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Research Communication

Effects of Sulpiride on mRNA Levels of Steroid 5α-Reductase Isozymes in Prostate of Adult Rats

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Summary

Prolactin (PRL) is implicated in prostate growth and in the development and regulation of benign prostatic hypertrophy (BPH) and prostate cancer (PCa). PRL may exert its effects on prostate in synergism with androgens. The most active androgen in the prostate is the 5α-dihydrotestosterone (DHT) obtained from testosterone by the 5α-reductase (5α-R) enzyme, which is expressed in the prostate as two isozymes, 5α-R1 and 5α-R2. In this study, sulpiride, a prolactin-secretion inductor, was administered to male rats. mRNA levels of 5α-R1 and 5α-R2 were measured in prostate of controls and sulpiride-treated rats, using one-step quantitative RT-PCR coupled with laser-induced fluorescence capillary electrophoresis (LIF-CE). Results demonstrated that sulpiride-induced hyperprolactinemia is associated with an increase in mRNA levels of both 5α-R1 and 5α-R2 in prostate of adult rats. Although a direct effect of sulpiride on prostate gland cannot be ruled out, hyperprolactinemia may be a factor to be considered in aging males, in whom prostatic diseases such as BPH and PCa are more frequent.

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Keywords 5α-reductase isozymes; transcriptional regulation; hyperprolactinemia; prostatic diseases; rat.

Abbreviations PRL, prolactin; BPH, benign prostatic hypertrophy; PCa, prostate cancer; DHT, 5α-dihydrotestosterone; 5α-R, 5α-reductase; LIF-CE, laser-induced fluorescence capillary electrophoresis; 6-FAM, 6-carboxyfluorescein; IS, internal standard; DEPC, diethyl pyrocarbonate.

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INTRODUCTION

The growth, differentiation, and programmed cell death of prostate cells are regulated by androgens (*1–4*). For this reason, treatments currently used in hormonotherapy for prostate disease are aimed only at inhibiting the effect of androgens on prostate cell growth (*5*). It is well established that the most active androgen in the stimulation of prostate cell proliferation is the 5α-dihydrotestosterone (DHT) (*6*) obtained from testosterone by 5α-reductase (5α-R) enzyme (*7*). 5α-R is expressed as two isozymes, 5α-R1 and 5α-R2, and it has been demonstrated that both isozymes are present in rat prostate by our group and other investigators (*8, 9*). Other hormones besides androgens are also implicated in prostate growth, for example, estrogens (*10*).

It is becoming clear that prolactin (PRL) is implicated in prostate growth (*11–15*) and in the development and regulation of BPH and prostate cancer (*16–20*). Some authors reported that PRL may promote the growth and proliferation of prostate cells in synergism with androgens (*21*), whereas others suggested that PRL has an independent action on prostatic growth and metabolism (*22–24*). We previously reported that stress hormones such as ACTH and CRH increase 3α,5α-reduced neurosteroids levels in plasma and brain of rats (*25*), and 5α-R enzyme plays a key role in the biosynthesis of these steroids. We also recently observed (unpublished data) that stress situations increase transcription of 5α-R genes in brain. PRL is another hormone secreted in stress situations (*26, 27*). Therefore, it is reasonable to speculate that the 5α-R enzyme may also mediate the action of PRL in the prostate gland.

The aim of this study was to investigate the effects of sulpiride-induced hyperprolactinemia, a specific D2 inhibitor (*28, 29*), on mRNA levels of 5α-R1 and 5α-R2 in prostate of adult rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 260–280 g were housed in an air-conditioned room with fluorescent lights on from 7:00 a.m. to

7.00 p.m. and given standard laboratory pellet chow and water *ad libitum*. Experiments were made strictly in accordance with the NIH guide for the "Care and Use of Laboratory Animals."

Chronic hyperprolactinemia was induced by daily intraperitoneal (i.p.) injections of 40 mg/kg aqueous sulpiride solution (Sigma) according to Van Coppenolle *et al.* (*30*). The rats were always injected at 9:00 a.m. At 30 min, after the final injection, the rats were euthanized by decapitation to avoid possible adverse effects of anesthesia. Blood samples were collected in heparinized tubes. The blood was centrifuged at 2000 rpm for 10 min. The plasma was separated and stored at −20°C until hormonal measurements were performed. The prostate was removed, weighed, frozen in liquid nitrogen, and stored at −80°C until analysis was performed on the whole prostate. Each study group (sulpiride-treated and controls) comprised 10 animals.

Hormone Assays

Plasma PRL levels were measured by ELISA using an enzyme immunoradiometric kit (Milenia, Rat Prolactin, Germany). All samples were assayed in duplicate and in the same assay. Intra-assay coefficient of variation was 4.3%, and the sensitivity was 0.6 ng/ml.

Plasma testosterone (T) concentrations were measured using a radioimmunoassay (RIA) kit (Sorin, Milan, Italy). All samples were assayed in duplicate and in the same assay. Intra-assay coefficient of variation was 7.4%, and the sensitivity was 0.05 ng/ml.

Oligonucleotides Used for Amplifications

Sequences of rat 5α-R (EC 1.3.99.5) isozymes were obtained from GeneBank® (J05035, M95058), and the sequence of plasmid pEGFP-C1 was obtained from the Clontech web page. These sequences were used to design the primer pairs. All forward primers were end labeled with 6-carboxy-fluorescein (6-FAM). Oligonucleotides were synthesized by PE-Applied Biosystems (Warrington, UK). Primer sequences (5′–3′) and PCR product sizes were obtained as previously described (*31*).

Construction of Internal Standard Templates

Two synthetic internal standard (IS) DNAs of 300 bp were synthesized from the sequence of plasmid pEGFP-C1 (Clontech, Palo Alto, CA) as previously described (*31*). Briefly, both competitive molecules, IS-1 (competitor DNA of 5α-R1) and IS-2 (competitor DNA of 5α-R2), were obtained after two consecutive amplifications from pEGFP-C1, with 5′ and 3′ ends modified to contain the same nucleotide sequences as SRD5A1 or SRD5A2 (*31*).

Reverse Transcription-Polymerase Chain Reaction

Total RNA was extracted from 25 mg of rat prostate tissues by acid-guanidinium thiocyanate-phenol-chloroform (*32*). The RNA was resuspended in diethyl pyrocarbonate (DEPC)-treated water and spectrophotometrically quantitated for analysis. First-stranded cDNA was carried out according to Torres and Ortega (*31*). The PCR profile was as follows: denaturing, 94°C for 30 seconds; annealing, 55°C for 30 seconds; and extension, 72°C for 30 seconds. The number of cycles was 35 in all cases. PCR was carried out in a PerkinElmer 2400 Thermal Cycler.

Analysis of PCR Products

A capillary electrophoresis (CE) system with laser-induced fluorescence (LIF) detection (ABIPRISM 310 Genetic Analyzer, Applied Biosystems) was used to characterize RT-PCR products. CE conditions were used as previously reported (*31*).

Fluorescence ratios of both 5α-R/IS were plotted against the amount of the appropriate competitive DNA, and the concentration of target DNA in the sample was calculated following Torres and Ortega (*31*).

Statistical Analysis

SPSS (version 13) software package was used for the statistical analysis. Comparison between groups was studied by means of Student's *t* test. Data are expressed as mean ± SE.

RESULTS

Plasma Hormone Levels

Plasma PRL levels were significantly higher in sulpiride-treated rats than in control rats (Fig. 1A). There was no significant difference in plasma T levels between sulpiride-treated and control rats (Fig. 1B).

Effects of Hyperprolactinemia on Wet Weight of Prostate

The wet weight of the prostate was measured after 30 days of sulpiride treatment and was significantly higher in sulpiride-treated rats than in controls (Fig. 2).

Quantitation of 5α-R1 mRNA Levels in Prostate

The amount of mRNA was expressed as number of mRNA copies per micrograms of total RNA. After the cDNA was generated from total RNA by RT reaction, it was co-amplified in the presence of decreasing amounts of competitive DNA ($64 \times 10^6 - 0.5 \times 10^6$ molecules). 5α-R1 cDNA and competitive-standard DNA IS-1 were co-amplified using the same pair of primers. With decreasing amounts of competitive DNA, the relative intensity of amplified product of target DNA increased. The ratio of fluorescence of 5α-R1 /IS-1 was plotted against the amount of competitive DNA IS-1.

As shown in Fig. 3, 5α-R1 mRNA levels were significantly higher in prostate of sulpiride-treated rats *versus* controls.

Quantitation of 5α-R2 mRNA Levels in Prefrontal Cortex

Likewise, 5α-R2 cDNA and competitive-standard DNA IS-2 were co-amplified using the same pair of primers. With decreas-

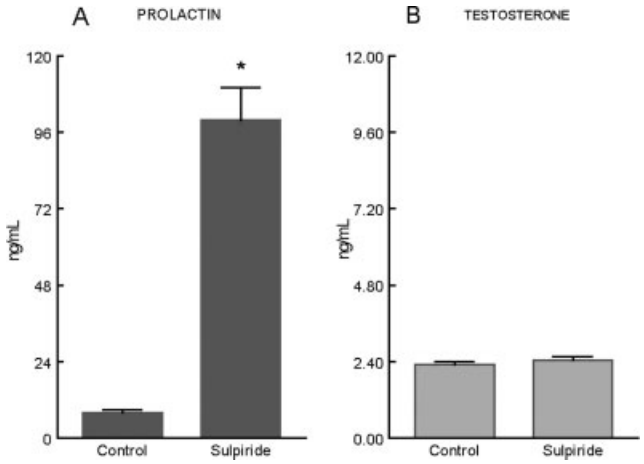

Figure 1. A: Plasma prolactin levels in controls and sulpiride-treated rats. $*p < 0.001$ *versus* control. B: Plasma testosterone levels in controls and sulpiride-treated rats. No significant differences were found.

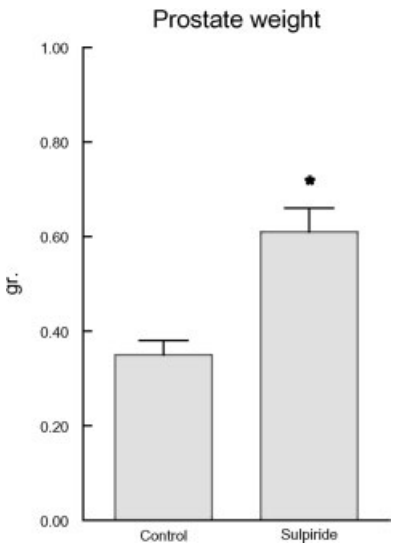

Figure 2. Effects of hyperprolactinemia on prostate weight (g) of controls and sulpiride-treated rats. $*p < 0.01$ *versus* control.

ing amounts of competitive DNA, the relative intensity of the amplified product of target DNA increased. The ratio of fluorescence of 5α-R2 /IS-2 was plotted against the amount of competitive DNA IS-2.

As shown in Fig. 3B, 5α-R2 mRNA levels were significantly higher in prostate of sulpiride-treated rats *versus* controls.

DISCUSSION

The results of this experiment clearly demonstrated that the administration of sulpiride is associated with an increase in mRNA levels of both 5α-R1 and 5α-R2 isozymes. These effects

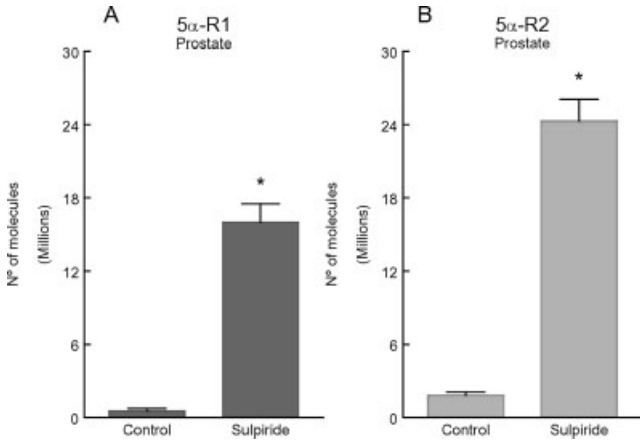

Figure 3. A: Effects of hyperprolactinemia on steroid 5α-reductase type 1 (5α-R1) mRNA levels in prostate of controls and sulpiride-treated rats. $*p < 0.001$ *versus* control. B: Effects of hyperprolactinemia on steroid 5α-reductase type 2 (5α-R2) mRNA levels in prostate of controls and sulpiride-treated rats. $*p < 0.001$ *versus* control.

of sulpiride on prostate gland may be exerted via sulpiride-induced hyperprolactinemia, although a direct effect of sulpiride on 5α-R isozyme mRNA cannot be ruled out because sulpiride is a specific inhibitor of dopamine D2 receptor (*28, 29*), present in rat prostate gland (*33*). It has long been known that PRL promotes the growth and proliferation of prostate cells (*11–15*), and sulpiride-induced hyperprolactinemia has been associated with increased prostatic weight (*34*). Likewise, ovine PRL administration enhanced prostatic weight and 5α-R activity (*35*), indicating a direct effect of PRL on prostate gland.

PRL may exert its effects on the prostate independently of androgens, as was demonstrated in *in vitro* models of the prostate of PRL-transgenic mice (*13, 16, 22–24, 36*). PRL may also act in prostate via androgens (*21*). PRL augments free steroid concentrations in blood and testosterone uptake in prostate cells (*37*). Other investigators demonstrated that hyperprolactinemia affected prostate enlargement in T-treated castrated animals but not in similar untreated animals (*30*), demonstrating that androgens mediate the effects of PRL on prostate growth.

The main androgen in the prostate gland is 5α-DHT, produced from T by 5α-R enzyme (*7*). The prostate gland expresses both 5α-R isozymes (5α-R1 and 5α-R2), as previously demonstrated by our group and other researchers (*8, 9*). In stress situations, PRL is hypersecreted (*26, 27*), and there is an increase in both 5α-R isozymes in rat brain (unpublished observations). Hence the effects of PRL on prostate growth may be exerted by an increase in both 5α-R isozymes in this gland, among other factors. Our group previously reported that both 5α-R isozymes may contribute to the increased DHT in prostate, which acts in both an autocrine and a paracrine manner in the

prostate, stimulating its differentiation and growth (*8*). The demonstration by Van Coppenolle *et al.* (*30*) that hyperprolactinemia is effective to induce prostate enlargement in castrated DHT-implanted animals is not contradicted by the present findings. Besides, augmenting the amount of prostatic DHT by increasing both 5α-R isozymes, PRL probably enhances the effects of DHT in this gland.

Several animal and human studies have indicated that hyperprolactinemia is related to plasma-androgen concentrations, although findings are controversial (*38–40*). Because both 5α-R1 and 5α-R2 genes are positively regulated by T, the most abundant androgen (*41*), we measured plasma T levels in controls and sulpiride-treated rats. The lack of a significant difference between these groups rules out T as responsible for the increased mRNA levels of 5α-R isozymes in sulpiride-treated rats.

This study offers new data on the effects of sulpiride-induced hyperprolactinemia on the prostatic gland, including an increase in the mRNA levels of both 5α-R isozymes. Increased 5α-R1 is associated with PCa, and increased 5α-R2 is associated with PCa and BPH, which are highly frequent diseases in developed countries (*42*). Therefore, high-PRL levels in ageing males may be considered as a new risk factor for prostatic diseases. Thus, Yatani *et al.* (*43*) showed an association between elevated PRL levels and PCa in humans.

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REFERENCES

1. Horton, R. (1992) Benign prostatic hyperplasia. New Insights. *J. Clin. Endocrinol. Metab.* **74**, 504A–504C.
2. Geller, J. (1989) Pathogenesis and pathological treatment of benign prostatic hyperplasia. *Prostate* **2**, 95–104.
3. Isaacs, J. (1984) Antagonistic effect of androgen on prostatic cell death. *Prostate* **5**, 545–557.
4. Walsh, P. (1984) Benign prostatic hyperplasia: etiological considerations. *Prog. Clin. Biol. Res.* **145**, 1–25.
5. Carraro J. C., Raynaud, J. P., Koch, G., Chisholm, G. D., Di Silverio, F., Teillac, P., Da Silva, F. C., Cauquil, J., Chopin, D. K., Hamdy, F. C., Hanus, M., Hauri, D., Kalinteris, A., Marencak, J., Perier, A., and Perrin, P. (1996) Comparison of phytotherapy (Permixon) with finasteride in the treatment of benign prostate hyperplasia: a randomized international study of 1,098 patients. *Prostate* **29**, 231–240.
6. Wright, A. S., Thomas, L. N., Douglas, R. C., Lazier, C. B., and Rittmaster, R. S. (1996) Relative potency of testosterone and dihydrotestosterone in preventing atrophy and apoptosis in the prostate of the castrated rat. *J. Clin. Invest.* **98**, 2558–2563.
7. Rusell, D. W., and Wilson, J. D. (1994) Steroid 5α-reductase. Two genes/two enzymes. *Annu. Rev. Biochem.* **63**, 25–61.
8. Torres, J. M., Ruíz, E., and Ortega, E. (2003) Development of a quantitative RT-PCR method to study 5α-reductase mRNA isozymes in rat prostate in different androgen status. *Prostate* **56**, 74–79.
9. Mahendroo, M. S., Cala, K. M., Hess, D. V., and Rusell, D. W. (2001) Unexpected virilization in male mice lacking steroid 5α-reductase enzymes. *Endocrinology* **142**, 4652–4662.
10. Pelletier, G. (2002) Effects of estradiol on prostate epithelial cells in the castrated rat. *J. Histochem. Cytochem.* **50**, 1517–1524.
11. Lane, K. E., Leav, I., Ziar, J., Bridges, R. S., Rand, W. M., and Ho, S. M. (1997) Suppression of testosterone and estradiol-17β-induced dysplasia in the dorsolateral prostate of Noble rats by bromocriptine. *Carcinogenesis* **18**, 1505–1510.
12. Nevalainen, M. T., Valve, E. M., Ingleton, P. M., Nurmi, M., Martikainen, P. M., and Harkonen, P. L. (1997) Prolactin and prolactin receptors are expressed and functioning in human prostate. *J. Clin. Invest.* **99**, 618–627.
13. Wennbo, H., Kindblom, J., Isaksson, O. G. P., and Tornell, J. (1997) Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland. *Endocrinology* **138**, 4410–4415.
14. Costello, L. C., and Franklin, R. B. (1994) Effect of prolactin on the prostate. *Prostate* **24**, 162–166.
15. Tangbanluekal, L., and Robinette, C. L. (1993) Prolactin mediates estradiolinduced inflammation in the lateral prostate of Wistar rats. *Endocrinology* **132**, 2407–2416.
16. Crépin, A., Bidaux, G., Vanden-Abeele, F., Dewailly, E., Goffin, V., Prevarskaya, N., and Slomianny C. (2007) Prolactin stimulates prostate cell proliferation by increasing endoplasmic reticulum content due to SERCA 2b over-expression. *Biochem. J.* **401**, 49–55.
17. Dagvadorj, A., Collins, S., Jomain, J. B., Abdulghani, J., Karras, J., Zellweger, T., Li, H., Nurmi, M., Alanen, K., Mirtti, T., Visakorpi, T., Bubendorf, L., Goffin, V., and Nevalainen, M. T. (2007) Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. *Endocrinology* **148**, 3089–3101.
18. Nevalainen, M., Valve, E., Ahonen, T., Yagi, A., Paranko, J., and Harkonen, P. (1997) Androgen-dependent expression of prolactin in rat prostate epithelium in vivo and in organ culture. *FASEB J.* **11**, 1297–1307.
19. Janssen, T., Kiss, R., and Schulman, C. (1995) Organ culture of human tissue model of hormonal and pharmacological regulation of benign prostatic hyperplasia and of prostate cancer. *Acta Urol. Belg.* **14**, 7–14.
20. Kadar, T., Ben-David, M., Pontes, J., Fekete, M., and Schally, A. (1988) Prolactin and luteinizing hormone receptors in human benign prostatic hyperplasia and prostate cancer. *Prostate* **12**, 299–307.
21. Prins, G. (1987) Prolactin influence of cytosol and nuclear androgen receptors in the ventral, dorsal and lateral lobes of the rat prostate. *Endocrinology* **120**, 1457–1464.
22. Reiter, E., Lardinois, S., Klug, M., Sente, B., Hennuy, B., Bruyninx, M., Closset, J., and Hennen G. (1995) Androgen-independent effects of prolactin on the different lobes of the immature rat prostate. *Mol. Cell. Endocrinol.* **112**, 113–122.
23. Price, D. (1963) Comparative aspects of development and structure in the prostate. *Natl. Cancer Inst. Monogr.* **12**, 351–369.
24. Smith, C., Assimos, D., Lee, C., and Grayhack, J. (1985) Metabolic action of prolactin in regressing prostate: independent of androgen action. *Prostate* **6**, 49–59.
25. Torres, J. M., Ruiz, E., and Ortega, E. (2001) Effects of CRH and ACTH administration on plasma and brain neurosteroid levels. *Neurochem. Res.* **26**, 555–558.
26. Poletini, M. O., Szawka, R. E., Franci, C. R., and Anselmo-Franci, J. A. (2006) Ovarian steroids but not the locus coeruleus regulate stress-induced prolactin secretion in female rats. *J. Neuroendocrinol.* **18**, 938–948.
27. Maruyama, M., Matsumoto, H., Fujiwara, K., Noguchi, J., Kitada, C., Fujino, M., and Inoue, K. (2001) Prolactin-releasing peptide as a novel stress mediator in the central nervous system. *Endocrinology* **142**, 2032–2038.

28. Debeljuk, L., Rozados, R., Daskal, H., Velez, V., and Mancini, A. (1975) Acute and chronic effects of sulpiride on serum prolactin and gonadotropin levels in castrated male rats (38581). *Proc. Soc. Biol. Med.* **148**, 550–552.
29. Nakagawa, K., Obara, T., Matsubara, M., and Kubo, M. (1982) Relationship of changes in serum concentrations of prolactin and testosterone during dopaminergic modulation in males. *Clin. Endocrinol.* **17**, 345–352.
30. Van Coppenolle, F., Slomianny, C., Carpentier, F., Le Bourhis, X., Ahidouch, A., Croix, D., Legrand, G., Dewailly, E., Fournier, S., Cousse, H., Authie, D., Raynaud, J. P., Beauvillain, J. C., Dupouy, J. P., and Prevarskaya, N. (2001) Effects of hyperprolactinemia on rat prostate growth: evidence of androgeno-dependence. *Am. J. Physiol. Endocrinol. Metab.* **280**, 120–129.
31. Torres, J. M., and Ortega, E. (2004) Quantitation of mRNA levels of steroid 5α-Reductase isozymes: a novel method that combines quantitative RT-PCR and capillary electrophoresis. *Int. J. Biochem. Cell Biol.* **36**, 78–88.
32. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159.
33. Amenta, D., Cavallotti, C., and Amenta, F. (1987) Dopamine receptors mediating the stimulation and the inhibition of adenylate cyclase in rat prostate gland. *Neurosci. Lett.* **1**, 66–70.
34. Van Coppenolle, F., Le Bourhis, X., Carpentier, F., Delaby, G., Cousse, H., Raynaud, J. P., Dupouy, J. P., and Prevarskaya, N. (2000) Pharmacological effects of the lipidosterolic extract of Serenoa repens (Permixon) on rat prostate hyperplasia induced by hyperprolactinemia: comparison with finasteride. *Prostate* **43**, 49–58.
35. Yamanaka, H., Kirdani, R. Y., Saroff, J., Murphy, G. P., and Sandberg, A. A. (1975) Effects of testosterone and prolactin on rat prostatic weight, 5α-reductase, and arginase. *Am. J. Physiol.* **229**, 1102–1119.
36. Van Coppenolle, F., Skryma, R., Ouadid-Ahidouch, H., Slomianny, C., Roudbaraki, M., Delcourt, P., Dewailly, E., Humez, S., Crepin, A., Gourdou, I., Djiane, J., Bonnal, J. L., Mauroy, B., and Prevarskaya, N. (2004) Prolactin stimulates cell proliferation through a long form of prolactin receptor and K+ channel activation. *Biochem. J.* **377**, 569–578.
37. Farnsworth, W. (1988) Prolactin effect on the permeability of human benign hyperplastic prostate to testosterone. *Prostate* **12**, 221–229.
38. Ruiz, E., Ortega, E., Mendoza, C., and Osorio, C. (1984) Effects of sulpiride on levels of FSH, LH and steroid hormones. *Rev. Esp. Fisiol.* **40**, 243–248.
39. Huang, W. J., Yeh, J. Y., Kan, S. F., Chang, L. S., and Wang, P. S. (2001) Effects of hyperprolactinemia on testosterone production in rat Leydig cells. *J. Cell. Biochem.* **80**, 313–320.
40. Bartke, A., Smith, M. S., Michael, S. D., Peron, F. G., and Dalterio, S. (1977) Effects of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. *Endocrinology* **100**, 182–186.
41. Torres, J. M., and Ortega, E. (2003) Differential regulation of steroid 5α-reductase isozymes expression by androgens in the adult rat brain. *FASEB J.* **17**, 1428–1433.
42. Novelli, G., Margiotti, K., Sangiulo, F., and Reichardt, J. K. (2001) Pharmacogenetic of human androgens and prostate disease. *Pharmacogenomics* **2**, 65–72.
43. Yatani, R., Kusano, I., Shiraishi, T., Miura, S., Takanari, H., and Liu, P. I. (1987) Elevated prolactin level in prostates with latent carcinoma. *Ann. Clin. Lab. Sci.* **17**, 178–182.