Age-Dependent and Lobe-Specific Spontaneous Hyperplasia in the Brown Norway Rat Prostate


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ABSTRACT

We showed previously that exogenously administered testosterone caused age- and lobe-specific overgrowth of the prostate in Brown Norway rats. A common feature observed in testosterone-treated animals was cell hypertrophy in each of the ventral, dorsal, and lateral lobes of both young (6 mo old) and old (24 mo old) rats. By contrast, hyperplasia was seen only in the dorsal and lateral lobes of old rats treated with testosterone. These observations prompted us to examine whether age- and lobe-specific overgrowth might also occur in untreated rats as a consequence of the endogenous hormonal milieu. To this end, blood and prostates were collected from a large number (25–30 rats per group) of 4- to 6-mo-old (young) and 21- to 24-mo-old (old) Brown Norway rats. Both serum testosterone (−45%) and estradiol (−22%) concentrations decreased significantly with age, but the greater magnitude of the decrement in testosterone relative to estradiol led to a reduction in the serum testosterone:estradiol ratio. Paradoxically, although the prostate is androgen dependent, the wet weight, protein, and DNA contents increased significantly with age in the dorsal and lateral lobes of old rats despite the decrease in testosterone level. Histologic examination revealed that the increased weights and DNA contents of the dorsal and lateral lobes in old rats coincided with an increased number of epithelial cells in the distal and intermediate segments of these lobes, indicative of hyperplasia but independent of change in cell size. Taken together, these results show a spontaneous age-related overgrowth of cells in the dorsal and lateral prostatic lobes of old Brown Norway rats despite diminished serum testosterone concentrations. The aging Brown Norway rat, therefore, may be a useful model for studies of some aspects of the pathogenesis underlying spontaneous age-related prostatic hyperplasia.

INTRODUCTION

Rodents have been used extensively in prostate research, but in general they are not considered useful for studies of age-dependent spontaneous overgrowth of the prostate. However, several reports have suggested that prostatic hyperplasia can be induced in rodents by means of hormones or chemicals [1–5]. Moreover, we recently showed in the prostates of Brown Norway rats that maintenance of steady-state physiologic to pharmacologic concentrations of testosterone by its exogenous administration for 3 mo caused age-dependent and lobe-specific increases in wet weight, protein content, cell number, and cell size [6]. In particular, increased weights and protein contents were accompanied by epithelial cell hypertrophy in each of the ventral, dorsal, and lateral lobes in both young (6 mo old) and old (24 mo old) rats in response to testosterone. However, significant increases in DNA content were observed only in the dorsal and lateral lobes, not the ventral lobe, of old rats as a function of testosterone dose.

On the basis of our observations of the effects of both pharmacologic and physiologic concentrations of androgen, we hypothesized that age-dependent spontaneous hyperplasia also might occur in this rat strain under the influence of the endogenous hormonal milieu and, if this was the case, that the Brown Norway rat might be a potentially useful model for understanding the pathogenesis of age-related abnormal prostate growth. Herein we report that significant lobe-specific increases in wet weight, protein content, and DNA content and alterations in morphology consistent with cell hyperplasia occur in the prostate of aged Brown Norway rats despite an age-related decline in serum testosterone concentration.

MATERIALS AND METHODS

Animals

Viral antibody-free male Brown Norway rats of ages 4–6 mo (young) and 21–24 mo (old) were obtained from Charles River Breeding Laboratory (Wilmington, MA) under special arrangement with the National Institute on Aging (Bethesda, MD). The rats were fed Purina (Ralston-Purina, St. Louis, MO) lab chow and water ad libitum. Animal protocols were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Hygiene and Public Health.

Measurement of Serum Testosterone and Estradiol Concentrations

At the time animals were killed, trunk blood was collected and allowed to clot for 2 h at room temperature. The serum was separated by centrifugation and stored frozen (−20°C) until assayed. Serum aliquots of 1 ml were extracted twice with 5 ml of anhydrous ethyl ether and then were dried to dryness under nitrogen. Testosterone concentrations were determined by RIA as described by Cochran et al. [7].

For the measurement of estradiol concentrations, an ultra-sensitive estradiol RIA system (Diagnostic Systems Laboratories Inc., Webster, TX) was used. Serum aliquots of 200 μl were used directly in the assay system according to the manufacturer’s directions. The sensitivity of this assay is 5 pg/ml.

Dissection of Prostatic Lobes

The urogenital complex was dissected from the abdominal cavity of each animal and immersed in ice-cold Hanks’ solution.
Balanced Salt Solution (HBSS; Gibco Laboratories, Grand Island, NY), pH 7.4. The tissue was further rinsed and transferred to a Petri dish containing fresh, ice-cold HBSS. The ventral, dorsal, and lateral lobes were separated under a dissection microscope, blotted onto filter paper, and weighed. Each lobe was subsequently divided into two portions. One portion was weighed and snap frozen in liquid nitrogen for subsequent determination of protein and DNA content. The other portion was fixed in Bouin’s fixative and embedded in paraffin for morphological analysis. For the latter, 5-μm longitudinal full-face sections were cut so that distal, intermediate, and proximal segments were seen under a light microscope after staining with hematoxylin and eosin. To enable the proximal segment to be recognized easily, a portion of the urethra was retained to provide a landmark. Moreover, the proximal ducts are unbranched and are surrounded by a very thick stromal layer. The distal segments, which were recognized by infolded columnar epithelial cells surrounded by very thin stromal layer, lie furthest from the urethra at the ductal tips. The branched ducts in between the distal and proximal segments were considered as the intermediate segment.

**Determination of Protein and DNA Content**

Frozen portions of ventral, dorsal, and lateral prostatic lobes from young and old rats were homogenized in ice-cold PBS (1:10 w:v) with a Polytron (Kinematica AG, Luzern, Switzerland) homogenizer. An aliquot of the homogenate was diluted 10-fold, and 10 μl was removed to assay protein content by the method of Smith et al. [8], using the Micro BCA protein assay reagent kit (Pierce Chemical Co., Rockford, IL). BSA was used as the standard. DNA was precipitated with an equal volume of 0.4 M perchloric acid from 1 ml of the tissue homogenate. The DNA pellet was washed in perchloric acid, and after hydrolysis at 70°C in 1 ml 0.8 M perchloric acid, a 250-500-μl aliquot was assayed by the diphenylamine method of Burton [9]. Calf thymus DNA was used as the standard. Total protein and DNA contents per ventral, dorsal, and lateral prostatic lobes were determined by multiplying the amount of protein or DNA per aliquot by the dilution factor adjusted for the total volume of tissue homogenate and tissue weight [10].

**Statistical Analyses**

Data are expressed as the mean ± SEM. Statistical differences between young and old groups were compared by Student’s t-test (p < 0.05).

**RESULTS**

**Age-Dependent Change in Serum Testosterone and Estradiol Levels**

Comparisons of serum testosterone levels between young and old Brown Norway rats are shown in Figure 1. Figure 1A shows the serum testosterone levels for individual rats from the young and old groups. Serum testosterone concentrations in young rats varied between 0.7 and 2.4 ng/ml, whereas in old rats it varied from 0.4 to 1.6 ng/ml. Although there was some overlap, mean serum testosterone levels were significantly (p < 0.05) lower in the old (0.8 ng/ml) than in the young (1.4 ng/ml) rats.

Serum estradiol levels varied widely in both young (range = 7.9–23.9 pg/ml; mean = 15 pg/ml) and old (range = 5.4–26.5 pg/ml; mean = 12 pg/ml) rats (Fig. 1B). The mean values differed significantly (p < 0.05), with the serum estradiol concentration being 22% less in old than in young rats.

Figure 1C shows the serum testosterone:estradiol ratio in young and old Brown Norway rats. Each dot represents the value for an individual young or old rat. The horizontal line indicates the mean value for each age group (young, n = 30; old, n = 25).

**Age-Dependent Change in Wet Weight, Protein, and DNA Contents**

The wet weights, protein contents, and DNA contents of the ventral, dorsal, and lateral prostate lobes from young and old rats are shown in Figure 2. With respect to wet weights, no significant change was seen in the ventral lobe as a function of age (Fig. 2A), whereas wet weights of the dorsal (Fig. 2B) and lateral (Fig. 2C) lobes increased significantly with age by 50% and 70%, respectively. Lobe-specific changes in protein contents were consistent with
wet weights; no change was observed in the ventral prostate (Fig. 2D), but 40% and 50% increases were seen in the dorsal (Fig. 2E) and lateral (Fig. 2F) lobes, respectively, of old rats \( (p < 0.05) \). DNA contents increased significantly with age in the dorsal (Fig. 2H) and lateral (Fig. 2I) lobes, by 50% in both cases, but not in the ventral lobe (Fig. 2G). The DNA content data suggest that spontaneous, age-related increases in cell number associated with hyperplasia occur in the dorsal and lateral lobes of old rats.

Age-Dependent Change in the Histology of Prostatic Lobes

To examine the morphological consequences of the age-related increases in DNA contents in the dorsal and lateral lobes, we examined the histology of the distal, intermediate, and proximal segments of these lobes in comparison to comparable segments of the ventral lobe. Figure 3 shows a comparison of the histology of the ventral lobe between young (A–C) and old (D–F) rats. The distal and intermediate segments of the ventral lobe of young rats were characterized by acini containing tall columnar epithelial cells arranged in a regular, monolayer pattern surrounded by a thin layer of stromal cells (Fig. 3, A and B). Highly infolded glandular acini with nuclei usually located at the base of the epithelial cells were seen predominantly in the distal segment (Fig. 3A). Ducts in the proximal segment of the ventral lobe of young rats were lined with cuboidal epithelial cells surrounded by several layers of stromal cells (Fig. 3C). In old rats, ducts of the distal (Fig. 3D) and proximal (Fig. 3F) segments were indistinguishable from their young counterparts, but in the intermediate segment (Fig. 3E) of each old rat examined, cellular atrophy was typified by the decreased height of the secretory epithelial cells lining the glandular lumen.

Figure 4 shows the dorsal lobe histology. The distal (Fig. 4A) and intermediate (Fig. 4B) segments of this lobe in young rats were composed of ducts lined with low columnar epithelial cells with nuclei located at their base, arranged in a monolayer with some epithelial infoldings and surrounded by a thin stromal layer. Ducts of the proximal segment were lined with cuboidal epithelial cells surrounded by several layers of stromal cells (Fig. 4C). In striking contrast, ducts of the distal (Fig. 4D) and intermediate (Fig. 4E) segments of the dorsal lobe of old rats showed an abundance of luminal infoldings containing an increased number of densely packed columnar epithelial cells with readily
FIG. 3. Low-power photomicrograph (×70) of a portion of the distal (A, D), intermediate (B, E), and proximal (C, F) segments of ventral prostatic ducts from young and old Brown Norway rats. Insets in upper right corner of each micrograph show epithelial cells in higher magnification (×280). A–C Young rats; D–F old rats. Note the profound atrophy of epithelial cells in the intermediate segment of old rats (E). Reproduced at 69%.

...evident darkly stained nuclei. The abundance and density of nuclei suggested that cells within these hyperplastic regions were no longer directly attached to the luminal basement membrane. The epithelial component in the proximal segments of young and old rats was not different (Fig. 4, C and F). Stromal cells also did not differ between young and old rats.

Similar to observations in the dorsal lobe, the distal (Fig. 5A) and intermediate (Fig. 5B) segments of the lateral lobe of the young rats were composed of ducts lined with low columnar epithelial cells surrounded by a thin layer of stromal cells. The epithelial cells were arranged in a monolayer with occasional villous projections. In contrast, the distal (Fig. 5D) and intermediate (Fig. 5E) segments of the lateral lobe of old rats showed an abundance of luminal infoldings with considerable crowding of epithelial cells. The ducts of the proximal segments were lined with epithelial cells arranged in luminal infoldings and surrounded by thick layers of stromal cells in both young and old rats (Fig. 5, C and F).

DISCUSSION

Few reports have supported the rodent prostate as a model for spontaneous overgrowth of this gland [11, 12]. This is not surprising, because most studies have examined responses only of the ventral lobe, where, in fact, age-dependent atrophy has frequently been observed. Moreover, the ventral lobe does not show hyperplasia even after prolonged administration of pharmacological doses of testosterone [13, 14]. In a previous study comparing young and old Brown Norway rats, we reported the dose-dependent effects of chronic steady-state testosterone treatment for 3 mo on the three lobes of the prostate. The ventral lobe responded to exogenous testosterone treatment with a dose-dependent, but age-independent, increase in wet weight that was due to cellular hypertrophy, without evidence for cellular hyperplasia [6]. The dorsal and lateral lobes of young and old rats responded to testosterone similarly to the ventral lobe, with an increase in wet weight and cell size; but interestingly, the DNA content, and hence the cell number, increased in the dorsal and lateral lobes of only the old rats. These results suggested that the secretory capacity, reflected in the cell size of epithelial cells, could be stimulated by testosterone in a dose-dependent fashion in all three lobes of both young and old rats. By contrast, the increased cell number in the dorsal and lateral lobes of old animals suggested that cellular hyperplasia was related to an age-dependent and lobe-specific hypersensitivity to exogenous testosterone treatment. In that study, however, we did not determine whether the hypersensitivity was due to testosterone alone or to a combination of testosterone and its potential in vivo metabolites, which include estradiol. Although the effects of testosterone were dose dependent and appeared to be most dramatic at the highest serum concentrations of testosterone, we also noted that serum testosterone concentrations within the physiologic range at the low-
est dose of exogenous testosterone, released by 3-cm Silastic capsules, also caused cellular hyperplasia in the dorsal and lateral lobes of old rats.

These results prompted us to examine more closely the age-dependent, lobe-specific morphology of the prostate under the endogenous hormonal environment. Herein we show that age-dependent, lobe-specific increases in prostate weight occur in response to the endogenous hormonal milieu in the Brown Norway rat, a condition we refer to as spontaneous overgrowth. In striking contrast to the ventral lobe, the weight of which did not change with age, the dorsal and lateral lobes in old rats increased in weight to 1.5-fold and 1.7-fold, respectively, of young rats. Furthermore, we found that this increase in wet weight was coincident with an increase in DNA content, indicative of increased cell number due to hyperplasia. The occurrence of spontaneous hyperplasia despite a dramatic fall in the serum testosterone concentration of 45% in old rats, together with an age-related but lesser reduction in the serum estradiol level, suggested that the altered androgen:estrogen ratio could have a profound impact on the growth of the prostate.

Benign prostatic hyperplasia (BPH) in aging humans and dogs is associated with a reduced serum androgen:estrogen ratio. Human, dog, and rat prostate epithelial cells express both androgen and estrogen receptors and, consistent with these findings, there is evidence in both dogs [15, 16] and rats [17–19] that estradiol acts synergistically with testosterone to cause overgrowth of the prostate. In fact, we have observed a synergistic effect of combined treatment with estradiol and testosterone on dorsal and lateral prostate overgrowth in Brown Norway rats [20]. The synergistic effects of androgen and estrogen on prostate growth may depend on the strain of rat, age, and the presence of testes, as demonstrated by Hildebrand et al. [21]. A recent study also demonstrated that a small increase in free serum estradiol during fetal life could induce cell proliferation in the mouse prostate, whereas high concentrations of estradiol decreased growth [22].

The age-dependent morphologic changes indicative of epithelial cell hyperplasia in Brown Norway rats are peculiar to the dorsal and lateral lobes, and more specifically, to the intermediate and distal segments within these lobes. By contrast, the intermediate segment within the ventral lobe of old rats actually displayed evidence of cellular atrophy. These results suggest that age-dependent histologic changes do not occur throughout the entire prostate but instead are lobe specific and localized to certain regions despite their exposure to similar concentrations of endogenous steroid hormones and other circulating factors. The histologic appearance of spontaneous hyperplasia in the dorsal and lateral lobes of Brown Norway rats that we observed was very similar to that of the chemically induced atypical hyperplasia reported previously in other strains of rats [4, 5]. The age-dependent epithelial cell hyperplasia in Brown Norway rats differs from BPH in humans, which involves primarily stromal cells within the periurethral region,
whereas canine BPH is characterized by diffuse epithelial or glandular proliferation throughout the prostate.

The lobe-specific nature of the overgrowth in the Brown Norway rat prostate is interesting because in some strains, chemically induced or spontaneous carcinoma develops predominantly in the dorsolateral lobe of the rat prostate [1, 23–28]. Why the dorsal and lateral lobes are predisposed to the development of carcinoma in situ, or overgrowth, is not known. The ventral, dorsal, and lateral lobes of the rat prostate each possess unique biochemical and structural characteristics [10, 29, 30], including differential sensitivity to several hormones [6, 31, 32]. The reason for these unique lobe-specific differences and the basis of this differential hormonal responsiveness are not clear at this time. It is, however, interesting to note that some investigators have suggested that biochemical and anatomical homologies link the human prostate to the dorsolateral lobes in the rat prostate [33, 34], and therefore, our findings may have particular relevance to the pathogenesis of human prostatic disease involving abnormal growth.

The factors that initiate age-related hyperplasia in the dorsal and lateral lobes of the Brown Norway rat prostate are presently unknown. One possibility is that increased rates of cell proliferation, decreased rates of cell death (apoptosis), or both occur during aging. We recently reported age-dependent and region-specific differences in telomerase activity of the prostatic lobes that possibly are related to cell proliferation [35, 36]. In addition, androgen ablation leads to cell death in the ventral, but not in the dorsal or lateral, lobes of the rat prostate [10]. Whether changes in expression of apoptosis inducing or suppressing genes occur during aging in the various lobes of the Brown Norway rat prostate is currently being investigated.

A second possibility is that age-related and lobe-specific changes occur in sensitivity to androgens and/or estrogens, perhaps related to changes in tissue levels of androgen and/or estrogen receptors, 5α-reductase activity, or aromatization of testosterone to estradiol. Androgen receptors are expressed predominantly in epithelial cells of the adult rodent prostate, and the number of receptors is quantitatively greater in the ventral lobe than in the dorsal and lateral lobes. Recent studies suggest that prostatic epithelial cells express predominantly estrogen receptor β, which is abundant in all three lobes, whereas expression of estrogen receptor α appears to be in stromal cells, with higher levels in lateral > dorsal > ventral lobes [37]. Age-related decreases in androgen receptor levels and 5α-reductase activity have been reported previously in rat prostate [38–42]. However, the age-related efficacy of estrogens and the expression of estrogen receptors have not been reported. Based upon this information, the relative prostatic responsiveness to androgens might be predicted to decrease with age, whereas the relative estrogenic effects cannot be predicted.

Thirdly, we and others have demonstrated the hormonal regulation of the expression of growth factors and their receptors, as well as their region-specific expression within the prostate [43–49]. Age-related changes in the mitogenic
pathways linked to growth factor activity may also play a role in prostate growth. Finally, age-dependent alterations in cell function induced by oxidative damage related to oxygen free radical generation may affect DNA repair mechanisms leading to increased cell proliferation and/or cell atrophy. An age-related decline in the activities of several antioxidant enzymes, particularly in the dorsolateral rather than ventral lobes of Noble rats, was suggested as a mechanism underlying the hormonal induction of dysplasia in this strain [50]. We suggest that the Brown Norway rat might represent an appropriate model for study of these and other possible mechanisms underlying spontaneous age- and lobe-specific hyperplasia in the prostate.

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