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Transgenic Mice Overexpressing the Prolactin Gene Develop Dramatic Enlargement of the Prostate Gland*

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ABSTRACT

An altered endocrine status of elderly men has been hypothesized to be important for development of prostate hyperplasia. The present study addresses the question whether increased PRL expression is of importance for development of prostate hyperplasia in mice. Three lines of PRL transgenic mice were generated having serum levels of PRL of approximately 15 ng/ml, 100 ng/ml, and 250 ng/ml, respectively. These mice developed dramatic enlargement of the prostate gland, approximately 20 times the normal prostate weight and they had a 4- to 5-fold increased DNA content. Histologically, the prostate glands in the transgenic mice were distended from secretion, and the amount of interstitial tissue was increased. The levels of testosterone and IGF-I were increased in the PRL transgenic animals. In mice overexpressing the bovine GH gene, displaying elevated IGF-1 levels, the prostate gland was slightly larger compared with normal mice, indicating that the effect of PRL was not primarily mediated through elevated plasma IGF-I levels. The present study suggests that PRL is an important factor in the development of prostate hyperplasia acting directly on the prostate gland or via increased plasma levels of testosterone. (*Endocrinology* **138:** 4410–4415, 1997)

HYPERPLASIA of the prostate gland is a very common disease among older men affecting over 50% of men older than 50 yr of age and more than 95% of the male population after 70 yr of age (1). The altered endocrine status of aging men is likely to be of importance for development of the disease.

The level of PRL increases with age (2, 3), coinciding with development of prostate hyperplasia. PRL is a multifunctional pituitary hormone involved in a wide variety of physiological processes. In vertebrates, many diverse effects have been attributed to PRL, including reproduction, immune response, osmoregulation, cellular proliferation, and differentiation (4, 5). The most studied functions of PRL in mammals are the effects on mammary gland development, mammary growth, and stimulation of transcription of milk protein genes (6). The prostate has PRL receptors (PRLR) (7, 8) and both trophic and differentiating effects have been observed (9).

The role for PRL in disease of the prostate is, however, not clear. In the human, conflicting results concerning the PRL levels in patients with prostate hyperplasia have been reported demonstrating both increased serum levels (10, 11), unchanged levels (12), and increased levels only in patients with prostate cancer (12). These discrepancies may partially be explained by the recent observation that PRL is locally produced in the human prostate (13). A possible effect of PRL on the prostate is also suggested by the observation that hypophysectomy has an additive effect when combined with castration and adrenalectomy compared with only castration and adrenalectomy in patients with metastatic prostate cancer (14).

Androgens are known to be very important for development of the prostate gland. However, the level of testosterone is decreasing with age (15, 16), indicating that other factors than androgens could be important for development of prostate hyperplasia.

The aim of the present study was to study the effects of increased levels of PRL on the prostate gland by creating PRL-transgenic mice. We now report that PRL-transgenic mice develop dramatic prostate enlargement.

Materials and Methods

Construction of the metallothionein promoter-rat PRL plasmid

The rat PRL expression vector, Mt.rPRL-WBO2 was based on the pRPRL-*Hin*dIII A and B plasmids described earlier (17) and the methallothionein-1 (Mt-1) promoter from MtbGH 2016 plasmid (18). The Mt-1 promoter was subcloned as a 650-bp fragment into a BsmF1 site 5′ of the start codon in the rat PRL gene inserted in a pGEM-7Z vector (Promega) resulting in the Mt.rPRL-WBO2 plasmid (see Fig. 1). The Mt-1-rPRL fragment was excised by digestion with *Bst*EII, located in the Mt-1 promoter, and *Bam*HI located 3′ in the polylinker of pGEM-7Z.

Transgenic mice

Transgenic mice were generated in C57BL/6JxCBA-f2 embryos by standard microinjection procedures (19). The DNA fragment to be injected was excised from the plasmid Mt.rPRL-WBO2 by restriction enzyme cleavage with *Bst*EII and *Bam*HI, separated by gel electrophoresis through a 0.7% agarose gel, cut out, isolated using isotachophoresis (20), and precipitated with ethanol. The bovine GH (bGH) DNA fragment was isolated from the plasmid MtbGH 2016 (generously provided by Dr.

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R. D. Palmiter) as a *Bst*EII-*Eco*RI fragment, separated by gel electrophoresis through a 1% agarose gel, cut out, isolated using Genclean II kit (Bio 101).

To identify transgenic animals, DNA was extracted from 0.5 cm sections of tails from 3-week-old mice by digestion with 400 mg of proteinase K in 0.6 ml of 1 M urea, 100 mM NaCl, 50 mM Tris HCl (pH 8.0), 10 mM EDTA, 0.5% SDS at 55 C for 16 h. The digested tails were frozen for 2 h in −70 C, then precipitated with isopropanol and washed with ethanol. The presence of the Mt-1 rPRL transgene was detected with PCR (94 C for 5 min and 30 cycles of sequential incubations at 94 C for 30 sec, 54 C for 30 sec, and 72 C for 120 sec) using one primer located in the Mt-promoter (5′-GCGAATGGGTTTACGGA-3′) and one in the rPRL gene (5′CCATGAAGCTCCTGATGCT-3′). Mice that had integrated the bGH transgene were identified with PCR (the same incubation conditions as for rPRL) using the same Mt-promoter primer and one primer located in the bGH gene (5′-CTCCAGGGACTGAGAACA-3′). The animals were housed under standard conditions and tap water and pelleted food were freely available.

RNA analysis

Total RNA was isolated from frozen tissues by acid guanidinium thiocyanate-phenol-chlorophorm extraction described by Chomczynski and Sacchi (21). Specific RNA was analyzed using an RT-PCR assay. The RT reaction was performed with 0.5 μg RNA as a template in the presence of 0.25 μg oligo-(deoxythymidine) primer (Promega), 5 U AMV-RT (Promega), 20 U RNAsin (Promega), and dNTP (Pharmacia) at a final concentration of 1 mM per nucleotide. RT buffer (50 mM Tris-HCl; pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM spermidine, and 10 mM DTT) was added to a total volume of 20 μl. After denaturation at 70 C for 5 min and annealing in room temperature for 10 min, the elongation was carried out for 60 min at 42 C. The RT reaction was terminated by heat inactivation (95 C for 7.5 min). Rat PRL-specific RNA was analyzed by amplifying an aliquot of complementary DNA (cDNA) by PCR (94 C for 5 min and 30 cycles of sequential incubations at 94 C for 30 sec, 60 C for 30 sec and 72 C for 120 sec) using a sense primer located in exon 4 (5-TCCATGAAGCTCCTGATGCT-3′) and an antisense primer located in exon 5 (5-GGATGGAAGTTGTGACCA-3′) specific for rat PRL (see Fig. 3). The PCR products were analyzed by electrophoresis in 1% agarose gel. The size of the fragment amplified from spliced RNA should be 152 bp and that from unspliced RNA or contaminating DNA 1252 bp. The fragments were transferred to Hybond-N nylon membranes (Amersham) and the membranes were baked in 80 C for 2 h and prehybridized in hybridization buffer (0.2 M NaH_2PO_4, pH 7.4, 8% SDS, 1 mM EDTA, 1% BSA fraction V) at 60 C for 2 h. As probe, a 823 bp *Pst*I fragment (22) containing the rat PRL cDNA was used, labeled with a random priming kit (Amersham) and P^{32}dCTP. The hybridization was carried out in the same buffer at 60 C for 12–16 h and washed with 2 × SSC, 0.5% SDS at 60 C for 1–2 h and with 0.1 × SSC, 0.1% SDS at 60 C for 0,5–2 h.

Mouse PRL specific RNA was analyzed by amplifying cDNA by PCR (94 C for 5 min and 30 cycles of sequential incubations at 94 C for 30 sec, 56 C for 30 sec and 72 C for 120 sec) using a sense primer located in exon 1 (5′-GTCACCATGACCATGAAC-3′) and an antisense primer located in exon 5 (5′-GGATGGAAGTTGTG ACCA-3′) for controls and in exon 4 (5′-TGGCTCTTGATAGGATGTAT-3′) for transgenic animals. The size of the fragment amplified from spliced RNA should be 558 bp and 442 bp. The transfer, hybridization, probe labeling, and washing were carried out as above. To use as a probe, mouse PRL cDNA was amplified from mouse pituitary cDNA by the same protocol and primers used for detection of mouse PRL expression in prostate. The PCR fragment was subcloned into a pCRTMII vector (Invitrogen) and identified as mouse PRL by digestion with restriction enzymes. As probe we used the entire subcloned fragment from the vector.

Specific RNA for the long form of the mouse PRL receptor was amplified by PCR (94 C for 5 min and 30 cycles of sequential incubations at 94 C for 30 sec, 56 C for 30 sec and 72 C for 120 sec.) using a sense primer located in the extracellular part of the receptor (5′-GACTCGCT-GCAAGCCAGACC-3′) and an antisense primer located in the intracellular part of the long form of the receptor (5′-TGACCAGAGTCACT-GTCAGG-3′). The size of the fragment amplified from spliced RNA should be 440 bp. The transfer, hybridization, probe labeling, and washing were carried out as above. An *Eco*RI-*Xho*I fragment of the plasmid 4A314 (R. Ball, Basel, Switzerland, unpublished data) containing the long form of the mouse PRL receptor cDNA was used as a probe.

DNA content analysis

Total nucleic acids (TNA) were extracted by homogenization of frozen tissues in 1% SDS, 20 mM Tris-HCl (pH 7.5), and 4 mM EDTA, followed by a 45-min digestion with proteinase-K in 45 C and extraction with phenol-chloroform (23). The DNA content in the TNA preparations were measured with a fluorescence spectrophotometer (450 nm excitation and 555 nm emission) after addition of Hoecht's dye H 33258 (0.2 μg/ml in 2 M NaCl, 1 mM EDTA and 10 mM Tris, pH 7.4).

Measurement of rat PRL

Serum levels of rat PRL were measured by rat PRL RIA (Amersham UK) according to a protocol from the manufacturer. Mouse PRL does not cross-react with the antibody raised against rat PRL according to the manufacturer. Serum was either collected from the mouse tail in heparin coated glass capillaries or by heart puncture in heparin coated syringes when killed. All samples were analyzed in duplicates.

Measurement of IGF-I

The IGF-I concentration in serum was determined by a RIA after acid ethanol extraction according to the manufacturer's protocol (Nicols Institute Diagnostics, San Juan Capistrano, CA) in a single assay.

Measurement of testosterone

Serum testosterone was measured by RIA according to the manufacturer's protocol (ICN Biomedicals, Inc., Costa Mesa, CA)

Histology

Tissue pieces were fixed in 4% paraformaldehyde in PBS (pH 7.4) overnight or longer, dehydrated, and embedded in paraffin. Sections were stained in hematoxylin/eosin.

Statistics

Statistical differences were calculated using the Wilcoxon rank sum test. Significance levels less than 0.05 were considered significant. Values are given as mean ± SEM.

Results

Generation of rat PRL transgenic mice

The rat PRL gene has been described and contains 5 exons. The entire gene is approximately 11 kb, and the cDNA is 800 bp. In the Mt.rPRL-WBO2 plasmid used to generate rPRL transgenic mice, the metallothionein-1 (Mt-1) promoter was inserted 33 bp upstream of the first exon into a BsmF1 site in the rPRL gene. The linearized fragment from the plasmid outlined in Fig. 1 was microinjected into 250 mouse zygotes obtained after superovulation and implanted into 9 foster mothers resulting in 40 newborn mice. Three mice were identified as carrying the Mt-rPRL construct using PCR analysis. The founder animals, two females and one male, were mated for establishing lines of transgenic mice. Transgenic lines were successfully established from the female founder animals mated to normal male mice.

Heterozygot male offspring from a bGH transgenic founder were used. Male mice from this line have serum levels of bGH higher than 5 times the normal peak values in mice (24).

The transgenic mice had elevated serum levels of rPRL

The rPRL levels in the founder animals were measured by RIA at 35 days of age. One female founder (L1) had very high levels of PRL (470 ng/ml), and the other female founder (L2) expressed the transgene at lower levels (11 ng/ml). The rPRL levels in the male founder (L3) was 32 ng/ml (no line was established from this founder due to infertility). Rat PRL levels were also measured in all the animals included in the study when killed (Table 1). Serum levels of rPRL were stable over the life span of the animals. Offspring generated from transgenic line L1 showed consistently high serum rPRL-levels, whereas offspring generated from L2-line expressed the transgene at lower levels (Table 1).

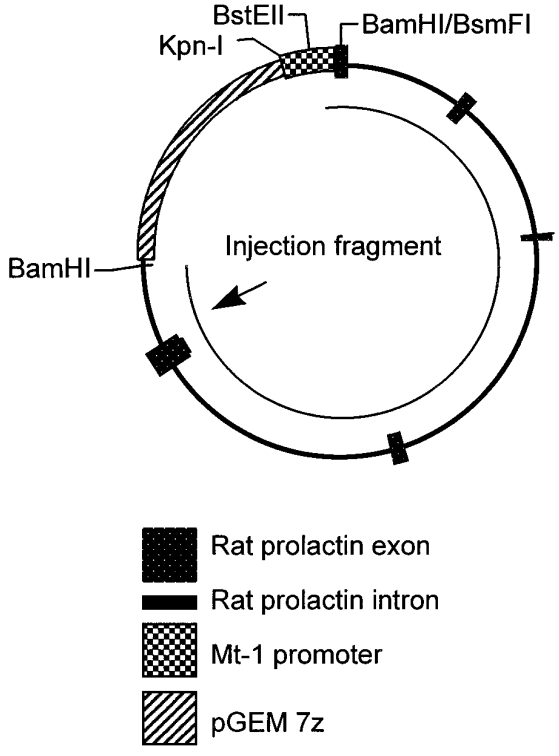

FIG. 1. The Mt-rPRL-WBO2 plasmid. The metallothionein promoter, the rat PRL gene, and the injection fragment are indicated.

Rat PRL transgenic mice developed marked enlargement of the prostate

The weight of prostate was examined when the animals were killed at 10–15 months of age. Histological examinations were carried out on all prostates. All prostates from PRL transgenic mice had a higher weight compared with age matched controls (Table 1). On average, the dorso-lateral lobes from the prostate were 20 times larger (wet weight) than the controls, and the ventral parts were 9 times larger (wet weight) (Table 1). The bGH transgenic mice had 1,6-times larger dorso-lateral lobes of the prostate glands (Table 1) and also increased body weight (1.4 times larger than controls, data not shown). In contrast, the body weight of the PRL transgenic mice were not increased (data not shown).

The DNA content in the prostate glands were measured in rPRL transgenic animals (n = 5) and controls (n = 5) when killed. The total DNA in the dorso-lateral lobe was increased 4.7 times (155 ± 34 μg DNA/prostate lobe *vs.* 33 ± 5 μg DNA/prostate lobe in the controls, $P < 0.01$) and in the ventral lobe 4.2 times (96 ± 11 μg DNA/prostate lobe *vs.* 23 ± 5 μg DNA/prostate lobe in the controls, $P < 0.01$).

Histologically, in contrast to controls (Fig. 2a) all prostates from PRL transgenic mice showed hyperplasia and glands distended by secretion mingled with nests of small glands (Fig. 2b). The amount of stroma was increased in the rPRL transgenic animals compared with controls (Fig. 2, a and b).

TABLE 1. Age when sacrificed, plasma levels of rat prolactin, testosterone and IGF-I and weight of different lobes of the prostate gland in rat PRL and bGH transgenic mice and normal control animals

Animal	Age (Months)	Rat PRL (ng/ml)	Dorso-lateral prostate (g)	Weight compared with normal (%)	Ventral prostate (g)	Weight compared with normal (%)	Testosterone (nmol/ml)	IGF-I (ng/ml)
PRL transgenic animals (n = 9)								
2 (L1)	14	235	0.65	2000	0.19	943	34	569
14 (L1)	10	260	0.97	2985	0.11	546	34	397
16 (L1)	11	281	0.60	1846	0.17	844	16	513
1 (L2)	14	17	0.56	1723	0.24	1190	13	238
3 (L2)	14	16	1.07	3292	0.19	943	25	472
4 (L2)	14	17	0.51	1569	0.21	1043	34	440
5 (L2)	12	12	0.57	1754	0.20	993	5.0	358
7 (L2)	14	15	0.48	1477	0.13	645	26	311
6 (L3)	15	103	0.26	800	0.15	745	3.7	433
Mean	13		0.63[a,b]	1938	0.18[a,b]	877	21[a]	415[c]
SEM	1		0.08		0.01		3.0	34
GH transgenic animals (n = 5)								
Mean	11.6		0.05[c]	159	0.02	100	8.3	495[a]
SEM	0.25		0.01		0.005		2.0	57
Normal animals (n = 14)								
Mean	12.6		0.03		0.02		6.2	317
SEM	0.46		0.002		0.002		2.9	18

[a] = $P < 0.01$ and [c] = $P < 0.05$ PRL or bGH transgenic compared with control animals and [b] = $P < 0.01$ PRL transgenic animals compared with bGH transgenic animals.

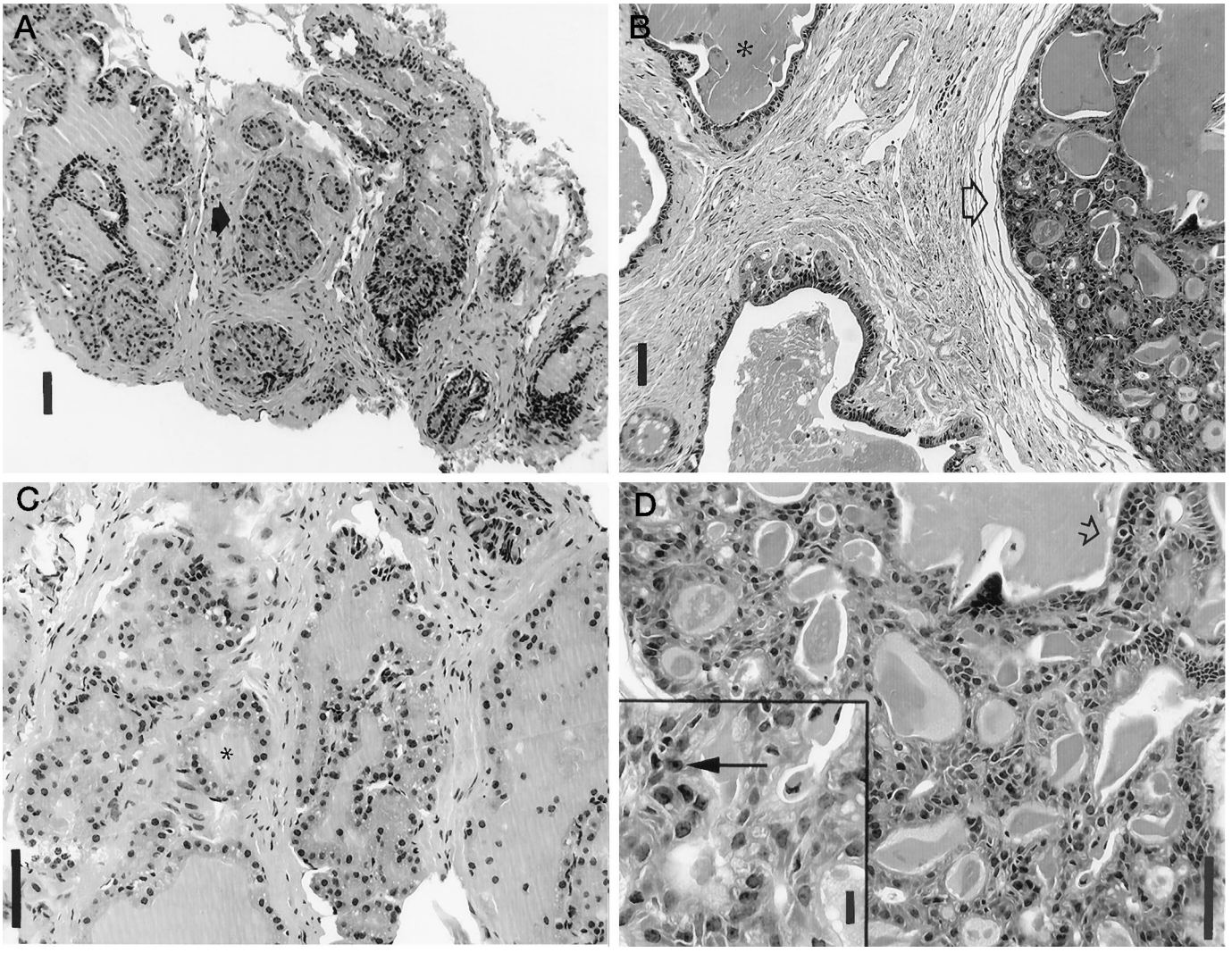

FIG. 2. Histology of the prostate. A, Prostate from a normal mouse killed at 14 months of age. Normal prostate gland showing lobules of mostly small, regular glands (*arrow*) separated by a moderate amount of collagenous stroma (*bar* represents 50 μm). B, Prostate gland from a PRL transgenic animal (animal no. 4 in Table 1). Prostate showing a mixture of dilated, secretion filled glands (*) and tightly packed groups of small proliferating glands (*arrow*). In contrast to the controls, the collagenous stroma is abundant (*bar* represents 50 μm). C, Prostate gland from a normal mouse. Normal prostate showing acini lined by a single layer of cuboidal or low columnar epithelium (*). Acini are separated by a moderate amount of stroma (*bar* represents 50 μm). D, Prostate gland from a PRL transgenic animal (animal no. 4 in Table 1). The glandular epithelium is hyperplastic and shows a moderate polymorphism (*arrow, bar* represents 50 μm). *Inset* shows focally prominent nucleoli (*arrow, bar* represents 10 μm).

The glandular epithelium of the transgenic mice was hyperplastic and revealed a moderate nuclear polymorphism and focally displayed prominent nucleoli compared with controls (Fig. 2, c and d).

The rPRL transgene, the endogenous mPRL gene and the PRLR were expressed in the prostate gland

Specific messenger RNA (mRNA) for the rPRL transgene was detected in both the dorso-lateral part of the prostate and in the ventral lobe (Table 2) in all of the lines (L1, L2, and L3) measured with RT-PCR. In normal and transgenic animals, expression of the mouse PRL gene was detected in all parts of the prostate gland (Table 2). Also, PRLR-specific mRNA was detected in the dorso-lateral lobe and the ventral lobe of the prostate (Table 2). Several different tissues were analyzed for the presence of mRNA corresponding to rPRL using a RT-PCR assay. The primers were selected in a way that the PCR reaction could not amplify cDNA corresponding to expression of the mouse PRL gene. The transgene was expressed in the liver, kidney, pancreas, seminal vesicles, testis, thymus, and the prostate gland (Fig. 3).

Rat PRL transgenic mice had elevated levels of testosterone and of IGF-I. bGH transgenic mice had elevated levels of IGF-I

The serum levels of testosterone and IGF-I were measured when the animals were killed. PRL transgenic mice had higher levels of testosterone than controls (Table 1). The testosterone levels among the individual rPRL transgenic mice could not be correlated to the prostate weight in neither

the dorso-lateral lobe nor the ventral lobe (r = 0.50 and r = −0.20, respectively $P > 0.05$).

The IGF-I levels were elevated in the rPRL- and the bGH transgenic animals compared with controls (Table 1).

Discussion

In this study, we report creation of transgenic mice overexpressing the rat PRL gene. We observed that a particularly significant alteration in the transgenic phenotype was a dramatic enlargement of the prostate gland.

The enlargement of the prostate in the transgenic animals was a result of increased secretion of the prostate glands but also a true hyperplasia because the number of cells were increased by 4- to 5-fold. The proportion of connective tissue compared with epithelial cells were increased in the PRL transgenic animals, thus resembling the histological finding in benign prostate hyperplasia (BPH) of man (25). In the PRL transgenic animals, the dorso-lateral part of the prostate, corresponding to the part of the human prostate where BPH develops (26), showed a greater increase in weight and DNA content than the ventral part.

The effect of the PRL could either be direct mediated via PRLR in the prostate gland (7, 8), indirect via receptors (for references, see 5) in other organs (*e.g.* by stimulation of testosterone synthesis from the testis; 27) or a combination of both.

TABLE 2. Expression of the rat PRL transgene, the mouse PRL gene, and the PRL receptor in the prostate measured by RT-PCR as described in *Materials and Methods*

Animal	Prostate lobe	Transgenic rat PRL	PRL receptor	Mouse PRL
Transgenic animals				
L1	Dorso-lateral	+	+	+
	Ventral	+	+	+
L2	Dorso-lateral	+	+	+
	Ventral	+	+	+
L3	Dorso-lateral	+	+	+
	Ventral	+	+	+
Normal animals				
Normal	Dorso-lateral	na	+	+
	Ventral	na	+	+

Different lobes of the prostate glands were collected at 11 to 15 months of age. Nine transgenic animals from 3 different lines and 4 normal animals were analyzed. +, Positive; na, not analyzed.

The importance of androgens for growth of the prostate gland is indeed unquestionable, and removal of androgens from patients with prostate cancer has beneficial effects. In the prostate, PRL can increase cytosol and nuclear androgen receptor levels in the rat (28) and also increase the uptake of testosterone in man (29), suggesting that PRL can increase the tissue concentration or sensitivity for testosterone. However, long-term elevation of testosterone in rats does not result in an increased growth of the prostate above normal size (30), not supporting that elevated testosterone levels are the major cause of the enlargement and hyperplasia in the PRL transgenic mice in this study. Moreover, testosterone levels in the PRL transgenic mice could not be correlated to the prostate weight.

The PRL-transgenic mice had elevated IGF-I levels. However, serum IGF-1 is not likely to be the major mediator of the effects of PRL because mice transgenic for bGH with normal or slightly enlarged prostate glands in relation to body weight (31 and the present study), had at least as high serum IGF-I levels as the PRL-transgenics.

Because PRL was produced in the normal prostate gland (13 and the present study) and rat PRL was expressed in our transgenic model, it could be possible that PRL acts in a paracrine or autocrine fashion to stimulate the prostate growth in addition to an endocrine mechanism of action. This hypothesis needs to be further clarified in future studies.

The additional beneficial effects of hypophysectomy combined to orchidectomy and adrenalectomy compared with orchidectomy and adrenalectomy alone on the growth of prostate cancer metastases in man suggest that some pituitary hormone(s) is involved in stimulation of the prostate gland. Experiments using isografted pituitaries have shown a stimulation of prostate growth (32). However, isografts also secrete other pituitary hormones, *e.g.* GH (33). GH is structurally related to PRL and both the GH-receptor and the PRLR belong to the cytokine receptor superfamily (34). The

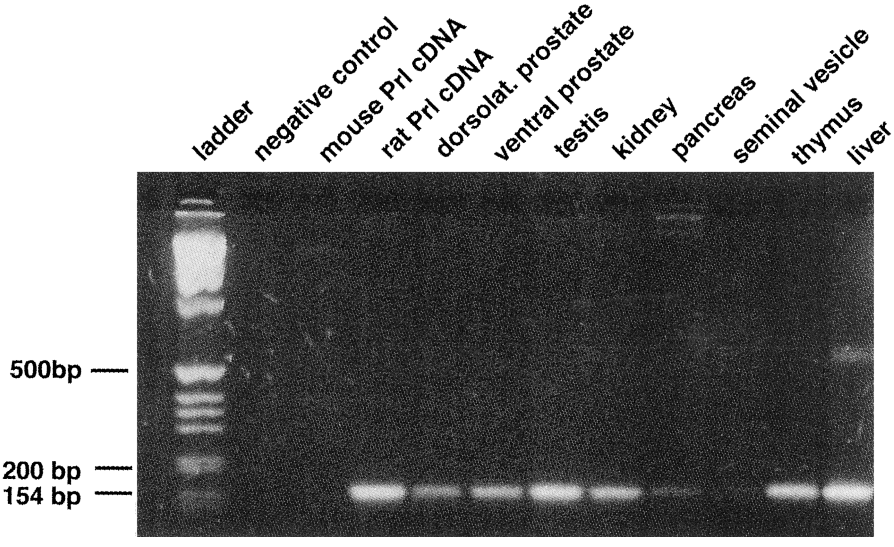

FIG. 3. Expression of the PRL transgene in different organs were analyzed with RT-PCR as described in *Material and Methods*. Tissue samples were collected from the line L1 at 14 months of age. The different organs analyzed are indicated. An expected, 152 bp fragment was detected in all organs analyzed. As a negative control the PCR reaction was run without any template added. Appropriate lanes from the same ethidiumbromide gel were selected and shown.

prostate gland of mice transgenic for bovine GH (bGH) were slightly larger than in controls (31 and the present study). In contrast to studies using pituitary isografts, all effects observed in PRL transgenic animals can be attributed directly or indirectly to the elevated PRL levels.

PRL transgenic animals may serve as a useful model for studying prostate hyperplasia. In addition to previously described transgenic models using expression of int-2 (35) and large-T (36, 37), the present model represents a hormone-dependent hyperplasia and might therefore be closer to the human patophysiology. Another similarity to BPH in man is that the stroma compartment of the prostate in the rat PRL transgenic mice is enlarged, in contrast to the int-2 and large T models (35–37).

In the human, the influence of PRL on prostate growth is not clear, but the present study suggests that the question whether PRL is an important factor for development of prostate hyperplasia in man should be addressed.

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