9.03 ng/ml) animals. In our experiments, the rats were killed on the day after the last sulpiride injection, so the prolactinemia measured does not represent the PRL peak, but rather the chronic PRL level after 60 days of treatment with sulpiride.

Our experiments in castrated-adrenalectomized rats show that the contribution of the adrenals to endogenous androgen level support is negligible. Thus the adrenals do not interfere with rat prostate growth. These results are in agreement with van Weerden et al. (61): these authors measured a very low level of androstenedione and no detectable plasma concentrations of...
dehydroepiandrosterone in rat adrenal gland cell suspensions. On the contrary, in humans, the adrenals significantly contribute to the control of the androgen level (34).

In a previous work (60), we demonstrated that hyperprolactinemia induced rat LP enlargement. In the present study we characterize one of the possible mechanisms of action of PRL on the prostate: the overexpression of Bcl-2. The effects of hyperprolactinemia were observed only in the LP of the rat prostate, affecting wet weight, histological structure, and Bcl-2 expression. The VP and DP were insensitive to the rise in PRL and did not show any Bcl-2 overexpression. The LP is considered the most hormone-sensitive part of the prostate (30, 54, 55). It has been shown that the dorsolateral lobes are the parts of the rat prostate that give rise to spontaneous and experimental tumors (45–47) with varied hormone responsiveness. Moreover, the rat LP and DP are considered to be the most homologous to the human prostate (48). Furthermore, it has been suggested that PRL plays an important role in BHP and human prostate cancer development (24, 25, 37, 39).

Hyperprolactinemia, stimulated by daily sulpiride injections, induced a marked enlargement of the LP in noncastrated animals (4.1 times control after 30 days and 2 times control after 60 days). PRL had no effect on castrated animals. However, in castrated, T-implanted, and castrated DHT-implanted rats, the rise in PRL levels also increased the weight of the LP (by a factor of 2.4 for T and 1.6 for DHT, respectively, after 30 days and by a factor of 3 for T and 3.2 for DHT, respectively, after 60 days), suggesting that PRL acts in synergy with the androgens.

In our experiments, 1-cm T implants partially restored (45%) normal T level. Robaire et al. (53) showed that 2.5-cm T implants are needed to restore the physiological T level in the rat. However, 1-cm DHT implants increased the DHT level by 336% compared with the physiological level in noncastrated rats. This difference in T and DHT level of recovery by use of 1-cm implants is explained by the fact that the physiological level of DHT (formed by 5α-reductase from T) was lower (0.183 ± 0.032 ng/ml) than the T level (2.087 ± 0.272 ng/ml).

The T level was higher in noninjected rats than in animals treated with sulpiride (Table 2). Because it has been shown that hyperprolactinemia decreases the number of pituitary gonadotropin-releasing hormone receptors and LH secretion (4, 8), the T level, controlled by LH, is therefore also reduced. Thus, in our experiments, the rise in weight of the LP in the noncastrated sulpiride-injected animals could be explained by the rise in PRL level.

In men, the PRL level increases (17, 62) and the T level diminishes with age (10, 36). Some research has demonstrated the same changes in rat PRL (8) and T (4) levels. In our study, in castrated T-implanted groups, the T level was 55% less than control. We used 1-cm T implants, which deliver one-half a physiological T level, to mimic the T level of aging rats. In our study, the hyperprolactinemia induced by sulpiride produced LP hyperplasia. Thus, in old rats and in aging men, a rise in prolactinemia could be sufficient to induce prostate hyperplasia, even with lower T levels. In this in vivo model, it is possible to develop a hormonal environment similar to that of aging rats and elderly men.

Our histological studies demonstrate that hyperprolactinemia induces glandular hyperplasia in the LP, but not the DP and VP, in the rats with increased PRL levels. An increase was observed in the proportion of large glands associated with inflammation. This was not the case in the LP of animals not injected with sulpiride. Robinette (54) reported that estradiol-17β had a specific action on LP growth. Estradiol-17β causes inflammation in dorsolateral (30) and lateral rat prostates (59) that can be reduced by treatment with bromocriptine (a dopaminergic agonist known to decrease PRL levels). Thus the potent involvement of estradiol-17β in prostate dysplasia and inflammation implies PRL action. Moreover, an enlargement of the dorsolateral prostate was shown (66) in transgenic mice with an overexpressed PRL receptor, suggesting the implication of PRL in the growth of the prostate gland.

The mechanism by which PRL affects prostate growth is not yet known. In our study we noticed synergistic effects between PRL and androgens. This phenomenon could be due to the fact that PRL enhances the T effect (14), as well as increasing cytosol and nuclear androgen receptor levels in rats (49). Furthermore, androgens (40, 49) and PRL (49, 50) upregulate PRL receptor levels in the rat prostate. Moreover, hyperprolactinemia induces the turnover of tissue DHT content in the LP (5, 50, 55). Some in vitro studies have demonstrated an independent action of PRL in prostate cells (48, 52, 58). PRL also has an androgeno-independent proliferative effect in the rat LP in organ culture (31). PRL receptors have been identified in the VP (43), as well as in both LP and DP rat and human prostates (39). Some of these receptors are located on the basal and lateral surfaces of the epithelial cells (39). These receptors may fix circulatory PRL and induce the growth of the LP in rats. Nevertheless, PRL receptors are also located on the apical surfaces of the secretory epithelial cells of prostatic acini (39). Because the epithelial prostatic cells are joined by tight junctions, PRL receptors located on the apical surface of these cells are not accessible to circulatory PRL, unlike basolateral cell membrane receptors. However, in recent experiments, Nevalainen and colleagues (37, 39) clearly demonstrated that prostatic epithelial cells were able to produce PRL. They used in situ hybridization to show that the epithelium of rat DP and LP expressed PRL mRNA and protein. Thus prostatic PRL may act in an autocrine/paracrine manner in the prostate through apical receptors, where it may mediate some androgen actions. The incidence of serum
hyperprolactinemia on prostatic PRL synthesis is unknown.

Interestingly, the experiments carried out by Nevalainen et al. (37) demonstrated that the expression pattern of PRL protein was different in the DP and LP. In the DP, the cytoplasm of sparsely located, single epithelial cells was very strongly stained. In contrast, in the LP, the majority of the epithelial cells were stained, but the staining was less intense than in the DP. These results probably explain the fact that, in our experiments, we did not observe the effect of PRL on the DP.

In addition, for the first time, we observed a rise in the expression of the antiapoptotic protein Bcl-2 in the LP epithelial cells of noncastrated animals treated with sulpiride. We did not notice this phenomenon either in the VP or in the DP. Bcl-2 is known to downregulate apoptosis in prostate cells (7, 28, 44, 51), as in other models (19, 21, 63, 65). Bcl-2 also provides resistance to androgen depletion in androgen-sensitive human prostate cancer cells LNCaP (51). Thus, in this in vivo model of hyperprolactinemia, the overexpression of Bcl-2 in noncastrated animals led to a decrease in the apoptosis level in the epithelial cells of the LP. This modifies the balance between proliferation and apoptosis, eventually causing the LP hyperplasia shown in the histological study. In the NB2 rat lymphoma cell line, PRL induced a 15-fold increase in the level of Bcl-2 mRNA within 3 h (32). The authors suggest that the trophic action of PRL results from suppression of cell death induced by the rise in the expression of Bcl-2 (27, 32). Recently, it was shown that PRL is a survival factor in LP epithelium in organ culture (2). In our study, the increase in weight of the LP may be explained by PRL-induced inhibition of apoptosis mediated by Bcl-2 overexpression. Furthermore, the tissue-specific modulation of Bcl-2 expression by PRL in the rat prostate may explain the lack of sensitivity of the VP to PRL, even if each rat prostate lobe has PRL receptors (3, 18, 26, 38). The antiapoptotic action of PRL on prostate cells requires further investigation and may be useful in developing a treatment.

In conclusion, the chronic hyperplasia model proposed in this work may serve as a useful approach for studying the development mechanism of prostate hyperplasia. This model, representing PRL-dependent hyperplasia, is probably close to the human pathology, where the implication of PRL is uncontested.

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